TITLE

COMPARATIVE STUDIES ON THE EFFECTS OF DIFFERENT MODIFICATIONS ON THE COLD WATER SOLUBILITY OF STARCH FROM SELECTED UNDERUTILIZED LEGUMES

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CERTIFICATION

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To Chief and Mrs R.J.C Arazu.

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ABSTRACT

The industrial utilization of native starch is limited by its imperfect nature, hence the need for modifications. The continued use of conventional crops such as cassava, maize, rice and potato as sources of starch for industrial uses adds to the demand pressure on these crops. On the other hand, the isolation of starch from underutilized legumes will not only reduce this pressure, but will also add value to boost the economic potential of these legumes. This work was aimed at comparing the effects of different modifications on the cold water solubility and functional properties of starch isolated from *Vigna subterranea, Sphenostylis stenocarpa, Cajanus cajan,* and *Mucuna pruriens var pruriens*. The recovered starch yield ranged between 70 – 99 %. Most of the modifications enhanced the desirable properties of the starches. The cold water solubility of the modified starches were in the range of 35 – 81 %, with acid-alcohol, alcohol- alkaline and acid hydrolysis modifications giving the best solubility, while heat moisture treatment and carboxymethylation modifications gave the least solubility. Most of the modified starches had high swelling power, with the exception of the acid treated starches. The pH of the modified starches were around neutrality (pH 7) excluding acid-treated which was neutralised with a 1 M NaOH solution. The water absorbing capacity of the starches increased with increasing solubility for most modified starches excluding the acid-treated starches. There were reductions in the amylose content of most of the modified starches with the exception of the acid hydrolysed, pyrodextrinized and osmotic pressure treated starches. The gelatinisation temperature of the modified starches reduced with increasing solubility. The gelatinisation temperature analyses of the starches ranged between 68 and 72 ^{0}C for the native starches and 34 and 60 ^{0}C for the modified starches. The moisture contents of the modified starches were relatively low. The clarity of the starches varied, with the oxidized and acid-alcohol treated starches giving the highest clarity. The modified starch yield ranged between 70 % and 99 %. Based on visual assessments, the samples were relatively clean and white with a minor contribution of colour from the seed coat of the grains. There were some variations in the transmittance properties of the pastes of the native and modified starches. These properties of modified starches suggest that starches from underutilized legumes could be used in the production of cold water soluble starch thereby increasing its value addition.

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LIST OF ABBREVIATIONS

AH- Acid hydrolysed

Acet- Acetylation

AYB- African Yam Bean

Combo- acid-alcohol + alcohol-alkaline treatments (Dual modification)

CWS- cold water solubility

WAC- water absorption capacity

SP- Swelling power

GT- Gelatinisation temperature

N- Native starch

C/ F- Pigeon pea (*Cajanus cajan*)

M/ E- *Mucuna pruriens var pruriens*

B/ O- Bambara groundnut (*Vigna subterranea*)

A- African yam bean (*Sphenostylis stenocarpa*)

HMT- Heat moisture treatment

ANN- annealing

Pyr- Pyrodextrinization

P- Phosphorylated starch

Ox- Oxidized starch

OP- Osmotic pressure treated starch

Succ- Succinylated starch

A/A- Alcohol-alkaline

Ac/Al- Acid-alcohol

CHAPTER ONE

INTRODUCTION

Starch is an abundant organic polysaccharide molecule in nature with two main components- amylose and amylopectin. The industrial utilisation of native starch is limited because of its imperfect nature therefore the importance of modifications for both industrial and food applications (Kavlani *et al*., 2012). Legumes are fruits or seeds of prime importance in human and animal nutrition and contain moderate amounts of carbohydrate and protein. The legumes in the study (*Cajanus cajan, Mucuna pruriens var pruriens, Sphenostylis stenocarpa, and Vigna subterranea*) is usually cultivated by the rural community. Such subsistence cultivation makes this legume, which show promise despite its shortcomings, underutilized and neglected. In order to increase legume production and utilisation, one of the approaches is to exploit its major components, starch through value-added product design and development strategy (Shimelis *et al*., 2006). Harnessing the potentials of underutilized legumes as invaluable sources of starch could be a way out (Adebowale *et al*., 2002; Adebowale and Lawal 2003; Shimelis *et al*., 2006). Producing starch and starch derivatives from conventional plants like cassava, maize, rice and potato places too much demand on them, particularly as they have to meet the needs of both domestic and industrial uses; thus, the need to include legumes more so, underutilized ones (Lawal, 2004). The industrial utilization of native starch is limited because of its imperfect nature such as water insolubility, low thermal resistance, tendency to easily retrograde, unstable pastes, low thickening power, and viscosity (Akpa and Dagde, 2012; and Ashogbon and Akintayo, 2014). Modification of starch is carried out to enhance the positive attributes, and to eliminate the shortcomings of native starch (Vaclavik and Christian, 2008). Starch modification could be by physical, chemical, enzymatic and genetic methods (Kavlani *et al*., 2012). Subsequently, studies have been performed by preparing cold water soluble starch by various modifications (Sair, 1964; Trubiano, 1987; Khalil *et al.*, 1990; Chen and Jane, 1994; Bello-Perez *et al.*, 2000; Atichokudomchai and Varavinit, 2003; Sanchez-rivera *et al.,* 2005; Sang *et al.*, 2007; Chatakanonda *et al.*, 2011; Xin *et al.*, 2012, Yu *et al.*, 2015).

This work was aimed at comparing different physical and chemical modifications on the cold water solubility of some underutilised legume starches alongside evaluating the functional properties of the starches to improve the method for industrial application.

1.1 Legumes

Legumes are second only to the *Graminiae* in their importance to humans. The seeds are of prime importance in human and animal nutrition due to their high protein content (20 – 50 %) which is significantly more than the level found in their root crop counterparts; yam and cassava (Ustimenko-Bakumovsky, 1983). Legumes are important ingredients of diet in many parts of the world and have been considered as the most significant food sources for people of low incomes (Bressani and Elias, 1979) Legumes contain about 60 % carbohydrates in which starch constitutes the major portion (Sathe and Salunkhe, 1981). Refined starches from several cereals, roots and tubers are used widely in industrial and food applications but legume starches have few commercial uses (Hoover and Sosulski 1989; 1991). Legume plants belong to the family variously referred to as *Fabaceae* or *Leguminosae* within the order Fabales. To date, approximately thirteen to eighteen thousand species of legume have been discovered (Aykroyd and Doughty 1964).

1.1.1 Underutilized Legumes

The concentration on a few major staple crops has resulted in an alarming reduction not only on crop diversity but also the variability within them, especially the neglected and underutilized species (NUS). NUS are indigenous, relatively common, available, accessible, well-adapted, easy and cheap-to- produce crops. Moreover, they are culturally linked to the people who use them traditionally (Jaenicke and Pasiecznik, 2009). Underutilized species are usually ignored by policy makers probably because their economic value is not apparent and hence are excluded from research and development agenda of research and academic institutions (Stifel, 1990).

Across the world, many of the plant species that are cultivated for food are neglected and underutilized while they play a crucial role in food security, nutrition, and income generation of the rural poor (CGIAR, 1999). The term neglected and underutilized species refers to a category of non-commodity cultivated and wild species, which are part of a large agro-biodiversity portfolio today falling into disuse for a variety of agronomic, genetic, economic, social and cultural factors (CGIAR, 1999). Neglected and underutilized species are traditionally grown by farmers in their centres of diversity. While these crops continue to be maintained by cultural preferences and traditional practices, they remain inadequately characterised and neglected by research and conservation. Lack of attention indicates that their potential value is underestimated and underexploited. It also places

them in danger of continued genetic erosion and disappearance which would further restrict development options for the poor. Many neglected and underutilized crop species (NUCS) are nutritionally rich (Dansi *et al.,* 2012); therefore their erosion can have immediate consequences on the nutritional status and food security of the poor while their enhanced use can bring about better nutrition and reduce hunger. A lot of NUCS are recorded to be adapted to difficult environments unfit for other crops where they can provide sustainable productions (Dansi *et al.,* 2012). In this way, they contribute significantly to maintain diversity rich and hence more stable agro-ecosystems. Only about 30 crop species provide 95 % of the worlds' food energy whereas over 7,000 species have been known to be used for food and are either partly or fully domesticated. This large array of plant species includes those recognized to be underutilized as well as those that are recognised as important minor crops. Uses also vary from place to place for instance, the legume Lathyrus is largely used for fodder in Turkey but in South Asia is mostly used as human food (Dansi *et al.,* 2012).

In developing strategic approaches there has been the tendency to build on successful experiences with underutilized crops; Africa hosts thousands of edible plants, but only a small number dominate agriculture. However, these crops decline rapidly and their potential is often overlooked. Worldwide, farmers are abandoning them as globalisation, population growth and urbanisation change agricultural and food systems (Dansi *et al.,* 2012). There is growing international recognition that nutritious NUS crops are important in improving the livelihoods of smallholder farmers in Africa and because many NUS are well adapted to marginal environments, they also offer opportunities for climate change adaptation (Dansi *et al.,* 2012).

Bambara groundnut, for instance, is drought tolerant, which helps farmers manage risks. It has high nutritional value yet its constrained by weak value chains that largely involve local markets. The knowledge acquired in developing value chains of this crop can be applied also to many other NUS. Commercialising NUS requires a holistic value chain approach, supportive policies and receptive markets. The need to harness the potentials of underutilized legumes as invaluable sources of starch and protein concentrates have been emphasised (Adebowale *et al*., 2002, Adebowale and Lawal, 2003). Producing starch and starch derivatives from conventional food crops like cassava, maize, rice and potato, places too much demand on them, particularly as they have to meet the nutritional needs of a high percentage of the population (Adebowale and Lawal, 2003).

1.1.1.1 *Cajanus cajan* (Pigeon Pea)

Though largely considered an orphan crop, pigeon pea has a huge untapped potential for improvement both in quantity and quality of production in Africa. Typically, the average nutritional composition of pigeon pea is 19.2 % protein, 57.3 % carbohydrates, 1.2- 1.5 % fat, 8.1 % fibre and 3.8 % ash (Smartt, 1976). In addition to the benefit of serving as a starch source, such efforts could lead to a reduction in the over-dependence on cassava starch for food and industrial purposes, reduce post- harvest losses and increase the utilization and potential of these largely underutilized crops in Nigeria and most parts of Sub-Saharan Africa (Smartt, 1976). In a study comparing the properties of thermal alkaline treated pigeon pea, Roskhrua *et al.* (2013) reported an increase in the granule morphology and gelatinisation temperature alongside a reduction in the swelling power and viscosity of the modified pigeon pea starch. Srijunthongsiri *et al.* (2014) also reported a decrease in viscosity and an increase in gelatinisation temperature of *Cajanus cajan* starch.

Plate 1- *Cajanus cajan*

Fig. 1: Image showing *Cajanus cajan* plant (Smartt, 1976)

1.1.1.2 *Mucuna pruriens var pruriens*

Mucuna pruriens var pruriens also known as velvet bean is a trailing vine yielding seeds which vary in colour and shape. The seed has a starch content of about 51.5 % (Sridhar and Seena, 2006).

Plate 2- *Mucuna pruriens var pruriens* (Burkill, 1995)

Fig. 2: Image showing *Mucuna pruriens* plant

M. pruriens had a great taxonomic confusion about its varietal difference but now it is accepted that there are two varieties namely, *M.pruriens var. utilis* and *M. pruriens var. pruriens* (Carsky *et al.,* 1998; Sridhar and Seena, 2006). The main differences are the pubescent hairs on the pods, the seed coat colour and duration of harvesting. *M. pruriens* (itching beans) have long stinging hairs on their pods and contact on them results in itching dermatitis, whereas *M. utilis* possess silky hairs on their pods; Interestingly, unconventional legumes as these are promising in terms of nutrition, provision of food security, agricultural development, crop rotation and industrial purposes in developing countries (Sridhar and Seena, 2006). *Mucuna pruriens var pruriens* is an annual perennial, herbaceous, vigorous climbing vine that grows to 3-18 cm in height. The *M. pruriens* is traditionally used as food by certain ethnic groups in a number of countries including India, Philippines, Nigeria, Ghana, Malawi and Brazil (Carsky *et al.,* 1998). Although the *M. pruriens* contains high levels of protein and carbohydrate, its utilization is limited due to the presence of a number of anti-nutritional/ anti-physiological compounds such as phenolics, tannins, L-Dopa, lectins and protease inhibitors, which reduce the nutrient utilization potential (Pugalenthi *et al.,* 2005). Adebowale and Lawal (2003) reported an increase in moisture content and a decrease in solubility for *M. pruriens* starch.

1.1.1.3 *Sphenostylis sternocarpa (*African Yam Bean)

The plant, *Sphenostylis stenocarpa*, is a tuberous underutilized legume of tropical Africa used in human and animal nutrition (Adebowale *et al.,* 2002; Eke, 2002). Like most grain legumes cultivated in Africa, African Yam bean is rich in protein (19.5 %), carbohydrates (62.6 %), fat (2.5 %), vitamins and minerals (Iwuoha and Eke, 1996). The protein is made up of over 32 % essential amino acids, with lysine and leucine being predominant (Onyenekwe *et al*., 2000). In spite of its composition, it has a low consumption rate. This is mainly due to its long cooking time of about 145 minutes (Nwokolo, 1996).

Plate 3– *Sphenostylis stenocarpa* (Iwuoha and Eke, 1996)

Fig. 3: Image showing *Sphenostylis stenocarpa* plant

African yam bean is a vigorous herbaceous climbing vine reaching 1.5 - 2 metres in height producing pods as well as small spindle shaped tubers about 5 - 8 cm long, just like sweet potato. It is usually cultivated as a secondary crop with yam in Ghana and Nigeria. A few farmers who still hold some seed stocks, especially the white with black-eye pattern, plant it at the base of yam mounds in June or July. The crop flourishes and takes over the stakes from senescing yam. It flowers and begins to set fruits from late September and October. The large bright purple flowers result in long linear pods that could house about 20 seeds (Potter, 1992). Adebowale *et al.* (2009) reported a decrease in moisture content making it a good source of starch with a capacity for prolonged shelf life; also, they reported spherical granule morphology.

1.1.1.4 *Vigna subterranea* (Bambara Groundnut)

Though considerably not popular throughout the world, cultivation of *Vigna subterranea* occur in some African countries like Zimbabwe, Ghana, South Africa and Nigeria alongside some countries like India, Sri Lanka, and Malaysia (Goli, 1997).

Plate 4- *Vigna subterranea*

Fig. 4: Image showing *Vigna subterranea* plant (De Kock, 2004).

Bambara groundnut is highly nutritive and contains high quantities of carbohydrate, protein and fat. The starch exhibits higher swelling power, breakdown and set back but lower gelatinization temperature, pasting temperature, water and oil absorption capacity (Gidley, 1987). Bambara groundnut contains 60 % carbohydrate, 20 % protein, 6 % oil and rich in micronutrients, they are reported to provide more methionine than other grain legumes (De Kock, 2004). A few studies have reported on the structure and functional properties for bambarra groundnut starch and flour. Adebowale et al. (2002) reported that the swelling capacity increased with increase in temperature for both starch and flour of bambarra groundnut. This bean flour had higher water absorption capacity than that of Great Northern bean, reported by Sathe and Salunkhe (1981). Piyarat (2007) reported an increase in swelling power and reductions in gelatinisation temperature and absorption capacity.

1.2 Starch

Starch is a natural glucose-based polymer; it is the major carbohydrate storage material which exists in form of granules in many higher plants, and is composed essentially of homopolymer of α-D-glucopyranosyl units and small amounts of non-carbohydrate components , particularly lipids, proteins, phosphorus (Liu, 2005; Xie, 2008).

1.2.1 Sources of Starch

Starch occurs mainly in the seeds, roots and tubers of higher plants (Ashogbon and Akintayo, 2014). Plants synthesize starch during photosynthesis. It is synthesized in plastids as a storage compound for respiration at dark periods, also, synthesized in amyloplasts found in tubers, seeds and roots as a long-term storage compound in which large amounts of starch accumulate as water-insoluble granules (El-Fallal *et al.,* 2012). The shape and diameter of these granules depend on the botanical origin. Regarding to commercial starch sources, the granule sizes range from 2 – 30 μm (maize starch) to 5 – 100 μm (potato starch) (Robyt, 1998). A variety of different enzymes are involved in the synthesis of starch. Sucrose is the starting point of starch synthesis. It is converted into the nucleotide sugar ADP-glucose that forms the actual starter molecule for starch formation. Subsequently, enzymes such as soluble starch synthase and branching enzyme synthesize the amylopectin and amylose molecules (Smith, 1999; El- Fallal *et al.,* 2012).

Starch-containing crops form an important constituent of the human diet. Besides the direct use of starch-containing plant parts as a food source, starch is harvested and chemically or enzymatically processed into a variety of different products such as starch hydrolysates, glucose syrups, fructose, starch or maltodextrin derivatives, or cyclodextrins. In spite of the large number of plants able to produce starch, only a few plants are important for industrial starch processing. The major industrial sources of starch are maize, cassava, potato, and wheat (El-Fallal *et al.,* 2012).

1.2.2 Structure and Properties of Starch

Starch, as extracted from various plant tissues, is obtained in the form of granules with typical particle sizes between 1-100 microns. However, the shape and size of the granules depend on the source (French, 1984; Chen *et al.*, 2010). These granules are present in the chloroplasts of green leaves and in the amyloplasts of storage organs such as seeds and tubers. Starch granule differences amongst various plant species are accounted for, not only

by the ratio of constituent molecules, but also by their location and interaction (Zobel, 1988a, 1988b). The crystalline composition consists of around 15 to 45 % of the starch granules. The diameter of starch granules ranges from 2 to 100 μm depending on its source (Whistler and Daniel, 1985). The crystallinity of native starch varies between 15 and 45 % depending on the origin and pre-treatment (French, 1984). According to the currently accepted concept, amylopectin forms the crystalline component whereas amylose exists mainly in the amorphous form (Zobel, 1992; Hanashiro *et al*., 1996; Marc *et al*., 2002; El-Fallal *et al.,* 2012). Granules are (in fact) insoluble in cold water but swell and form a gel if the outer membrane has been removed by grinding. On the other hand, if a granule is treated in warm water, a soluble portion of the starch diffuses through the granule wall and the remainder of the granules swells to such an extent that they burst (Ashogbon and Akintayo, 2013; 2014).

Starch is made up mostly of amylose and amylopectin chains (Table 1). The structural and molecular arrangement is peculiar for amylose and amylopectin fractions and their ratios in starch vary depending on the source (Ashogbon and Akintayo, 2014). The activity of the enzymes involved in starch biosynthesis may be responsible for the variation in amylose content of the various starches (Krossmann and Lloyd, 2000; Marc *et al.,* 2002). Starch in its native form is insoluble in cold water but can be solubilised by heating with excess water. The change in the conformation during application of moist heat results in the loss of the crystallisation of amylopectin followed by swelling, hydration & solubilisation (Krossmann and Lloyd, 2000). The process is called gelatinisation.

Table 1: Properties of amylose and amylopectin fractions of starch

Properties	**Amylose**	**Amylopectin**
Chain length	Long, straight	Branched
Arrangement	Densely packed	More open, lower density
Size	Small	Large
Rate of digestion	Slow	Faster

Source: (McWilliams, 2001)

1.2.3 Components of Starch

1.2.3.1 Major Components of Isolated Starch

1.2.3.1.1 Amylose

Amylose is a linear molecule consisting of α-(1-4) linked glucose residues with a small fraction of α-(1-6) linkages (Fig. 1). It makes up a minor fraction of the starch granule where it generally accounts for 20 - 30 % of the total starch content (Chen and Jane, 1994). Each macromolecule of the linked glucose residues bears a reducing and non-reducing end. Amylose is located in the granule as bundles between amylopectin clusters and randomly dispersed. They could be located therefore between the amorphous and crystalline regions of the amylopectin clusters (Robin *et al*., 1974). Though amylose is the minor component in most granules, it has a large influence on the properties of starch (Takeda *et al.,* 1992). The length of the amylose chain is not the same for every source; it varies among different plant species but usually ranges between 102 - 104 glucose units. The amylose is essentially linear but not purely and its properties when dissolved are generally regarded as typical of a linear polymer (Biliaderis, 1990). In a study on the properties of thermal alkaline treated pigeon pea, Roskhrua *et al.* (2013) reported a reduction in amylose content after modification.

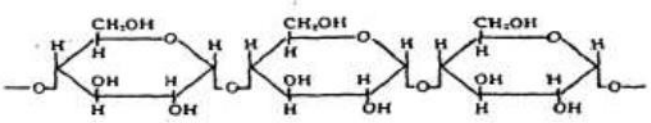


Fig. 5: Structure of amylose (Zhong *et al.,* 2006).

1.2.3.1.2 Amylopectin

Amylopectin is the major constituent of starch which consists of large highly branched molecules; it is composed of linear α- (1-4) linked glucose chains connected by α- (1-6) linkages (Manners, 1989). The average frequency of branching points in amylopectin is 5 % but varies with the botanical origin (Thompson, 2000). The complete amylopectin molecule contains about 2,000,000 glucose units; making it one of the largest molecules in

nature (Marc *et al*., 2002).The multiplicity in branching is a common feature of both amylopectin and glycogen. The outer chains are linked by glycosidic bonds at their potential reducing group through C_6 of a glucose residue to an inner chain (Fig. 2). Such chains are in turn defined as chains bearing other chains as branches. The single other chain per molecule likewise carries other chains as branches but contains the sole reducing terminal residue. The ratio of chain to chain is an important parameter which is also referred to as the degree of multiple branching (Marc *et al*., 2002). Roskhrua *et al.* (2013) reported an increase in the amylopectin content of *Cajanus cajan* starch after modification.

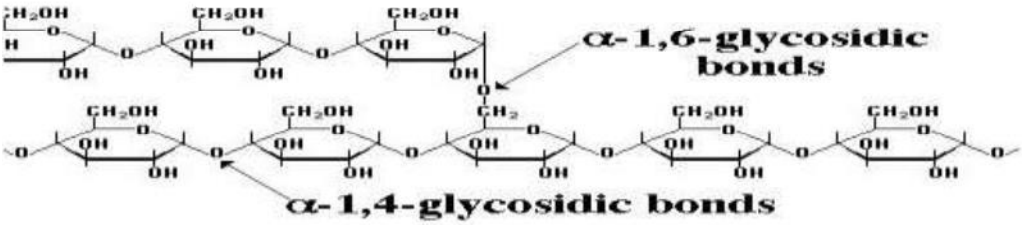


Fig. 6: Structure of amylopectin (Green *et al.*, 1975).

1.2.3.2 Minor Components of Isolated Starch

Besides proteins, other minor constituents including lipids, phosphorus, and trace elements, are commonly found in isolated starch (Champagne, 1996). The minor components are categorized into three groups: particulate material, surface components and internal components (Galliard and Bowler, 1987). Particulate materials are fragments of non-starch material that separate with starch, surface components are associated with the surface of granules and can be removed by extraction, and internal components are materials that are buried within the granule and are inaccessible to extraction unless the granule has been subjected to disruption (Galliard and Bowler, 1987). Starch proteins can be classified as surface proteins which can be extracted in aqueous solutions, or as integral proteins, which are extractable only when a starch solution is heated to temperatures near the gelatinisation temperature (Morrison and Karkalas, 1990; Majoobi *et al*., 2011). Starch contains several different minerals in small amounts, but the most important mineral is phosphorus (Buleón *et al*., 1998). Phosphorus plays an extremely important role in starch functional properties, such as, paste clarity, viscosity consistency, and paste stability. Phosphorus in starch is mainly present in two forms; phosphate-monoesters and phospholipids. In tuber starches, lipids are only found on the granule surface, while starches from cereal endosperm have

surface and integral lipids (Morrison *et al.*, 1984 and Davis *et al.*, 2003). It is well established that polar lipids, e.g. monoglycerides and fatty acids, form a helical inclusion complex with the amylose molecule (Zobel *et al.*, 1988a; Biliaderis, 1990; Rutschman and Solms, 1990).

1.2.4 Starch Biosynthesis

The soluble starch synthases (SSS), together with the starch-bound starch synthase(s) (GBSS) and starch branching enzymes (SBE) form the complex structures of the amylose and amylopectin components of starch (Schulman *et al*, 2008). The SSS synthesizes the linear (alpha-1, 4-glucoside) portion of the amylopectin molecule, which is then branched by the branching enzyme. The GBSS, however, is responsible for producing the amylose exclusively. Starch synthases, both SSS and GBSS, are present in multiple forms. The steps involved in starch biosynthesis include

1. Elongation of the α-1,4-Linked Glucan: Glucose is transferred from ADP-glucose to the non-reducing end of a growing α- 1,4-linked glucan, thus generating an extra glucose with simultaneous release of ADP (Fig. 3). The enzymes responsible for the process are called starch synthases, SS (Davis *et al*., 2003 and Schulman *et al*, 2008).
2. Formation of the α-1,6 Branch: Starch branching enzymes (SBEs) are involved in the synthesis of amylopectin where they create the branch points by hydrolysis of an α-1,4-linkage and subsequent formation of an α-1,6-glucosidic bond between the cleaved chain and a C6 hydroxyl group of an α-1,4-glucan (Andersson *et al*., 2002).
3. Other Enzymes: De-branching enzymes (DBE) hydrolyse the α-1, 6 glucan branches in amylopectin. Two classes of de-branching enzymes, pullulanase and isoamylase, have been identified in plants (Doehlert and Knutson, 1991). DBE is mainly associated with starch degradation, but it also necessary for normal starch biosynthesis (Ball *et al*., 1996). Several other enzymes have been implicated as being involved in starch biosynthesis, such as phosphorylases and the disproportionating enzyme (D- enzyme). D-enzyme catalyses the transfer of α-1, 4-linked oligosaccharides from the end of one glucan chain to another.

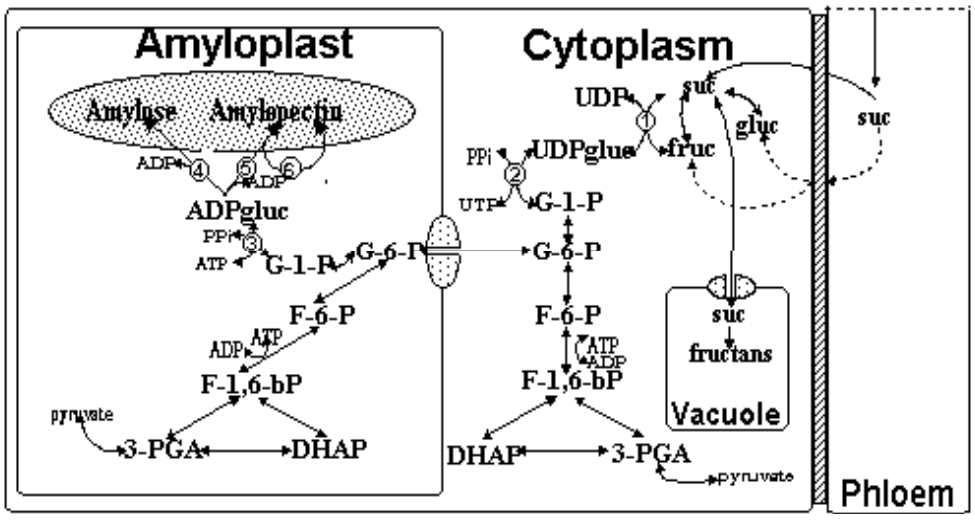


Fig. 7: Biochemical pathway of starch synthesis (Schulman *et al.*, 2008).

1. Sucrose synthase
2. UDP glucose pyrophosphorylase
3. ADP glucose pyrophosphorylase
4. Granule-bound starch synthases
5. Soluble starch synthases
6. Starch branching enzymes

1.2.5 Biodegradation of Starch

Starch is water-insoluble and cannot penetrate into cells thus, the biodegradation of starch occurs extracellularly. Amylases are a class of enzymes that are capable of digesting the glycosidic linkages found in starches. They are mainly secreted into the medium or are found membrane-bound (Vihinen and Mantsala, 1989). The process involves the diffusion of the amylase to the starch, formation of amylase-starch complex, catalysis of the hydrolysis reaction and diffusion of the soluble degradation product (glucose) from the starch to the solution (Slominska *et al.*, 2003). Some of the factors affecting the biodegradation of starch include the physicochemical properties (molecular weight, surface area, crystallinity) and chemical modification which involves either the removal or introduction of chemical groups in the starch chain (Slominska *et al.*, 2003). In a study on starch degradation in germinating lentils by Tarrago and Nicolas (1976), there was an initial slow degradation of starch due to the low amylase activity but after the fourth day, there was a rapid increase in degradation of starch.

1.2.6 Starch Extraction from Grains and Seeds

Grains can be separated into their anatomical parts (bran, germ, and endosperm) by dry milling. Wet milling goes a step further and separates a grain into its chemical components; starch and protein (Hoseney, 1998).

In wet milling, the grain is milled in the presence of water to release the starch, which is subsequently recovered from the water, purified, and dried. Similar to dry milling, the first step is to separate the bran, germ, and endosperm. In some cases the grain is conditioned before wet milling to improve separation. Steeping alters the characteristics of the proteins, making the grains softer, and the germ swollen and rubbery; this improves separation and the release of starch from the protein matrix (Hoseney, 1998). With some grains (e.g. rice) the protein-starch association is very strong and chemical treatments (e.g. soaking in sodium hydroxide) are needed to solubilize the protein to allow the release of starch (Hoseney, 1998).

1.2.7 Functional Properties of Starch in Legumes

The utilisation of starch is dependent on various functional properties such as swelling power, solubility, gelatinisation temperature, viscosity, paste clarity and amylose content (Olu-Owolabi *et al.*, 2014).

1.2.7.1 Solubility and Swelling Power of Starch in Legumes

Starch, a polysaccharide is insoluble in water due to the presence of hydrogen bonds between the hydroxyl group and starch molecule preventing its interaction with water. Also, there is molecular crowding from polymerization (steric hindrance) that decreases the ability of adjacent hydroxyl (-OH) groups from interacting with water (Shimelis *et al.,* 2006). At higher temperatures, these hydrogen bonds are weak, leading the interaction of the hydroxyl group with water leading to starch solubility. The solubility and swelling power provide evidence of the magnitude of interaction between starch chains within the amorphous and crystalline domains (Ashogbon and Akintayo, 2014). The extent of this interaction is influenced by the amylose to amylopectin ratio, and by the characteristics of amylose and amylopectin in terms of molecular weight/distribution, degree and length of branching and conformation (Hoover, 2001).

Swelling power is a measure of the hydration capacity of starch and is expressed as the weight of centrifuged swollen granules, divided by the weight of the original dry starch used to make the paste (Shimelis *et al.,* 2006). The swelling power (SP) is the ratio of the wet weight of the sediment gel to the dry weight of starch (Crosbie, 1991). When starch is heated in excess water, the crystalline structure is disrupted due to the breakage of hydrogen bonds, and water molecules become linked by hydrogen bonding to the exposed hydroxyl group of amylose and amylopectin (Ashogbon and Akintayo, 2014). This causes an increase in granule swelling and solubility (Shimelis *et al.,* 2006). The major factor that controls swelling behaviour and solubility of starch is the strength and character of the micelle network within the granule, which in turn is dependent on the degree and kind of interaction (Teli *et al*., 2009). The extent of this interaction is thought to be influenced by the sample's amylose content, amylose, amylopectin structure and degree of granulation. For example, amylose-lipid complexes have been shown to restrict swelling and solubilisation as reported by Sodhi and Singh (2003). They showed that in five rice starches, the one with the lowest amylose content (7.8 %) had the highest swelling power and lowest solubility; whereas the sample with the highest amylose content had the lowest swelling power. Also, Lii *et al*. (1995) reported a higher swelling power for rice starch with lower amylose content. However, starch swelling power and solubility varies within varieties, the one with the highest swelling power is nearly two times that of the lowest one, but the differences in solubility were much less (Wang and Wang, 2004). In a study with *Vigna subterranea*, Lawal *et al.* (2004) reported an increase in swelling capacity and solubility

1.2.7.2 Gelatinisation of Starch in Legumes

Gelatinisation is an important property of starch which occurs through the collapse of molecular order during the heating process (Kaur *et al*., 2012). It is an important starch functional property that varies with respect to the composition (amylose to amylopectin ratio, phosphorus, lipids, proteins and enzymes, etc.), the molecular structure of amylopectin (unit chain length), extent of branching, molecular weight and granule architecture (crystalline to amorphous ratio), granule morphology and size distribution of starches (Sodhi and Singh, 2003; Kaur *et al*., 2012).

Starch granules are insoluble in cold water. When starch is heated in water, granules absorb water and swell (Ratnayake and Jackson, 2006). The absorption of water by

amorphous regions within the granules destabilizes their crystalline structure, resulting in the loss of birefringence (Donovan, 1979; Biliaderis, 1990; Parker and Ring, 2001). Gelatinisation occurs initially in the amorphous regions as opposed to the crystalline regions of the granule because hydrogen bonding is weaker in these reforms. When starch slurry is heated to its gelatinisation temperature, the granules swell rapidly by absorbing water. The absorbed water enters the amorphous regions of the granules resulting in the disassociation of the intermolecular hydrogen bonds holding the granules together; the granules swell to many times their original volume leading to the disruption of the granular units which collapse with continued heating and agitation (Ratnayake and Jackson, 2006). Srijunthongsiri *et al.* (2014) reported an increase in gelatinisation temperature of heat moisture modified *Cajanus cajan* starch. Also, Lee (2007) reported a gelatinisation temperature range of 58.9 ^{o}C for lentil bean and 65.7 ^{o}C for mung bean with corresponding enthalpy values. Also, Adebowale *et al.* (2009) reported an increase in gelatinisation temperature and viscosity of *Sphenostylis stenocarpa* starch.in a study on the modification and properties of using heat moisture and annealing treatments

1.2.7.3 Retrogradation of Starch in Legumes

Retrogradation describes the process in which a heated starch paste cools below the melting temperature of starch crystallites, and the amylose and amylopectin re-associate and unite with the swollen starch grains in an ordered structure that results in viscosity increase, gel firming, and textural staling of predominantly starch-containing systems (Hoover, 2001). The molecular interactions (hydrogen bonding between starch chains) after cooling of the gelatinised starch paste have been called retrogradation (Hoover, 2001). During retrogradation, amylose forms double-helical associations of 40 - 70 glucose units (Jane and Robyt, 1984) whereas amylopectin crystallization occurs by association of the outermost short branches. The rapid initial rate of retrogradation relates to the loss of networked amylose, the development of amylose aggregates, and binding of granule remnants into assemblies by amylose and amylose aggregates. Thus, amylose is responsible for short-term (less than one day) changes during retrogradation (Zhou *et al*. 2003).

1.2.7.4 Pasting Properties of Starch in Legumes

The physicochemical properties of amylose and amylopectin are quite diverse and it is understandable that these two major components of starch contribute in different ways to

the pasting properties of starch (Be-Miller and Whistler 1996). When starch is heated with water the insoluble granules are disrupted by the energy supplied, resulting in a loss of the molecular organization responsible for the crystallinity and the penetration of water. Swelling of starch granules results in an increase in viscosity, a complete loss of crystallinity, and the loss of birefringence occurs when the heating is continued. The temperature at which birefringence is first lost is called the gelatinisation temperature. For wheat, the temperature range over which this occurs is 53 – 64 ^{0}C, a little lower than for most other starches (Be-Miller and Whistler, 1996).

1.2.7.5 Paste Clarity of Starch in Legumes

Starch clarity is very important for many food applications. The clarity of gelatinised starch suspensions varies among different botanical sources and may be attributed to amylose content and granular size (Sodhi and Singh, 2003). The decrease in clarity has been attributed to leached amylose and amylopectin chains that lead to the development of functional zones that scatter light (Perera and Hoover, 1999).

1.2.7.6 Amylose Content of Starch in Legumes

Amylose was probably the first biopolymer for which a helical structure was proposed (Hanes, 1937). The well-known deep blue complex formed with iodine was later proved to involve amylose in a helical conformation (Rundle and French, 1943). The colour and intensity of the complex depends on the chain length (CL) of the amylose (Bailey and Whelan, 1961). The amylose content of starch can be determined by various methods that tend to produce differing results; this can be by measuring the iodine content potentiometrically or spectrophotometrically as the colour and intensity of the complex depend on the chain length (CL) of the amylose (Bailey and Whelan, 1961). At CL >80 the wavelength maximum (max) of the absorption of light is > 610 nm. The max shifts to lower wavelengths and the colour shifts to red for shorter chains (Bailey and Whelan, 1961). Thus, the short chains of amylopectin possess a maximum absorption of 530-575 nm. Amylose contents of starches can also be estimated directly by high-performance size-exclusion chromatography (HPSEC) (Bradbury and Bello, 1993). The content of amylose and amylopectin varies for the different starches (Chen *et al.,* 1997). In a study on the properties of thermal alkaline treated pigeon pea, Roskhrua *et al.* (2013) reported a reduction in amylose content after modification.

1.3 Modification of Starch

Starch is a natural occurring polymer but has limited applications due to its poor functional properties like its insolubility in cold water, poor pasting properties, low resistance to processing conditions, poor thermal, shear and acid stability, and high rates and extent of retrogradation (Ayoub and Rizvi 2009; Ashogbon and Akintayo, 2014). Therefore, there is the need for modification and enhancement of these properties. Sathe and Salunke (1981) and Dolmatova *et al*. (1998) associated the variation in gel formation of different leguminous flours and starch to the relative ratios of the different constituents (proteins, carbohydrates and lipids) that make up the legumes. Native starch granules are inert, insoluble at ambient temperature, highly resistant to enzymatic hydrolysis and therefore lack specific functional properties. Native starches are often modified to develop specific properties such as solubility, texture, adhesion, and heat tolerance, so as to be suitable for industrial applications (Anderson *et al.,* 2002; Watcharatewinkul *et al.,* 2010). Modification of starch is an ongoing process as there are many possibilities. There is a huge market for the many new functional and value added properties resulting from these modifications (Kaur *et al.,* 2012). Subsequently, studies have been performed by preparing cold water soluble starch by various modifications (Sair, 1964; Trubiano, 1987; Khalil *et al.*, 1990; Chen and Jane, 1994; Bello-Perez *et al.*, 2000; Atichokudomchai and Varavinit, 2003; Orozco Martinez and Betancur-Ancona, 2004; Pukkahuta *et al.*, 2007 Sanchez-rivera *et al.,* 2005; Sang *et al.*, 2007; Chatakanonda *et al.*, 2011; Xin *et al.*, 2012; Yu *et al.*, 2015). Generally, there are four broad classifications of starch modifications: chemical, physical, enzymatic, and biotechnological/ genetic (Jobling, 2004; Tharanathan, 2005; Hu 2007; Shi *et al*. 2009; Uthumporn *et al*. 2012; Ashogbon and Akintayo, 2013).

1.3.1 Physical Modification of Starch

Physical modification is simple, cheap, and safe because it requires no chemicals or biological agents (Ashogbon and Akintayo, 2014). Physical modification of starch can improve water solubility and reduce particle size. The methods involve the treatment of native starch granules under different temperature/moisture combinations, pressure, shear, and irradiation (Ashogbon and Akintayo, 2014). Though native starch has been used for food processing, the physical properties of these processed starches are limited in terms of the commercial applications for which they are suited (Laovachirasuwan *et al.,* 2010). Recently, new methods of physical modifications of starches from different botanical

sources have been investigated and include: superheated starch, iterated syneresis, thermally inhibited treatment (dry heating), osmotic pressure treatment, multiple deep freezing and thawing, instantaneous controlled pressure drop (DIC) process, mechanical activation with stirring ball mill, micronization in vacuum ball mill, pulsed electric fields (PEF) treatment and corona electrical discharges (Ashogbon and Akintayo, 2014).

1.3.1.1 Annealing of Starch

Annealing is a type of heat treatment for starch modification which involves the maintenance of starch at temperatures lower than the melting temperature (50 – 75 °C) in excess water (Hoover and Manuel, 1996b; Jacobs and Delcour, 1998). This process is accompanied by an increase in the gelatinisation transition temperature, the enthalpy of gelatinisation and by a decrease in the gelatinisation temperature range. The effects of annealing are due to interactions between amylose and the outer branches of amylopectin, resulting in an increase in double helices and close packing of the crystallites within the granule (Hoover and Manuel, 1996b). Annealing treatments induce the rapid migration or rearrangement of the amylose molecules in the granules to form intermolecular bonds between the amylose molecules and/or between the amylose molecules and the amylopectin molecules (Xie *et al.,* 2005). Often annealing is applied unintentionally, such as the steeping step used in the maize wet-milling process (Jacobs and Delacour, 1998)

1.3.1.2 Heat-Moisture Treatment of Starch

Heat-moisture treatment of starch is a physical treatment in which starches are treated at varying moisture levels (< 35 %) for a certain period of time at a temperature above the glass transition temperature but below the gelatinisation temperature. However, the temperature is often chosen without considering the gelatinisation temperature (Jacobs and Delcour, 1998). Changes in X-ray pattern, crystallinity, starch chain interaction, granule swelling, viscosity, gelatinisation parameters, retrogradation have been shown to occur in cereals, tubers and legume starches (Hoover and Vasanthan, 1994; Takaya *et al*., 2000). In some cases, for cereal starches, amylose-lipid complexes are formed by heat-moisture treatment as indicated by a decrease in the apparent amylose content (Jacobs and Delcour, 1998). Adebowale *et al.* (2009) in a study on the modification and properties of *Sphenostylis stenocarpa* using heat moisture and annealing treatments reported a reduction in moisture content and an increase in gelatinisation temperature and viscosity of the legume starch.

1.3.1.3 Non-Thermal Physical Modification of Starch

Different non-thermal treatments affect the physicochemical properties of starch differently. Some of the non-thermal processes are conducted at high hydrostatic pressure (HHP), using ultrasound effect, PEF treatment, and microwave treatment of starches from different botanical sources (Blaszczak *et al.,* 2005; Liu *et al.,* 2005; Blaszczak *et al.,* 2007; Marselles- Fontanet and Martin-Belloso, 2007; Lida *et al.,* 2008; Han *et al.,* 2009; Vallons and Arendt, 2009). Generally, HHP treatment restricts the swelling power of starch granules, so that their viscosity is lower compared to heat processed starches (Nasehi and Javaheri, 2012).

High power ultrasound (HPU) represents a non-thermal processing method that has been rapidly researched and used in the last 12 years. It offers the opportunity to modify and improve starch. Ultrasound is generated with either piezoelectric or magneto-strictive transducers that create high- energy vibrations (Herceg *et al.,* 2010). These vibrations are amplified and transferred to a sonotrode or probe, which is in direct contact with the fluid. HPU is very important in the following areas of food processing; extrusion, de-foaming, viscosity alteration, separation, filtration, crystallization, homogenization, emulsification and extraction (Herceg *et al.,* 2010). Sujka and Jamroz (2013) reported that starches treated by ultrasound showed depolymerization of the granules and in the modification of the paste properties, water was a better medium for starch modification.

1.3.1.4 Pre-gelatinisation of Starch

Pre-gelatinised starches (PGS) refer to cooked starches that are prepared by complete gelatinisation and drying (Ashogbon and Akintayo, 2014) The main properties of PGSs are increased water absorption and water solubility upon dispersion in cold water and this leads to "instant starch slurries" without heating. Their functional properties are highly dependent upon the cooking, botanical sources and drying conditions (Anastasiades *et al.*, 2002). The most important property of pre-gelatinised starch is that it instantly hydrates and swells in water at room temperature. However, finely ground products of pre-gelatinised starches are difficult to disperse in water homogeneously since they hydrate rapidly at contact with water and form lumps. Pre-gelatinised starch is used as a thickening agent for pie fillings, puddings, sauces and baby foods (Zuo *et al.*, 2012).

Pre-gelatinisation can be brought about by drum drying, spray drying, and extrusion cooking (Mounsey and Riordan, 2008; Majzoobi *et al.,* 2011). In a spray cooking process,

the technique involves the injection of starch slurry through an atomization aperture in a nozzle assembly to form a fine spray (Pitchon *et al.,* 1981). Thereafter, steam is injected through another aperture in the nozzle assembly into the atomized starch spray to gelatinise the starch. This entire operation takes place in an enclosed chamber (Schara and Katcher, 1989). Spray drying is a well-known technology in the food industry and is presently the most commonly utilized microencapsulation method for ingredient (Shefer and Shefer, 2003; Reineccius, 2006). Pre-gelatinised starch can be made by the drum drying method, in which a cooked starch sheet is produced from starch slurry on a hot drum and thereafter, the starch is ground after drying to a desired particle size (Seib, 1996).

Extrusion modification of starch is a process that uses the molten phase of high solid concentration to transform starch while maintaining a macro- molecular structure (Pitchon *et al.,* 1981). Extrusion cooking is considered to be a high temperature short time (HTST) process; the temperature in the extruder can be as high as 200 °C, while the residence time is short (~10 to 60 seconds) (Colonna and Mercier, 1989; Ashogbon and Akintayo, 2014). Extrusion process can be used to produce pre-gelatinised starch, in which granular starch with different moisture content is compressed into a dense compact mass, and disrupted, by the high pressure, heat and shear during the process. The extruded starch is further dried and ground to a desired particle size for food applications (Colonna and Mercier, 1989). The gelatinised starch is recovered essentially as granules.

Drum drying is the most widely used technique of pre-gelatinisation at industrial scale. The overall pre-gelatinisation process occurs in one or two steps. In the one step process, the starch slurry is fed onto the drums that are used to gelatinise and dehydrate starch paste at the same time. In the two-step process, the slurry is primarily cooked in a heat exchanger or a high-temperature jet cooker and dehydrated by drum drying (Loisel *et al.,* 2004). Starches that generate a pulpy texture are used to modify the texture of soups, gravies and sauces (Thomas and Atwell, 1999; Zuo *et al.*, 2012).

1.3.1.5 Osmotic Pressure Modification of Starch

Osmotic Pressure Treatment (OPT) is carried out in the presence of high salt solutions like sodium sulphate (Pukkahuta *et al.,* 2007). A uniform heat distribution is provided by using this method when compared to heat-moisture treatment and modified starch can now be produced in a large scale (Kavlani *et al.,* 2012). Osmotic pressure treated starches show changes in starch chain interactions, granule swelling, amylose leaching, viscosity,

gelatinisation temperatures, retrogradation, acid and enzyme hydrolysis (Pukkahuta *et al*., 2007).

1.3.2 Enzymatic Modification of Starch

Enzymatic modification of starch most commonly involves hydrolysis and transglycosylation by carbohydrate enzymes. Such enzymes can catalyse intermolecular glucan transfer reactions, as well as intramolecular disproportionation transfers within a single linear glucan molecule (Takaha and Smith, 1999). Another example is the branching enzyme (BE, 1, 4-α-glucan, 1, 4-α-glucan 6-glucosyltransferase), which is also widely used to enzymatically modify starch. Branching enzyme (BE) catalyses the formation of α-1, 6 branching points and converts amylose into branched glucans containing cyclic forms (Takata *et al*., 1996). A third enzyme known as maltogenic amylase, an α-microbial glycoside hydrolase hydrolyses starch to maltose by the cleavage of α-1, 4-glycosidic bonds (Lee *et al*., 2005). The most common enzymes for starch modification include a-amylase, ß-amylase, glucoamylase, pullulanase, and isoamylase. These enzymes have been isolated from fungi, yeasts, bacteria, and plant kingdoms. Starch hydrolysis involves liquefaction and saccharification of starch. Liquefaction is a process by which a concentrated suspension of starch granules is converted to a solution of low viscosity by α -amylase. Saccharification converts starch or intermediate starch hydrolysis products to D-glucose by enzymes such as glucoamylase (amyloglucosidase) (Biwer *et al.,* 2002).

1.3.3 Biotechnological Modification of Starch

Biotechnological modification of crops may allow the production of desired starch product without chemical and/or physical modification. The structure of starch is controlled by the genetic makeup of the plant, but the exact effects of many genes are not very clear due to the complexity of the system. Many studies have been carried out to identify those genes responsible for the production of starch (Shannon and Garwood, 1984). Biotechnological developments in starch synthesis have a great impact on modifying the basic structural composition of starch (Gerard *et al.,* 2001). The pathway of starch synthesis is currently known to involve the inter-conversion of sugars, sugar phosphates, and nucleotide-sugars. Four enzymes involved in starch biosynthesis have been identified for maize: ADP-glucose pyrophosphorylase, starch synthase, starch branching enzyme, and starch debranching enzymes (Gerard *et al.,* 2001). Some isoforms of these enzymes were also identified. However, the exact role of each isoform in the biosynthesis pathway is not clear.

Nevertheless, amylopectin is synthesized by starch synthase and branched by a branching enzyme. Amylose is synthesized by granule bound starch synthase (GBSS). These enzymes have many isoforms that play various roles in starch synthesis. Thus, biotechnological modification could result in altering starch structure (Shannon and Garwood, 1984).

1.3.4 Chemical Modification of Starch

Chemical modification involves the introduction of functional groups into the starch molecules, resulting in markedly altered physicochemical properties. Such modification of native granular starches profoundly changes the proximate compositions, gelatinization, retrogradation, and pasting characteristics (Ashogbon and Akintayo, 2014). The chemical modification of starch results in enhanced molecular stability against mechanical shearing, acidic, and high temperature hydrolysis; obtaining desired viscosity; increasing interaction with ion, electronegative, or electropositive substances; and reducing the retrogradation rate of unmodified starch. The most common chemical modifications are oxidation, esterification, and etherification (Xie *et al.,* 2005).

The chemical modification is mostly made directly on the granular starch, which is in a convenient form to be collected and handled after the reaction. Therefore, the granular structure influences greatly the substitution pattern of the starch components (Steeneken and Woortman, 1994). The chemical reagents penetrate with different efficiencies through the surface or via channels in the granules into the interior (Gray and Be-Miller, 2001; Huber and Be-Miller, 2001). Generally, the amorphous parts of the granules are substituted more easily than the crystalline areas though granules from different plants can be affected in various ways depending on their texture, type of crystallinity, amylose content and the presence of minor components (Burgt *et al.,* 1998). Amylose tends to become more strongly substituted than amylopectin which is preferentially substituted at the branched regions (Hood and Mercier, 1978; Burgt *et al.,* 1998).

Modification is generally achieved through derivatization, such as acetylation, cationization, oxidation, acid hydrolysis and cross-linking. These techniques are however limited due to issues concerning consumers' safety and the environment. There is a new evolving trend called dual modification, which involves the combination of physical and chemical agents, e.g., microwave-assisted acetylation or HHP-assisted phosphorylation (Ashogbon and Akintayo, 2014).

1.3.4.1 Esterification of Starch

Esterification is a form of modification that involves the replacement of some hydroxyl groups with ester groups. The level of substituents of the hydroxyl groups along the starch chains is often expressed as average degree of substitution (DS). Some type of starch esters include: starch acetate, starch succinate and starch phosphate (Ashogbon and Akintayo, 2014).

1.3.4.2 Acetylation of Starch

Acetylation replaces the hydroxyl groups in native starch with acetyl groups. The introduction of these acetyl groups reduces the bond strength between starch molecules and thereby alters their properties. Under alkaline conditions, starch is indirectly reacted with carboxylic anhydride (Rutenberg and Solarek, 1984). An alkali starch complex forms first, which then interacts with the carboxylic anhydride to form a starch ester (Jarowenko, 1986). Normally, the resultant acetylated starch will possess good stability at low temperatures and exhibit improved resistance to retrogradation. Acetylation is therefore common for baked, dry, canned and frozen food products such as gravies, fruit pies, salad dressings and filled cakes (Aning, 2012).

Acetylated starches are commercially produced by acetylation with acetic acid, acetic anhydride, ketene, vinyl acetate, or a combination of these reagents (Kruger and Rutenberg, 1967). The reagents and solvents used during the acetylation process must yield acceptable products and satisfy the requirement of government regulations, especially when the resultant starch acetates are intended for food purposes (Betancur-Ancona *et al.,* 1997).

1.3.4.3 Succinylation of Starch

Starch succinate is a half-ester produced by the reaction of starch with succinic anhydride. The level of succinic anhydride legally permitted by the U.S. Food and Drug Administration in food starch modification is not to exceed 4.0 % (McIntire, 1950). Starch suspension is prepared at pH ~ 8; Succinic anhydride is added slowly to a starch suspension while maintaining pH. Finally, starch is isolated by washing and drying. High degree of substitution (DS) starch succinate can be prepared in glacial acetic acid containing sodium acetate at 100 °C or using pyridine as the medium (Bhandari and Singhal, 2002). Starch succinate contains a free carboxylate group that increases the water holding power and tendency to swell in cold water. The capability of cold water swelling

increases with greater levels of succinic anhydride. Cooked starch succinates show excellent viscosity, stability, and clarity, as well as freeze-thaw stability (Bhandari and Singhal, 2002).

In the applications of starch succinate, it has been used as a binder and thickener in soups, snacks, canned and refrigerated foods because of its desirable properties, such as high thickening power, freeze-thaw stability and low gelatinisation temperature. Corn starch succinates have been used as tablet disintegrants for pharmaceutical applications (Bhandari and Singhal, 2002).

1.3.4.4 Phosphorylation of Starch

Starch phosphate is usually prepared using a mixture of sodium tripolyphosphate, sodium sulphate and often a low level of sodium trimetaphosphate. The levels of residual phosphate in food grade modified starches are not to exceed 0.04 % for sodium trimetaphosphate and 0.4 % for sodium tripolyphosphate and sodium trimetaphosphate, respectively. FDA of the U.S. also allows monosodium orthophosphate (not to exceed 0.4 % as phosphorous) and phosphorus oxychloride (not to exceed 0.1 %) to be used for food grade starch modification (Code of Federal Regulations, 2001). Starch phosphates are strongly anionic polymers, which yield higher viscosity, more clear and stable dispersions with long cohesive texture and resistance to retrogradation. The viscosities are reduced by the presence of salts. The gelatinisation temperature decreases with an increasing degree of substitution, and the monoester becomes cold water swelling when degree of substitution is increased to 0.07. Dispersion of starch phosphates has superior freeze-thaw stability than other modified starches (Kerr and Cleveland, 1962). Starch phosphates are good emulsification agents because of the ionic properties (Albrecht *et al.,* 1960). Starch phosphates are also used in foods as emulsion stabilizers for vegetable oil in water and thickening agents with good freeze-thaw stability. A combination of starch phosphate, guar gum, and propylene glycol has been used as an emulsion stabilizer for vinegar and vegetable oil in water (Solarek, 1986). In the production of cold water swelling starch phosphate, the phosphate is dry mixed with sugar and a flavouring agent, which is added to cold milk to form puddings with a smooth, soft, even texture, and superior eating quality (Neukom, 1958). Some starch phosphates have been used in breads to improve the baking quality (Solarek, 1986).

1.3.4.5 Etherification of Starch

Ether linkages are more stable even at high pH. Etherification gives starch excellent viscosity and stability. Hydroxyl-alkyl starches, including hydroxyl-ethyl and hydroxyl-propyl starches are mainly produced for industrial applications. Hydroxy-ethyl starch has not yet been approved as a direct food additive, but can be used as an indirect food additive, such as a sizing agent for paper contacting food, whereas hydroxyl-propyl starch is primarily used for the food industry (Ashogbon and Akintayo, 2014).

1.3.4.6 Propylation of Starch

The reaction of propylene oxide with starch under alkaline conditions is a substitution, nucleophilic, bimolecular, or SN_2 type (Tuschoff, 1986). Depending on the degree of modification, hydroxy-propylation of starch is achieved in one of two ways. At degree of substitution 0.25, starch is slurred in aqueous sodium hydroxide (pH ~ 11.5). Sodium sulphate, 5 – 15 % (based on dry starch weight) is added to protect the starch from swelling. Propylene oxide is added up to 10 % (based on starch dry weight). The mixture is warmed (45 to 50 °C) and stirred for approximately 24 hours (Xie *et al*., 2005). After the etherification reaction, cross-linking with phosphoryl chloride is usually done to give hydroxypropyl distarch phosphate. Without cross-linking, the hydroxypropyl derivatives of starches swell excessively and give stringy pastes (Seib, 1996; Xie *et al*., 2005). Hydroxypropyl starches are being widely used in food products where they provide viscosity stability and freeze-thaw stability. These starches are normally combined with cross-linking to provide the desired viscosity, texture, and stability for processing and storage. Hydroxypropyl starches are used as thickeners in fruit pie fillings, puddings, gravies, sauces, and salad dressings amongst other applications (Xie *et al.,* 2005).

1.3.4.7 Cationization of Starch

Cationic starch is produced from the reaction of starch with reagents containing amino, imino, ammonium, sulphonium or phosphonium groups. The free hydroxyls of the native starch is commonly altered by using cationic monomer such as 2,3 epoxypropyltrimethylammonium chloride (ETA) or 3-chloro-2-hydroxypropyltrimethyl ammonium chloride (CTA) under wet or dry processes or a process in between the two processes (Radosta *et al.,* 2004). In the dry-cationization process (absence of a liquid phase) reagent is sprayed to the dry starch during extrusion; in the semi-dry method,

reagent is sprayed onto the starch and the mixtures are thermally treated (Heinze *et al.,* 2004).

The cationic substitution by aqueous methods is believed to occur mainly at the amorphous region, as evidenced by the unchanged X-ray diffraction patterns and persistent birefringence under polarized light microscopy (Wang *et al.,* 2009). Generally, physicochemical properties as well as granular morphology of starch were always altered after cationization, especially those involving high degree of substitution. Furthermore, lower pasting temperature, higher peak viscosity and various changes in setback were observed in the rapid visco-analyser of different kinds of starches (Siau, *et al.,* 2004). There are many different applications of cationic starch; it is used for water treatment as flocculants (Krentz, *et al.,* 2006) and as an additive in textile and cosmetic products (Xie *et al.,* 2005). Furthermore, some researchers have found cationic starch to be useful as dynamic coating additive for protein analysis (Sakai-Kato, *et al.,* 2006).

1.3.4.8 Crosslinking of Starch

Cross-linking has been used to modify native starch utilising various cross-linking agents such as sodium trimetaphosphate, sodium tripolyphosphate, epichlorohydrin, and phosphoryl chloride (Ratnayake and Jackson, 2008). The type of cross-linking agent determines the changes in functional properties of a treated starch, because the molecular structures of the cross-linked systems produced by different cross-linking agents are different (Seker and Hanna, 2006). Starch contains hydroxyls which are able to react with reagents resulting in cross-linked starches.

Cross-linked starches are used in salad dressings to provide thickening with stable viscosity at low pH and high shear during the homogenization process. Cross-linked starches with a slow gelatinisation rate are used in canned foods where retort sterilization is applied; such starches provide low initial viscosity, high heat transfer and rapid temperature increase, which are particularly suitable for quick sterilization. Cross-linking is often employed in combination with other methods for starch modification, such as oxidation, hydrolysis, etherification and esterification (mono-substituents), to provide appropriate gelatinisation, viscosity and textural properties for food applications (Rutenberg *et al.,* 1975; Ashogbon and Akintayo, 2014).

1.3.4.9 Oxidation of Starch

Starch oxidation has been practiced since the early 1800s, and various oxidizing agents have been introduced, for instance, hypochlorite, hydrogen peroxide, periodate, permanganate, dichromate, persulfate, and chlorite (Rutenberg and Solarek, 1984).
Oxidation of starch entails introduction of carbonyl and carboxyl groups on glucose units within the matrix of the polymer. Previous studies have shown that such modification brings about improvement in whiteness of the starch, and restricted retrogradation or "setting up" on standing (Kuakpetoon and Wang, 2001; Adebowale *et al*., 2002).

The main uses for oxidized starch can be found in the paper and textile industries (Ashogbon and Akintayo, 2015). However, the application of oxidized starches in the food industry is increasing because of their low viscosity, high stability, clarity and binding properties. Oxidized starch for food use is mainly produced by the reaction of starch with sodium hypochlorite (Code of Federal Regulations, 2001). The major reactions of hypochlorite oxidation of starch include cleavage of polymer chains and oxidation of hydroxyl groups to carbonyl and carboxyl groups. Oxidized starches exhibit the same x-ray diffraction pattern as that of the native starch, which indicates that oxidation occurs mainly in the amorphous phases of the granule (Rutenberg and Solarek, 1984). Oxidized starches are normally whiter than unmodified starches because pigments on the granule surface are bleached. Oxidation of starch mainly causes the scission of the glucosidic linkages and oxidation of hydroxyl groups to carbonyl and carboxyl groups. The scission of the glucosidic linkage results in depolymerization of amylose and amylopectin hence decreases swelling power and paste viscosity. However, treatment with low levels of hypochlorite has been reported to increase paste viscosity. Formation of the carbonyl and carboxyl groups discontinuously along the chains reduces gelatinisation temperature, increases solubility and decreases gelation (Wurzburg, 1986). Since the bulkiness of the carboxyls and carbonyls sterically interfere with the tendency of amylose to associate and retrograde, oxidized starches produce pastes of greater clarity and stability than those of unmodified starch. Oxidized starches are used as binding agents in foods, such as batters applied to meats before frying. The tenacious coatings formed on frying may result from the salt bridges between the protein molecules and anionic oxidized starch or the reaction of amino groups with the aldehyde groups on the oxidized starch (Wurzburg, 1986).

1.3.4.10 Acid Hydrolysis of Starch

Acid hydrolysis involves suspending starch in an aqueous solution of hydrochloric acid (HCl) or sulfuric acid (H_2SO_4) and maintaining it at a temperature between ambient and just below the pasting temperature to prevent gelatinisation. When the desired reduction in viscosity is obtained, the acid is neutralized and the starch recovered by filtration. In addition to acid treatment in an aqueous solution, the acid hydrolysis of starch can also be prepared in anhydrous alcohols such as methanol, ethanol, 2-propanol and 1-butanol. These modified starches are different from those modified in aqueous conditions (Ma and Robyt, 1987). Acid modification includes a two-stage attack on the granules (Zuo *et al.*, 2013). At the early stage, a rapid initial attack occurs on the amorphous regions of starch containing branching points with α-1, 6 linkage; an increase in linear fraction in starch is found in this stage. A slower hydrolysis takes place on the more crystalline areas during the second stage. Factors influencing acid modification include acid type and concentration, temperature and time, type of alcohol and type of starch (Ma and Robyt, 1987). Acid modification is widely used in the starch industry to prepare thin boiling starches for use in food, paper and textile industries (Rohwer and Klem, 1984, Zuo *et al.*, 2013; Yu *et al.*, 2015).

1.3.4.11 Carboxymethylation of Starch

Carboxymethylation of polysaccharides is a widely studied conversion since it is simple and leads to products with a variety of promising properties. In general, the polysaccharide is activated with aqueous alkali hydroxide mostly sodium hydroxide and converted with monochloroacetic acid or its sodium salt yielding the carboxymethyl (CM) polysaccharide derivative (Heinze and Liebert, 2001). Not only cellulose and starch but also various polysaccharides from different sources are applied as starting materials. Carboxymethylation of starch is one of the most versatile functionalization procedures as it provides access to biomaterials with valuable properties. This process was carried out by substituting the hydroxyl groups (O-H) in the starch molecules with carboxymethyl groups (CH_2COOH) to produce carboxymethyl starch (CMS) (Heinze *et al.,* 2004). To prevent gelatinisation and to keep the granular structure intact, the reaction was usually carried out in an organic medium (Tijsen *et al.,* 2001)

.Most of the commercially produced CMS was found to have a high swelling power property, excellent solubility at ambient temperature, low intrinsic viscosity and reduced tendency to retrograde (Noor *et al.,* 2005).

1.3.4.12 Pyrodextrinization of Starch

Wang *et al.* (2009) showed that indigestible material content in dextrin obtained from starches modified by pyrodextrinization is inversely proportional to the amount of α-1, 4 glycosidic bonds, and that these dextrins exhibit characteristics similar to those of dietary fiber. Dextrin can be produced by dry pyroconversion, in which acid hydrolyses the starch and preferentially attacks the amorphous regions, leaving a highly crystalline starch.

1.3.4.13 Acid-Alcohol Modification of Starch

The use of acid-alcohol treatment for modifying starch is gaining popularity owning to higher recovery of modified starch and the use of less acid as compared to acid alone for hydrolysis of starch; thus, the importance in food and industrial applications (Yiu *et al.,* 2008; Sodhi *et al.*, 2009).

The mechanism of hydrolysis of hydrolysis of starch granules suspended in alcohol involves hydrolysis of glycosidic bonds with water inside the granules. In Acid-alcohol modification, the average degree of polymerization is dependent on the botanical source, acid concentration, type and treatment temperature (Ma and Robyt, 1987). In an experiment, Ma and Robyt (1987) observed the highest average degree of polymerization with methanol for acid-alcohol treated potato and waxy maize starches.

1.4 Applications of Starch

Starch has found wide usage in a number of industries namely the laundry, food, pharmaceutical, paper, textile and plastic industries. To enhance the functional properties of starch such as viscosity, texture, stability, solubility, clarity among many desired functional properties desired for starch utilization, starch is modified by chemical, physical or biotechnological means (Singh *et al.*, 2010).

Starch is used in textile industries in the sizing of yarns, stiffening and finishing of fabrics, thicken colours to obtain sharp and durable printed fabrics, laundering of clothes, used for weaving efficiency of fabrics, retaining print works on fabrics, it also removes dirt and sweat stains off clothes as they would stick to the starch than the fabric (Yu *et al.*, 2015).

In the food industry, modified starch can be used in the production of instant starches, gel preparation (example pudding), flakes, pulpy foods, to enhance paste consistency, thickening, smoothness, clarity of foods, to provide freeze-thaw stability, to encapsulate or preserve flavour of food products where succinylated starches can be used, enhance viscosity in dairy products and stabilise taste of canned foods. (Blenford, 1994; Wang *et al.*, 2012 and Yu *et al.*, 2015). Modified starches are used in different food types like baked food, baby foods, snacks and confectionaries (Ashogbon and Akintayo, 2014). The food industry generally recommends the use of modified starches with less degree of substitution.

In the pharmaceutical industry, starch can be used in drug development technology in the production of compressible excipient, tablet binder, dispersion agent and plasma volume expander which is useful for patients suffering from trauma, heavy blood loss and cancer treatment (Singh *et al.,* 2010; Chiu *et al.,* 1997).

Other uses include its use in the paper industry as retention aid and flocculants; plastic industry as a filler to enhance biodegradation of commodity plastics; adhesive industry where its high viscosity affords an appreciable binding capacity; in petroleum industry as it imparts desirable characteristics to the mud used in drilling oil wells; used in the production of fillers for animal feed; used in cosmetic production; used as filler in dry battery cell production; used in the production of rubber; used in the production of explosives; used in the fermentation industry as a colloid stabilizer for beverage production and in plywood manufacturing (Singh *et al.,* 2010; Wang *et al.*, 2012 and Yu *et al.*, 2015).

1.5 Problem Statement and Justification

A number of tropical legumes could be classified as underutilized because their economic potentials are not being optimally exploited. One of these potentials is their content of high amounts of starch which can be isolated, modified and utilized in a variety of industries. The tropical legumes used in this study namely *Cajanus cajan, Mucuna pruriens var pruriens, Sphenostylis stenocarpa, and Vigna subterranea* are usually cultivated by subsistent farmers in the rural communities who primarily depend on them as a source of food to their household.

It is thought that finding industrial uses for the starches from these legumes will add value and boost the utilization of these crops. In addition, it would increase the economic potential of these legumes which are currently classified as underutilized. Thus, the cultivation of these legumes will bring more reward to the subsistent farmers and encourage others to be involved in their cultivation and usage.

1.6 Aim and Objectives

This study is aimed at evaluating the effects of physical and chemical modification on the cold water solubility of starch from some underutilized legumes. The above aim was achieved through the following specific objectives:

1. Isolation of starch (native) from the different legumes namely *Cajanus cajan, Mucuna pruriens var pruriens, Sphenostylis stenocarpa, and Vigna subterranea*
2. Determination of the cold water solubility of the native starches from the legumes
3. Physical modification of the different starches
4. Chemical modification of the different starches
5. Determination of the cold water solubility of the modified starches
6. Comparison of the functional properties of native starches from the different legumes.
7. Comparison of the functional properties of modified starches from the different legumes.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

2.1.1 Reagents

The following chemicals/ reagents employed in the study were sourced as follows: Sodium trimetaphosphate (JHD, China), Sodium sulphate (May & Baker, England), Hydrochloric acid (HCl) (Sigma Aldrich, Germany), Sodium Hydroxide (Qualikem, India), Sodium hypochlorite (Labo Chemie, India), Succinic anhydride (Labo Chemie, India), Acetic anhydride (Sigma Aldrich, Germany), Acetic acid (Sigma Aldrich, Germany), Sodium tripolyphosphate (JHD, China), Methanol (Sigma Aldrich, Germany), ethanol (Sigma Aldrich, Germany), Monochloro acetate (JHD, China), Isopropyl alcohol (Sigma Aldrich, Germany), Hydrogen tetraoxosulphate (vi) (Labo Chemie, India), Iodine (Kermel, China), Potassium iodide (Kermel, China).

2.1.2 Equipment

The following apparatus were employed in the study: weighing balance (Ohaus Dial-O-Gram, Ohaus Cooperation, N.J. USA), Water bath (Model DK), magnetic stirrer (AM-3250B Surgi Friend Medicals, England), milling machine (Thomas Willey laboratory Mill Model 4, Anthor), centrifuge (Thomas Company, Philadelphia, USA), oven (Gallenkamp Hotbox, England), pH meter (model HI 8424- Ecosan pH meter, made in Singapore), sensitive weighing balance (B2404-5 mettler Toledo, made in Switzerland), Uv/visible spectrophotometer (Jenway 6405, England), microscope (WESO Light microscope), glass wares (Pyrex), thermometer, magnetic stirrer, hot plate.

2.1.3 Collection of Legumes

Cajanus cajan, Mucuna pruriens var pruriens, Sphenostylis sternocarpa, and Vigna unguiculata were obtained from Orie Igboeze Market, at Igboeze south Local Government Area and Ogige market in Nsukka, both in Enugu State. They were identified at the botany department of the University of Nigeria.

2.2 Methods

2.2.1 Legume Preparation and Starch Isolation

Starch was isolated by the modified method of Lim *et al.* (1999). The seeds of *Vigna subterranean*, *Cajanus cajan*, *Sphenostylis stenocarpa* and *Mucuna pruriens var pruriens* were de-stoned and dry milled to obtain fine legume flour, after which it was soaked in distilled water (1:3 weight/volume). It was filtered using a muslin cloth and the seed coat debris discarded and the solution left to stand for about three hours for the starch sedimentation process to occur. The starch was washed with distilled water and allowed to stand for 3 hours after which the supernatant was discarded. This process was repeated 5 times until a neutral pH of the supernatant was observed. The starch was collected and dried at 40 ^{0}C for 48 hours.

2.2.2 Starch Yield Percent

The percentage yield of the starch was determined by the method of Ji *et al.* (2004) and calculated as follows:

$$Starch\ yield\ (\%) = \frac{Recovered\ weight\ of\ starch}{Initial\ weight\ of\ starch} \times 100$$

2.2.3 Cold Water Solubility (CWS)

The CWS was determined by the method of Eastman and Moore (1984). A known quantity of 1 g starch powdered sample was added to a blender with 100 ml distilled water and mixed for 2 minutes. The starch suspension was centrifuged at 1,200 x g for 15 minutes. A 25 ml aliquot was transferred to a petri dish and dried in an oven at 110 ^{o}C for 4 hours. The CWS was calculated by the equation:

$$CWS(\%) = \frac{weight\ solute\ in\ supernatant\ \times 4}{weight\ of\ sample\ \ (g)} \times 100$$

2.2.4 MODIFICATON OF STARCH

2.2.4.1 Physical Modifications

2.2.4.1.1 Annealing

The method of Chatakanonda *et al.* (2011) was adopted. A measured volume, 75 ml of distilled water was added to 25 g of starch in a 500 ml beaker and the slurry was incubated at 50 ^{o}C for 72 hours in an air oven, after which it was filtered and dried at 50 ^{o}C for 12 hours.

2.2.4.1.2 Heat Moisture Treatment

The method of Sair (1964) was adopted. A measured volume, 70 ml of distilled water was added to 25 g of starch and was mixed into slurry in a glass beaker. The glass beaker was sealed and equilibrated at room temperature for 24 hours after which the jar was placed in an air oven for 16 hours at 100 ^{o}C. The beaker was left to cool, and the starch was dried at 30 ^{o}C for 48 hours in an air oven and the dried lumpy starch was ground to fine powder.

2.2.4.1.3 Osmotic Pressure Treatment

The method of Pukkahuta *et al.* (2007) was adopted. A known quantity, 50 ml of saturated Na_2SO_4 solution (50 g Na_2SO_4: 100 ml distilled water) was mixed with 25 g of starch in a 500 ml conical flask, and the solution was heated for 1 hour at a temperature of 120 ^{o}C. The flask was cooled to room temperature and then washed with distilled water to remove Na_2SO_4. This procedure was repeated twice after which the starch was dried at 60 ^{o}C for 12 hours.

2.2.4.2 Chemical Modifications

2.2.4.2.1 Acetylation

The method of Bello-Perez *et al.* (2000) was adopted. A known quantity, 25 g of starch was put in a 400 ml beaker and 70 ml of distilled water (25 ^{0}C) was added and stirred into slurry. The pH was adjusted to 8.0 using a 3 % NaOH solution drop-wise while shaking. Acetic anhydride was added slowly to the starch suspension in agitation simultaneously, and 3 % NaOH was added to maintain the pH at 8.0 - 8.4. The pH was adjusted to 4.5 with 1 N HCl, after which the slurry was filtered with Whatman no. 4 filter paper and air-dried.

2.2.4.2.2 Acid-Alcohol Modification

The method of Lin *et al.* (2003) was adopted. A measured quantity, 25 g of starch was suspended in 100 ml of absolute methanol in a 250 ml volumetric flask; the suspension was stirred and maintained at 25 ^{o}C. Reaction was started by adding 1 ml of concentrated (36 % by weight) HCl and allowed to proceed for 9 days. The reaction was stopped by adding 14 ml of 1 M sodium bicarbonate and then cooled in an ice bath; the starch was allowed to sediment and washed four times with absolute ethanol. The starch was oven dried at 40 ^{o}C and the percentage starch yield was calculated.

2.2.4.2.2.1 Acid-Alcohol 50 % Modification

This was determined by modifying the method of Lin *et al.* (2003). A measured quantity, 25 g of starch was suspended in 100 ml of 50 % methanol in a 250 ml volumetric flask; the suspension was stirred and maintained at 25 ^{o}C. Reaction was started by adding 1 ml of 20 % HCl and allowed to proceed for 9 days. The reaction was stopped by adding 14 ml of 1 M sodium bicarbonate and then cooled in an ice bath; the starch was allowed to sediment and was washed four times with 50 % ethanol. The starch was oven dried at 40 ^{o}C and the percentage starch yield was calculated.

2.2.4.2.2.2 Acid-Alcohol 70 % Modification

This was determined by modifying the method of Lin *et al.* (2003). A measured quantity, 25 g of starch was suspended in 100 ml of 70 % methanol in a 250 ml volumetric flask; the suspension was stirred and maintained at 25 ^{o}C. Reaction was started by adding 1 ml of 45 % HCl and allowed to proceed for 9 days. The reaction was stopped by adding 14 ml of 1 M sodium bicarbonate and then cooled in an ice bath; the starch was allowed to sediment and washed four times with 70 % ethanol. The starch was oven dried at 40 ^{o}C and the percentage starch yield was calculated.

2.2.4.2.3 Acid Hydrolysis

The method of Atichokudomchai and Varavinit (2003) was adopted. A measured quantity, 25 g of starch was incubated in 50 ml of 6 % HCl solution at 23 ^{o}C for 8 days without stirring. The suspension was neutralised with 10 % weight/volume NaOH solution and the slurry was washed 5 times with distilled water, dried in air oven at 40 ^{0}C for 24 hours and ground into fine powder.

2.2.4.2.4 Alcohol-alkaline Modification

The method of Chen and Jane (1994) was adopted. A measured volume, 100 ml of 40 % ethanol solution was added to a beaker containing 25 g of starch and stirred into slurry. 55 ml of 3 M NaOH solution was added at 4 ml/ minute and the mixture was allowed to stand for 15 minutes by gentle stirring. Further, 50 ml of 40 % ethanol was added slowly to the suspension and stirred for 10 minutes. The supernatant was carefully decanted and the starch washed with a fresh solution of ethanol and neutralised with 3 M HCl. The starch was washed further with 95 % ethanol solution, dehydrated and oven-dried at 50 ^{0}C.

2.2.4.2.5 Alcohol-alkaline Modification 20 % Treatment

This was determined by modifying the method of Chen and Jane (1994). A measured volume, 100 ml of 20 % ethanol solution was added to a beaker containing 25 g of starch and stirred into slurry. 55 ml of 1.5 M NaOH solution was added at 4 ml/ minute and the mixture was allowed to stand for 15 minutes by gentle stirring. Further, 50 ml of 20 % ethanol was added slowly to the suspension and stirred for 10 minutes. The supernatant was carefully decanted and the starch washed with a fresh solution of ethanol and neutralised with 1.5 M HCL. The starch was washed further with 95 % ethanol solution, dehydrated and oven-dried at 50 ^{0}C.

2.2.4.2.6 Alcohol-alkaline Modification 50 % Treatment

This was determined by modifying the method of Chen and Jane (1994). A measured volume, 100 ml of 50 % ethanol solution was added to a beaker containing 25 g of starch and stirred into slurry. 55 ml of 4 M NaOH solution was added at 4 ml/ minute and the mixture was allowed to stand for 15 minute by gentle stirring. Further, 50 ml of 50 % ethanol was added slowly to the suspension and stirred for 10 minutes. The supernatant was carefully decanted and the starch washed with a fresh solution of ethanol and neutralised with 4 M HCl. The starch was washed further with 95 % ethanol solution, dehydrated and oven-dried at 50 ^{0}C.

2.2.4.2.7 Carboxymethylation

The method of Khalil *et al.* (1990) was adopted. Isopropanol solution (isopropanol: water; 80:20) was added to 5 g of starch and the pH was adjusted with 2 N NaOH. 15 ml of 40 %

monochloroacetic (MCA) acid was added to the slurry and incubated at room temperature (25 ^{o}C) with intermittent shaking for 90 minutes.

2.2.4.2.8 Crosslinking

The method of Sang *et al.* (2007) was adopted. A solution containing 6 g of sodium trimetaphosphate and 5 g of sodium sulphate in 100 ml of distilled water was added to 25 g of starch and the pH of slurry was adjusted to 11.5 by the addition of 1.0 M NaOH. The mixture was stirred at 45 ^{0}C for 3 hours, cooled and pH adjusted to 6.5 using 1 M HCl. The product was washed with distilled water, centrifuged four times at 4000 x g for 10 minutes and dried at 40 ^{0}C. The starch was ground to fine powder.

2.2.4.2.9 Oxidation

The method of Sanchez-Rivera *et al.* (2005) was adopted. A measured quantity, 25 g of starch was put in a glass beaker and stirred into slurry with 100 ml of distilled water while maintaining the temperature at 35 ^{0}C. The pH was adjusted to 9.5 with 2 N NaOH. A quantity of 10 g of sodium hypochlorite was slowly added into the starch slurry over 30 minutes while maintaining the pH at 9.5 with 1 N NaOH for an additional 50 minutes. The pH was adjusted to 7.0 with 1 N H_2SO_4 and the slurry mixture was left to precipitate. The slurry was decanted and washed with distilled water. The starch was oven dried at 50 ^{o}C for 48 hours.

2.2.4.2.10 Phosphorylation

The method of Pieschel *et al*. (2004) was adopted. A measured quantity, 5 g of sodium tripolyphosphate was mixed with 50 ml of distilled water and 25 g of starch was added into a glass beaker, stirred for 10 minutes and then allowed to sediment. The residue was dried at a temperature of 45 ^{o}C and ground to fine powder. The dried mixture was transferred to a stainless beaker and the beaker placed in an oil bath, pre-heated at a temperature of 125 ^{o}C for 3 hours with continuous stirring and left to cool. The mixture was slurred in 750 ml of 50 % methanol and stirred for 30 minutes using a magnetic stirrer. The mixture was filtered, washed 3 times with 50 % methanol and left to dehydrate after washing with absolute ethanol. It was subsequently dried in an air oven.

2.2.4.2.11 Pyrodextrinization

The method of Orozco-Martinez and Betancur-Ancona (2004) was adopted. In the experiment, a known quantity, 25 g of starch was put in a 500 ml glass beaker and 10 ml of 2.2 M HCl was added. The mixture was allowed to react for 16 hours at room temperature, dried in an air oven at 110 ^{o}C for 3 hours and then ground to fine powder.

2.2.4.2.12 Succinylation

The method of Trubiano (1986) was adopted. Starch and distilled water (40 % weight/volume) were mixed into slurry. The pH was adjusted using 3 % NaOH in small portions, succinic anhydride (5 % weight/volume) was added simultaneously with NaOH to maintain the pH and the slurry was stirred continuously during the reaction time. The pH was adjusted to 6.5 using 0.5 N HCl. The product was recovered by filtration, after which it was washed with distilled water and re-filtered. The product was collected and dried at 55 ^{o}C overnight in an air oven.

2.2.5 Determination of the Functional Properties of Starch

2.2.5.1 Swelling Power

The method of Daramola and Osanyinlusi (2006) was adopted. A measured quantity, 0.1 g starch and 10 ml distilled water were mixed in a test tube. The slurry was heated in a water bath at 50 ^{0}C for 30 minutes with continuous shaking and was centrifuged at 1500 revolutions per minute (rpm) for 20 minutes so as to facilitate the removal of the supernatant which was carefully decanted and the weight of the starch paste recorded.

$$Swelling\ power = \frac{Weight\ of\ starch\ paste}{Weight\ of\ dry\ starch\ sample}$$

2.2.5.2 pH

The method of Akpa and Dagde (2012) was adopted as follows: A measured volume, 10 ml of distilled water was added to 1.5 g of starch and was stirred properly. The pH meter was inserted into the solution and the reading recorded.

2.2.5.3 Water Absorption Capacity (WAC)

The method of Medcalf *et al.* (1968) was adopted. A known volume, 5 ml of distilled water was added to 5 g of starch, stirred for 1 hour and centrifuged at 3000 rpm for 10 minutes. The starch paste was weighed and the absorbed water calculated by the formula:

$$WAC = \frac{Absorbed\ water\ (g)}{Weight\ of\ sample}$$

2.2.5.4 Amylose Content Determination

The method of Williams *et al.* (1970) was adopted. A quantity, 1 g of starch, 10 ml of 100 % (volume/volume) ethanol, and 90 ml of 1 N NaOH were mixed in a volumetric flask, the mouth was covered with an aluminium foil and mixed. The sample was heated for 10 minutes in a boiling water bath (to gelatinise the starch). It was left to cool, and then made up to mark with distilled water. The blank contained 10 ml of ethanol and 90 ml of NaOH; it was boiled and made up to the 100 ml mark with distilled water. 5ml of the starch solution was poured into a 100 ml volumetric flask, 1 ml of 1 N acetic acid and 2 ml of iodine solution were added and made up with distilled water. The absorbance was read using a spectrophotometer at 620 nm.

The Amylose content (%) was calculated as follows:

Amylose content (%) $= (3.06)\ (A)\ (20)$

Where A = absorbance value.

The Amylopectin content was determined by: 100 % - Amylose content.

2.2.5.5 Gelatinisation Temperature

The method of Association of Analytical Chemists (AOAC) (1984) was adopted. A measured quantity, 1.5 g of starch dissolved in 10 ml of distilled water in a beaker was continuously stirred over a water bath with a thermometer inserted in it. The temperature was read when a thickened milk-colour was formed.

2.2.5.6 Moisture Content

The method of Musa *et al.* (2010) was adopted. A measured quantity, 1 g of starch was dried in an air oven at 105 ^{0}C for 4 hours

W_0 = Initial weight before drying

W_1 = Weight of starch granules after drying

$$Moisture\ (\%) = \frac{Weight\ moisture\ in\ sample}{Weight\ of\ sample\ before\ drying} \times 100$$

2.2.5.7 Paste Clarity

The method of Kolawole *et al.* (2013) was adopted. Accurate concentrations of the starch slurry between 0.15 - 2.5 % weight/volume were made in different boiling tubes and heated in a water bath for 30 minutes. The transmittance was determined at 650 nm using a UV spectrophotometer.

Transmittance = 1 / Absorbance

CHAPTER THREE

RESULTS

3.1 Images of different isolated starches

The native legume starches appeared slightly coloured when compared to the commercial starch sample which served as standard. Similarly, some chemical modifications stained the starches causing them to have a faded white appearance (Plate 1 - 7).

(A)

Control **S - native** **C- native**

(B)

M- native **V- native** **S- Annealed**

(C)

C- Annealed

Plate 5 (A): Images of control and native starches after processing

(B): Images of native and annealed starches after processing

(C): Images of annealed starches after processing

(A)

M- Annealed **V- Annealed** **M- Acetylated**

(B)

V- Acetylated **C- Acetylated** **S- Acetylated**

(C)

V- Phosphorylated **C- Phosphorylated** **M- Phosphorylated**

(D)

S- Phosphorylated **C- Alcohol-Alkaline (A/A)** **M- A/A**

Plate 6 (A): Images of annealed starches after processing
(B): Images of acetylated starches after processing
(C): Images of phosphorylated starches after processing
(D): Images of phosphorylated and alcohol-alkaline (A/A) starches after processing

(A)

S- A/A **V- A/A** **V- Pyrodextrinization**

(B)

M- Pyrodextrinization **V- Pyrodextrinization** **S- Pyrodextrinization**

(C)

M- Oxidized **V- Oxidized** **C- Oxidized**

(D)

S- Oxidized **C- Oxidized** **M- Carboxymethylated**

Plate 7 (A): Images of alcohol-alkaline (A/A) and pyrodextrinized starches after processing

(B): Images of pyrodextrinized starches after processing

(C): Images of oxidized starches after processing

(D): Images of oxidized and carboxymethylated starches after processing

(A)

V- Carboxymethylated C- Carboxymethylated S- Carboxymethylated

(B)

M- Succinylated S- Succinylated C- Succinylated

(C)

S- Succinylated M- Acid-Alcohol (Ac/Al) C- Acid- alcohol

(D)

S- Acid- alcohol V - Acid-alcohol V - Osmotic Pressure Treatment (OPT)

Plate 8 (A): Images of carboxymethylated starches after processing
(B): Images of succinylated starches after processing
(C): Images of succinylated and acid-alcohol starches after processing
(D): Images of acid-alcohol and osmotic pressure starches after processing

S- OPT **M- OPT** **C- OPT**

V- Cross-linked **A- Cross-linked** **M- Cross-linked**

C- Cross-linked **M- Heat Moisture Treated (HMT)** **V- HMT**

S- HMT **C- HMT** **V- Acid alcohol 50 % treatment (Ac/Al 50 %)**

Plate 9 (A): Images of osmotic pressure treated starches after processing
(B): Images of cross-linked starches after processing
(C): Images of cross-linked and heat-moisture treated starches after processing
(D): Images of heat-moisture treated and acid-alcohol 50 % starches after processing

(A)

M- (Ac/Al 50 %) **C- (Ac/Al 50 %)** **S- (Ac/Al 50 %)**

(B)

V- Acid alcohol 70 % (Ac/Al 70 %) **M- (Ac/Al 70 %)** **C- (Ac/Al 70 %)**

(C)

S- (Ac/Al 70 %) **M- Alcohol-alkaline (A/ A) 20 % treatment** **V- (A/ A 20 %)**

(D)

S- (A/ A 20 %) **C- (A/ A 20 %)** **C- (A/ A 50 %)**

Plate 10 (A): Images of acid-alcohol (Ac/Al) 50 % treated starches after processing

(B): Images of acid-alcohol (Ac/Al) 70 % treated starches after processing

(C): Images of (Ac/Al) 70 % and alcohol alkaline (A /A) 20 % treated starches after processing

(D): Images of alcohol alkaline (A /A) 20 % and 50 % treated starches after processing

(A)

M- (A/ A 20 %) **S- (A/ A 20 %)** **V- (A/ A 20 %)**

(B)

C- Combo (A/A + Ac/Al) **S-Combo (A/A +Ac/Al)** **M- Combo (A/A + Ac/Al)**

(C)

V- Combo (A/A + Ac/Al)

Plate 11(A): Images of alcohol-alkaline (A/A) 20 % treated starches after processing

(B): Images of alcohol-alkaline + acid-alcohol (combo) treated starches after Processing

(C): Images of alcohol-alkaline + acid-alcohol (combo) treated starches after Processing

3.2 Results for Percentage Starch Yield

The percentage yield of starch isolated from *Cajanus cajan, Mucuna pruriens var pruriens, Sphenostylis stenocarpa, and Vigna Subterranea* flours based on a 1000 g of flour had 41.2 %, 40.6 %, 48.6 % and 46.5 % yields respectively (Table 2). The recovered starch after modification ranged from 72 % to 99 % (Table 2).

Table 2: Results for starch yield of native starches

	Recovered Starch (g)	**Initial weight**	**Starch Yield %**
NATIVE			
C_{native}	411.2	1000	41.20
M_{native}	406.3	1000	40.60
V_{native}	460.46	1000	46.05
S_{native}	486.99	1000	48.60

3.3 Yield Results of Modification of Starch with Acid-hydrolysis and Alcohol-alkaline

The percentage yield of starch isolated from *Cajanus cajan, Mucuna pruriens var pruriens, Sphenostylis stenocarpa, and Vigna Subterranea* flours after acid-hydrolysis and alcohol-alkaline modifications ranged from 76.77 % to 100 % (Table 3).

Table 3: Results for starch yield of acid-hydrolysis and alcohol-alkaline starches

	Recovered Starch (g)	**Initial weight**	**Starch Yield %**
$C_{\text{Acid hydrolysis}}$	18.171	20	90.86
$M_{\text{Acid hydrolysis}}$	17.068	20	85.34
$V_{\text{Acid hydrolysis}}$	16.79	20	83.95
$S_{\text{Acid hydrolysis}}$	18.597	20	92.99
$C_{\text{A/A}}$	19.19	20	95.95
$M_{\text{A/A}}$	19.15	20	95.75
$V_{\text{A/A}}$	19.826	20	99.13
$S_{\text{A/A}}$	18.825	20	94.13
$C_{\text{A/A 20\%}}$	21.56	20	107.84
$M_{\text{A/A 20\%}}$	18.05	20	90.25
$V_{\text{A/A 20\%}}$	18.457	20	92.29
$S_{\text{A/A 20\%}}$	19.104	20	95.52
$C_{\text{A/A 50\%}}$	15.353	20	76.77
$M_{\text{A/A 50\%}}$	17.876	20	89.38
$V_{\text{A/A 50\%}}$	18.292	20	91.46
$S_{\text{A/A 50\%}}$	18.172	20	90.86

3.4 Yield Results of Modification of Starch with Acid-alcohol

The percentage yield of starch isolated from *Cajanus cajan, Mucuna pruriens var pruriens, Sphenostylis stenocarpa, and Vigna Subterranea* flours after acid-alcohol modifications ranged from 88.64 % to 99.58 % (Table 4).

Table 4: Results for starch yield of acid-alcohol modified starches

	Recovered starch(g)	**Initial weight (g)**	**Starch Yield**
$C_{AC/AL}$	19.203	20	96.02
$M_{AC/AL}$	18.129	20	90.65
$V_{AC/AL}$	18.255	20	91.28
$S_{AC/AL}$	19.135	20	95.68
$C_{AC/AL\ 50\%}$	18.861	20	94.31
$M_{AC/AL\ 50\%}$	19.19	20	95.95
$V_{AC/AL\ 50\%}$	18.271	20	91.36
$S_{AC/AL\ 50\%}$	17.728	20	88.64
$C_{AC/AL\ 70\%}$	19.447	20	97.24
$M_{AC/AL\ 70\%}$	18.891	20	94.46
$V_{AC/AL\ 70\%}$	19.915	20	99.58
$S_{AC/AL\ 70\%}$	19.712	20	98.56

3.5 Yield Results of Modification of Starch with Acetylation, Annealing, Carboxymethylation, Cross-linking, Combo and Heat Moisture Treatments

The percentage yield of starch isolated from *Cajanus cajan, Mucuna pruriens var pruriens, Sphenostylis stenocarpa, and Vigna Subterranea* flours after modifications ranged from 80.99 % to 99.32 % (Table 5).

Table 5: Results for starch yield of acetylated, annealed and carboxymethylated, cross-linked, combo and heat moisture modified starches

	Recovered starch (g)	**Initial weight**	**Starch Yield(%)**
$C_{Acetylation}$	18.745	20	93.73
$M_{Acetylation}$	18.536	20	92.68
$V_{Acetylation}$	18.148	20	90.74
$S_{Acetylation}$	19.283	20	96.42
$C_{Annealing}$	19.413	20	97.07
$M_{Annealing}$	17.981	20	89.91
$V_{Annealing}$	17.93	20	89.65
$S_{Annealing}$	16.365	20	81.83
$C_{Carboxymethylation}$	19.864	20	99.32
$M_{Carboxymethylation}$	18.724	20	93.62
$V_{Carboxymethylation}$	16.532	20	82.66
$S_{Carboxymethylation}$	18.557	20	92.79
$C_{Crosslinking}$	19.017	20	95.09
$M_{Crosslinking}$	18.221	20	91.11
$V_{Crosslinking}$	19.294	20	96.47
$S_{Crosslinking}$	19.676	20	98.38
C_{Combo}	18.658	20	93.29
M_{Combo}	16.826	20	84.13
V_{Combo}	19.282	20	96.41
S_{Combo}	19.534	20	97.67

$C_{\text{Heat Moisture Treatment}}$	18.31	20	91.55
$M_{\text{Heat Moisture Treatment}}$	16.786	20	83.93
$V_{\text{Heat Moisture Treatment}}$	16.197	20	80.99
$S_{\text{Heat Moisture Treatment}}$	16.56	20	82.80

3.6 Yield Results of Modification of Starch with Osmotic pressure, Osidation, Phosphorylation, Pyrodextrinization and Succinylation Treatments

The percentage yield of starch isolated from *Cajanus cajan, Mucuna pruriens var pruriens, Sphenostylis stenocarpa, and Vigna Subterranea* flours after modifications ranged from 84.71 % to 100 % (Table 6).

Table 6: Results for starch yield of acid-alcohol modified starches

	Recovered starch (g)	**Initial weight**	**Starch yield %**
C $_{\text{Osmotic Pressure}}$	18.212	20	91.06
M $_{\text{Osmotic Pressure}}$	19.145	20	95.73
V $_{\text{Osmotic Pressure}}$	18.788	20	93.94
S $_{\text{Osmotic Pressure}}$	19.541	20	97.71
C $_{\text{Oxidation}}$	20.074	20	100.37
M $_{\text{Oxidation}}$	18.71	20	93.55
V $_{\text{Oxidation}}$	18.938	20	94.69
S $_{\text{Oxidation}}$	17.526	20	87.63
C $_{\text{Phosphorylation}}$	19.678	20	98.39
M $_{\text{Phosphorylation}}$	18.654	20	93.27
V $_{\text{Phosphorylation}}$	17.526	20	87.63
S $_{\text{Phosphorylation}}$	15.485	20	77.43
C $_{\text{Pyrodextrinization}}$	16.941	20	84.71
M $_{\text{Pyrodextrinization}}$	17.854	20	89.27
V $_{\text{Pyrodextrinization}}$	14.498	20	72.49
S $_{\text{Pyrodextrinization}}$	18.226	20	91.13
C $_{\text{Succinylation}}$	19.341	20	96.71
M $_{\text{Succinylation}}$	18.757	20	93.79
V $_{\text{Succinylation}}$	17.832	20	89.16
S $_{\text{Succinylation}}$	19.918	20	99.59

Where C= *Cajanus cajan*

M= *Mucuna pruriens var pruriens*

V= *Vigna subterranea*

S= *Sphenostylis stenocarpa*

A/A= Alcohol-alkaline;

AC/ AL= Acid-alcohol;

Combo= Dual modification (Alcohol-alkaline + Acid-alcohol

3.7 Effect of Modification on the Cold Water Solubility of starches.

The result of the experiment on the effect of modification on cold water solubility of starches is shown in figure 8 to 11. Most of the modification resulted in a significant ($p<0.05$) decrease in the cold water solubility of the starches.

Alcohol-alkaline, acid- alcohol, acid hydrolysed modified starches had the highest cold water solubility of 65 – 85 %. These values were comparable to those of the standard.

Considering the significant improvement in solubility of the alcohol alkaline and acid-alcohol treatments when compared to the other modifications, some modifications were made on these treatments where for the alcohol-alkaline treatment, first, the alcohol and alkaline were reduced to 20 % ethanol and 1.5 M NaOH respectively and for the second, the alcohol and alkaline were increased to 20 % ethanol and 4 M NaOH respectively. The two different modifications improved the solubility of the legume starches as compared to the first treatment of 40 % ethanol and 3 M NaOH. For the acid-alcohol treated, the modifications with the aim of achieving a more soluble yield involved first, reducing the acid concentration from a 36 % to a 20 % concentration alongside reducing the alcohol concentration from a 70 % to a 50 % concentration (Figure 8 - 11). On the other hand, the modification was altered again by increasing the concentration of the acid to 45 % and the alcohol maintained at 70 %. The both alterations of the modification improved the cold water solubility of the legume starches.

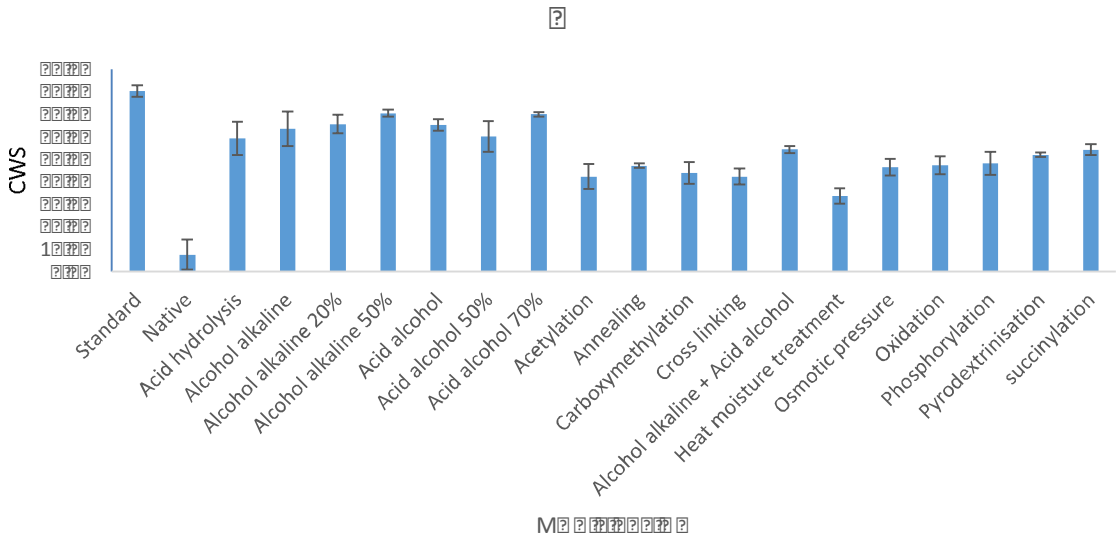


Figure 8: Cold Water Solubility for *Sphenostylis stenocarpa* (African Yam Bean)

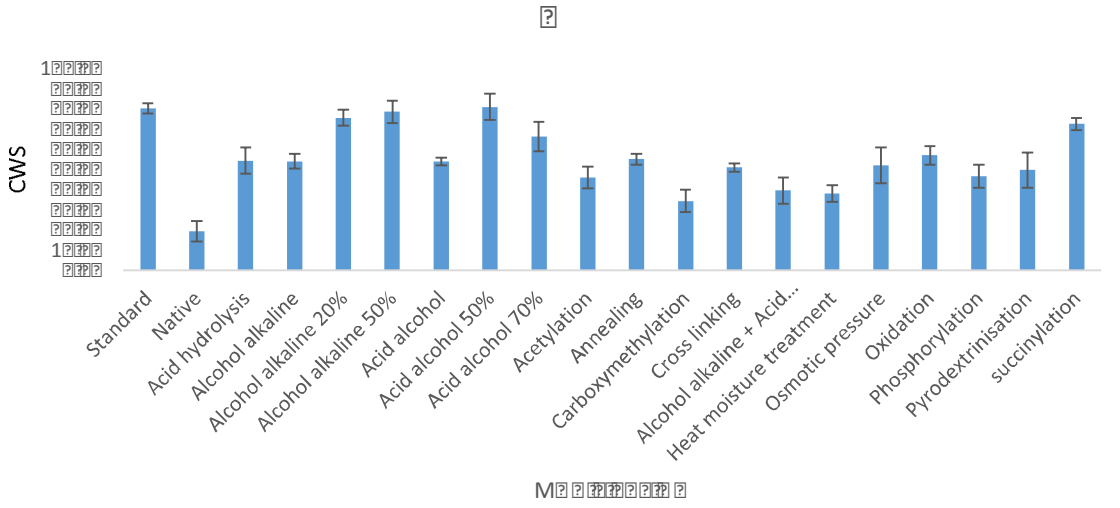


Figure 9: Cold Water Solubility for *Vigna subterranea* (Bambarra Groundnut)

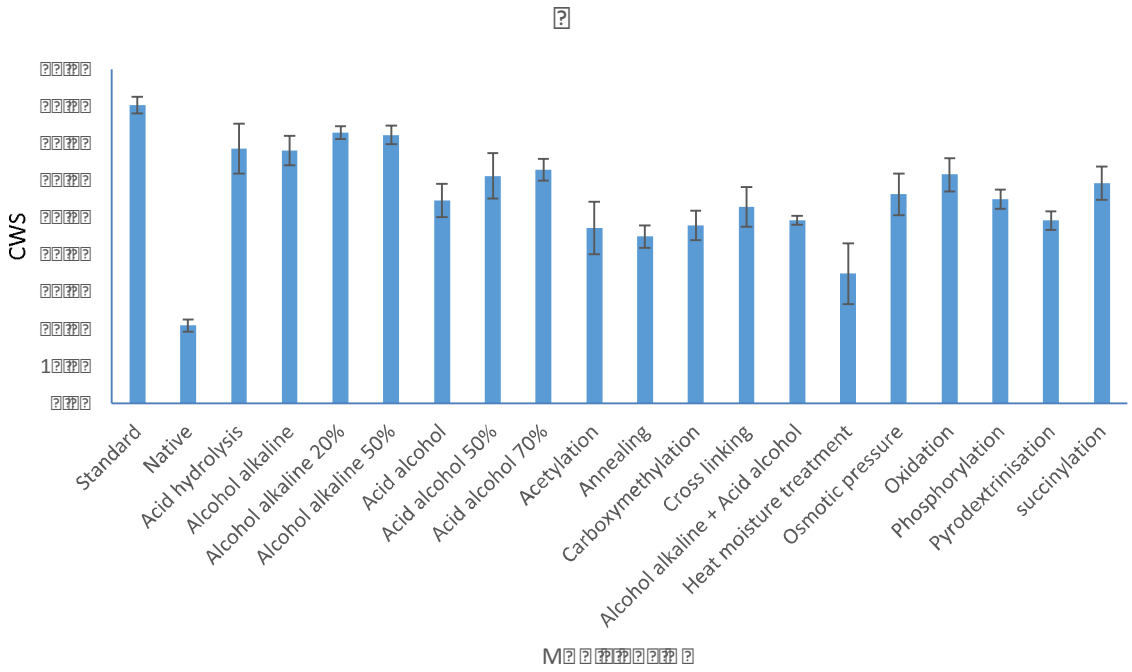


Figure 10: Cold Water Solubility for *Cajanus cajan* (Pigeon pea)

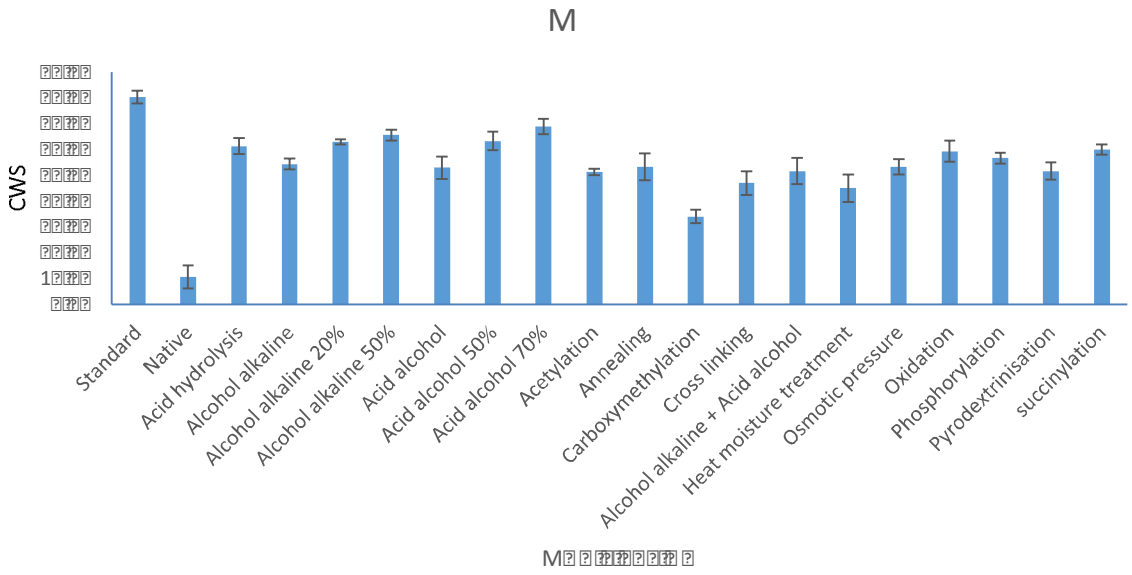


Figure 11: Cold Water Solubility for *Mucuna pruriens var pruriens*

3.8 Effect of Modification on the Swelling Power of starches.

The result of the experiment on the effect of the modification methods on the swelling power of the starches are shown in figure 12 – 15. The starches subjected to acid hydrolysis, acid-alcohol and pyrodextrinization had low swelling power, while the phosphorylated, succinylated, standard, dual modified, alcohol-alkaline treated, acetylated, carboxymethylated, native and annealed starches had high swelling power. After osmotic pressure treatment, starch from *Cajanus cajan* and *Mucuna pruriens var pruriens* had low swelling power while *Vigna subterranea* and *Sphenostylis stenocarpa* had high swelling power (Fig. 12 - 15).

Most of the modifications resulted in a significant ($p<0.05$) decrease in swelling power of the starches, with the exception of acetylation, annealing, carboxymethylation, osmotic pressure treatment and phosphorylation which resulted in a significant increase in swelling power. The effect of other modifications namely alcohol-alkaline 20 % and 50 %, dual-modification, phosphorylation and succinylation were not significant ($p>0.05$).

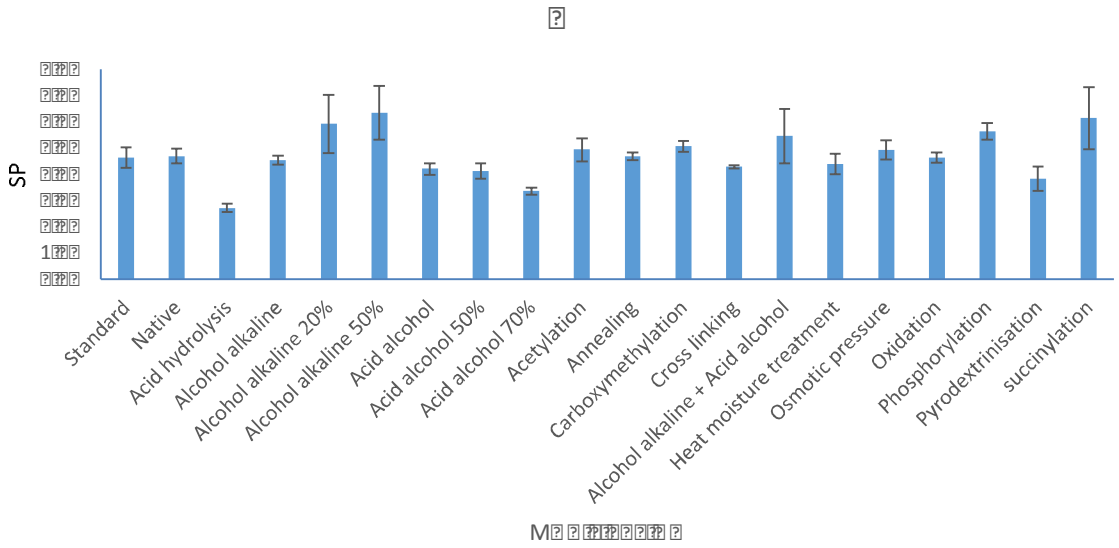


Figure 12: Swelling Power for *Sphenostylis sternocarpa* (African Yam Bean)

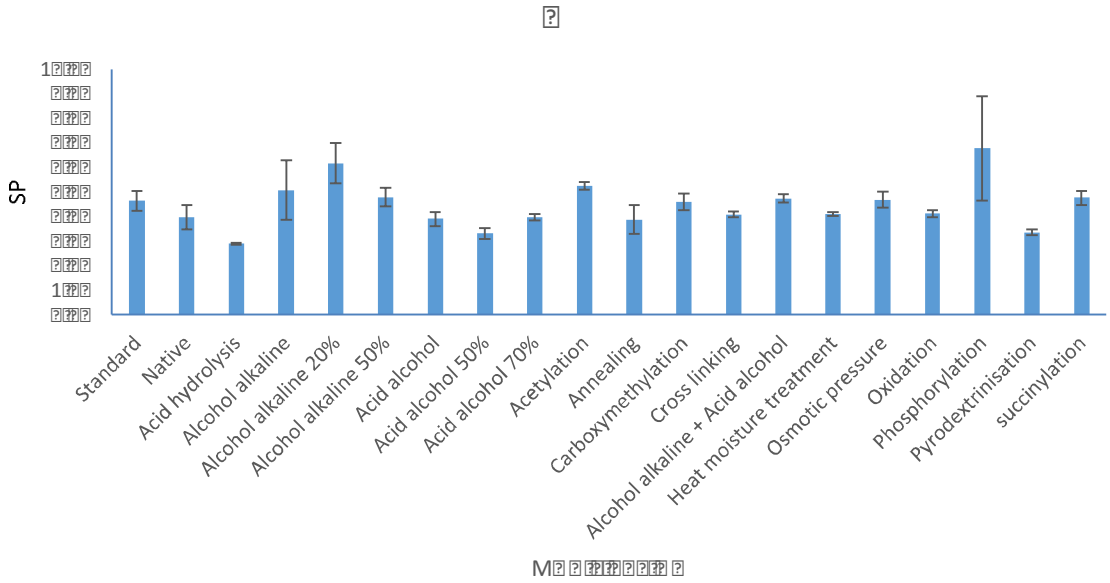


Figure 13: Swelling Power for Bambarra Groundnut (*Vigna subterranea*)

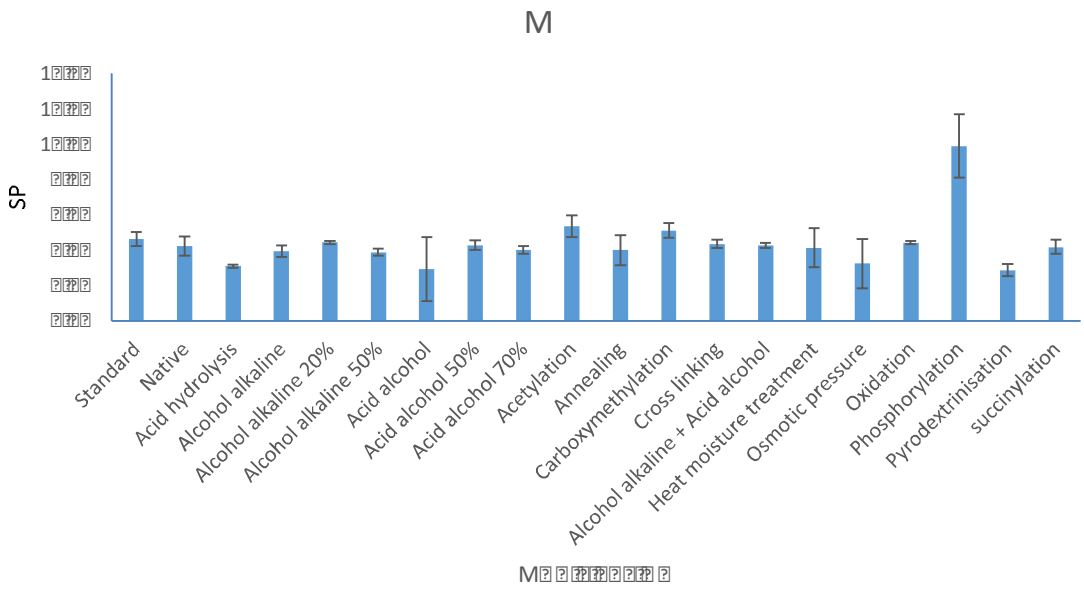


Figure 14: Swelling Power for *Mucuna pruriens var pruriens*

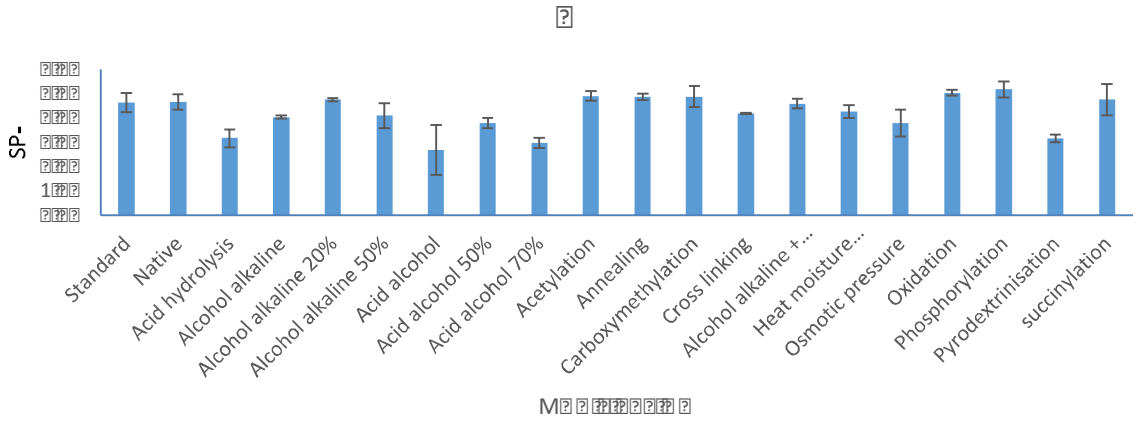


Figure 15: Swelling Power for *Cajanus cajan* (Pigeon pea)

3.9 Effect of Modification on the pH of starches.

Figures 16 – 19 represent the results of the experiments on the effects of modification on the pH of starch isolated from *Sphenostylis stenocarpa, Vigna subterranea, Cajanus cajan* and *Mucuna pruriens var pruriens* respectively. The pH values obtained were in the range of 5 – 9, with the exception of the acid-hydrolysed starches which had acidic pH range of 3.5 – 4.0.

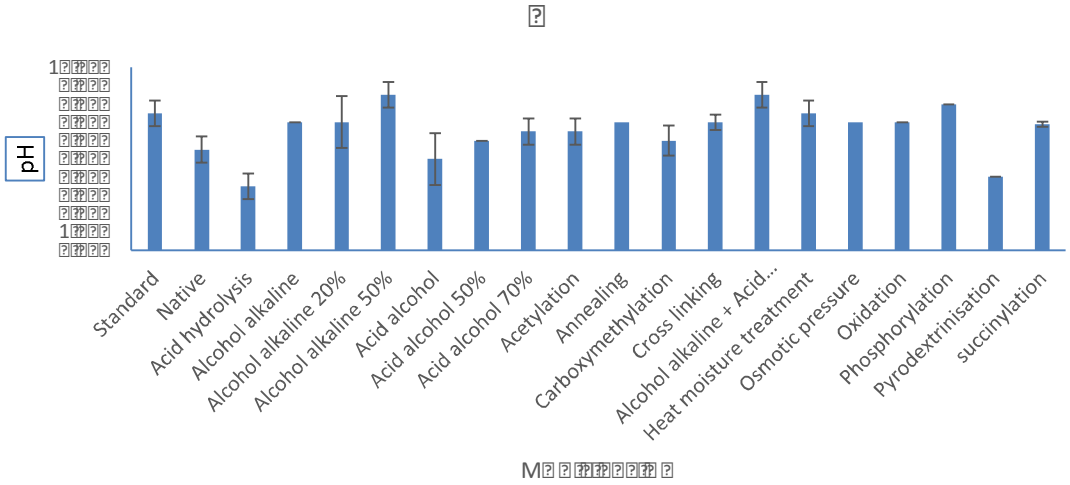


Figure 16: pH for African Yam Bean (*Sphenostylis stenocarpa*)

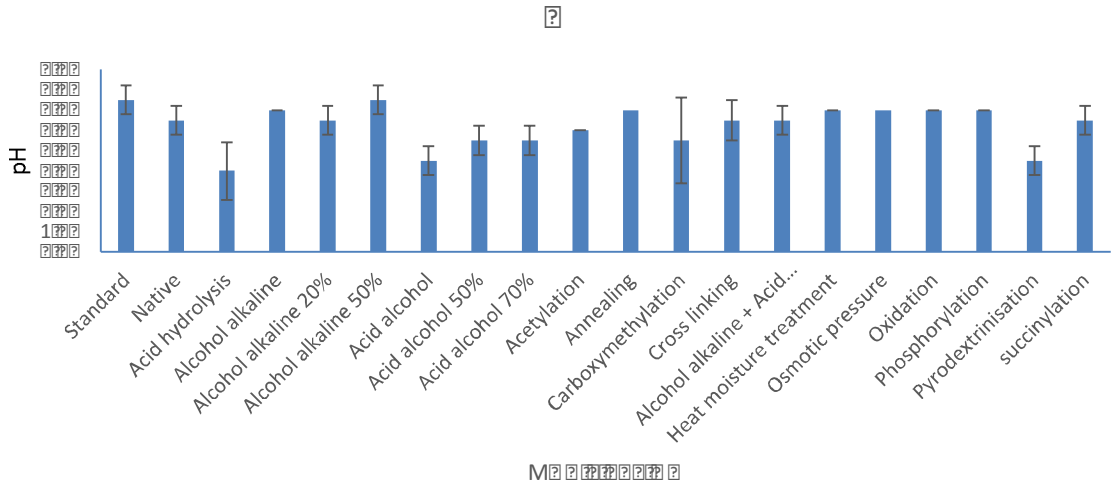


Figure 17: pH for Bambara Groundnut (*Vigna subterranea*)

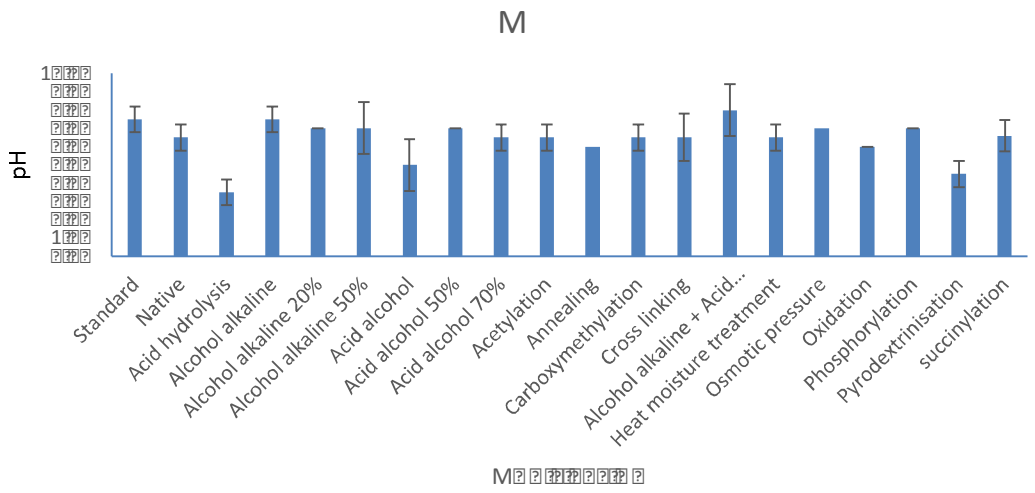


Figure 18: pH for *Mucuna pruriens var pruriens*

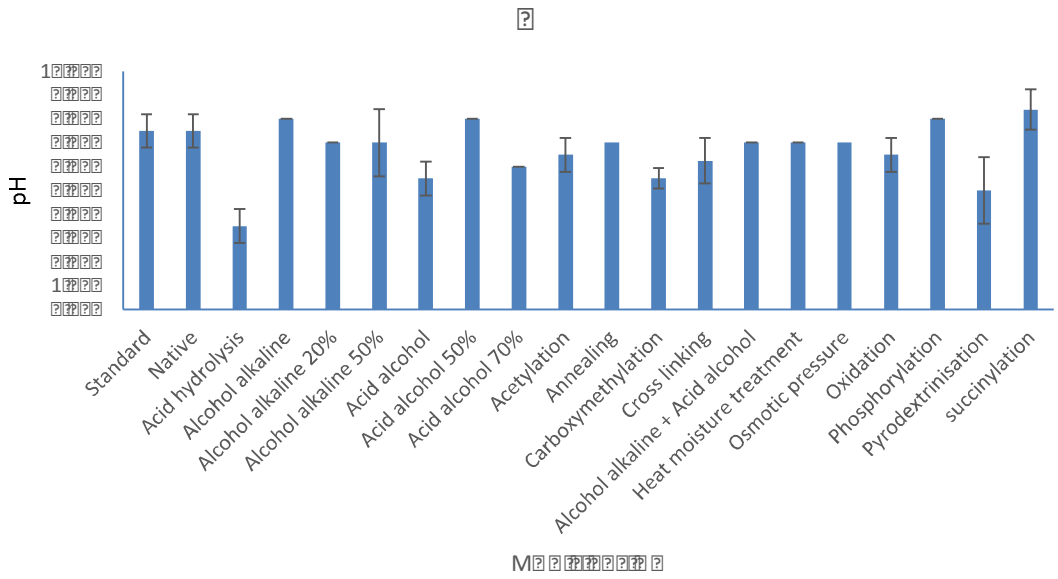


Figure 19: pH for *Cajanus cajan*

3.10 Effect of Modification on the Water Absorption Capacity (WAC) of starches.

The result of the experiment on the effect of the modification methods on the water absorption capacity of the starches is shown in Figure 20 – 23. From the figure, alcohol-alkaline treatment and dual modification did not have any impact on the water absorption capacity of the starch from *Sphenostylis stenocarpa*. However, starches subjected to pyrodextrinization, carboxymethylation, crosslinking oxidation and osmotic pressure treatments had a significantly ($p<0.05$) lower water absorption capacity than the standard.

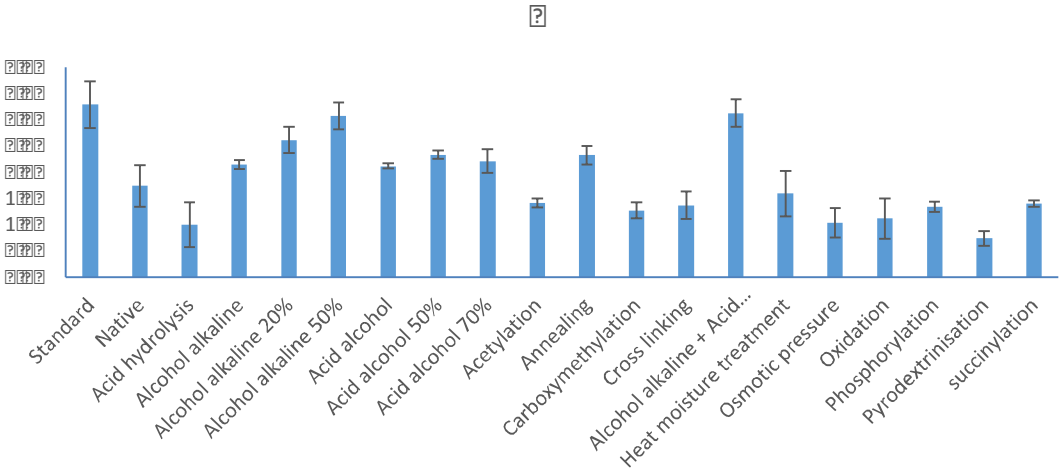


Figure 20: Water Absorption Capacity for African Yam Bean (*Sphenostylis stenocarpa*)

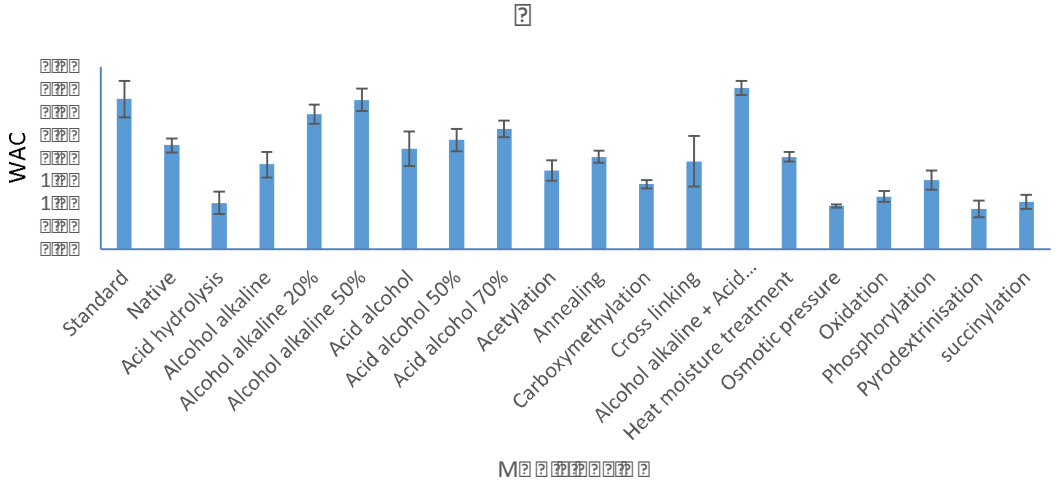


Figure 21: Water Absorption Capacity for Bambara groundnut (*Vigna subterranea*)

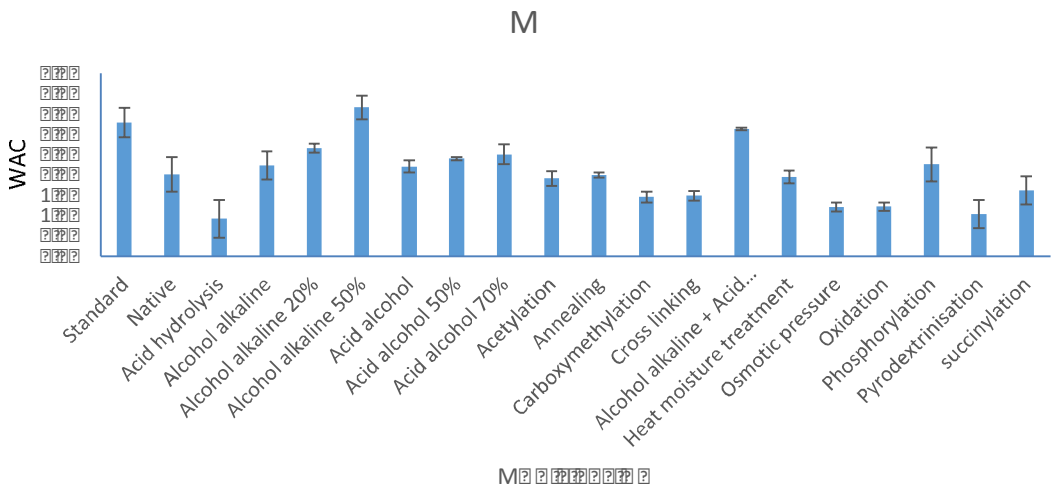


Figure 22: Water Absorption Capacity for *Mucuna pruriens var pruriens*

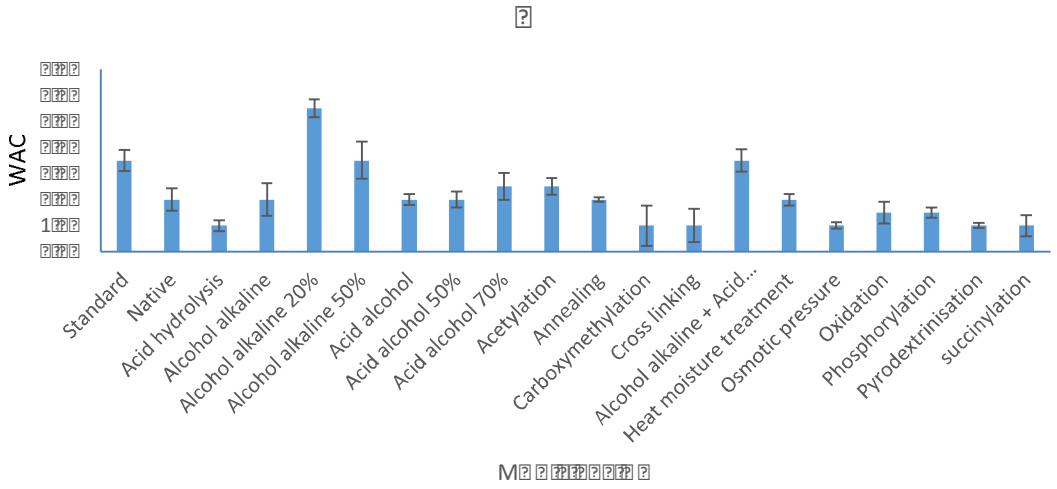


Figure 23: Water Absorption Capacity for *Cajanus cajan*

3.11 Effect of Modification on the Amylose and Amylopectin Content of Starches.

The result of the experiment on the effect of the modification methods on the amylose and amylopectin content of the starches are shown in figures 24 – 31. Modified starch from *Vigna subterranea* had comparable amylose content to that of the standard with the exception of starch hydrolysed by acid hydrolysis. Most of the modified starches had lower amylose content than their native starches except the acid hydrolysed, alcohol alkaline (excluding the 20 % and 50 % treated), osmotic pressure treated, pyrodextrinized and some succinylated starches (Fig. 24, 26, 28 and 30). The amylopectin content of the starches ranged from 71 – 86 %. (Fig. 25, 27, 29 and 31).

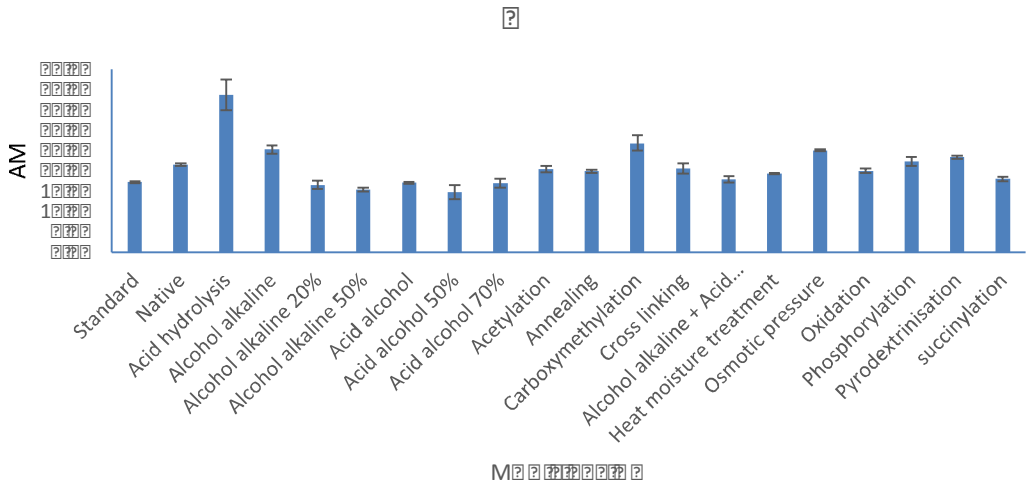


Figure 24: Amylose content result for *Vigna subterranean*

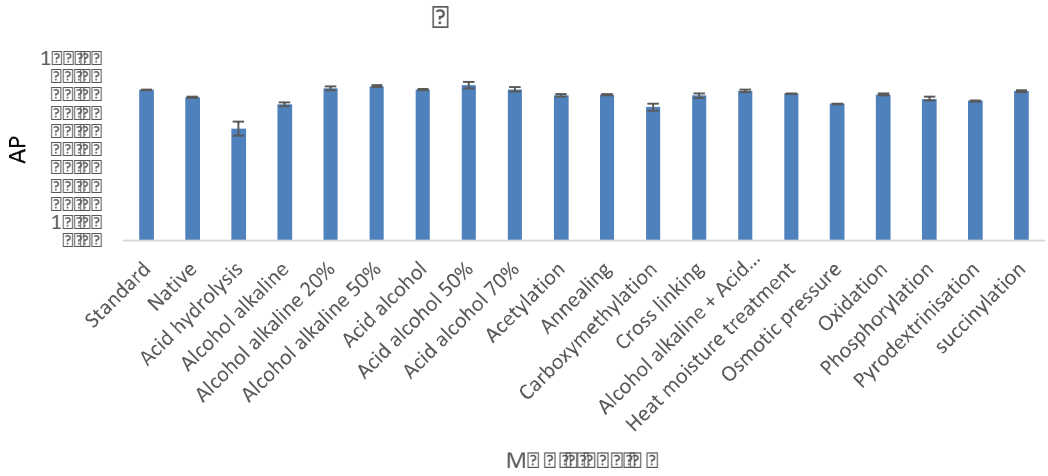


Figure 25: Amylopectin content result for *Vigna subterranean*

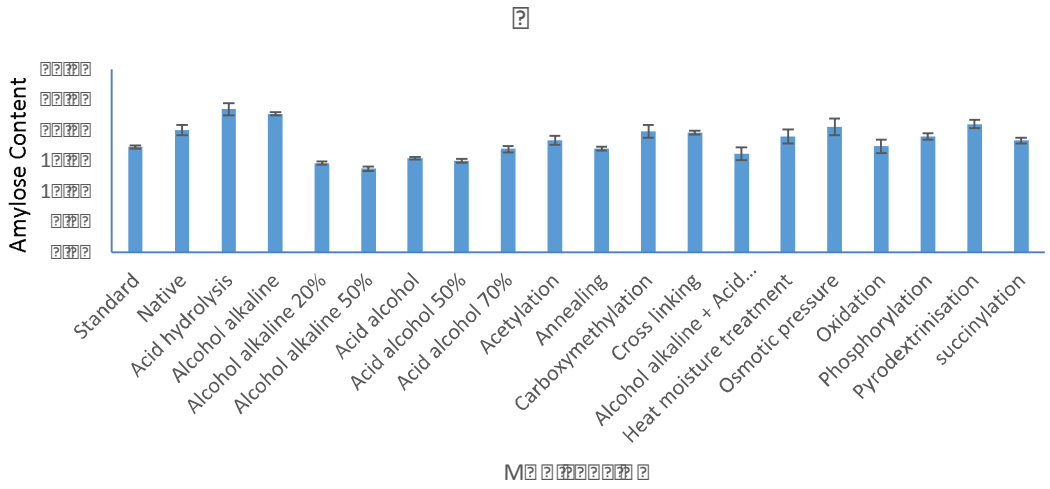


Figure 26: Amylose content result for *Cajanus cajan*

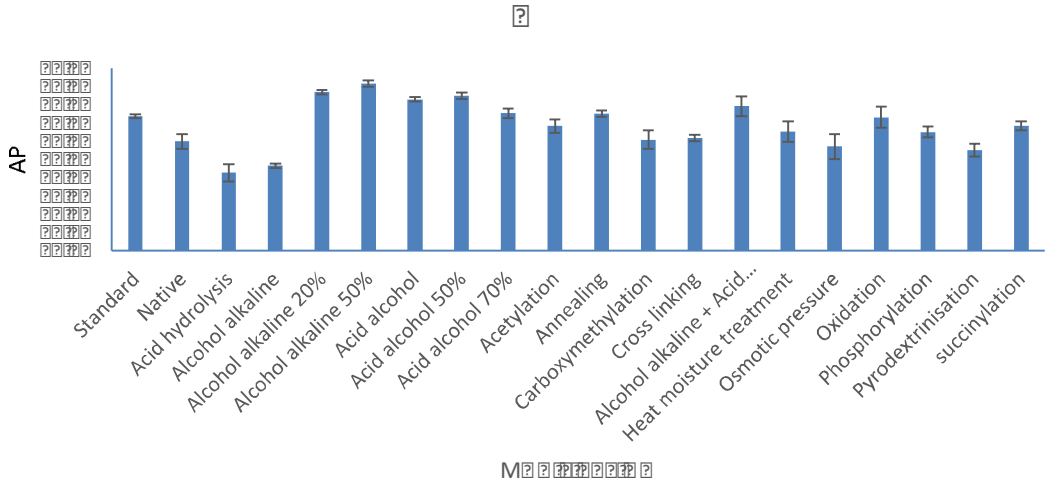


Figure 27: Amylopectin content result for *Cajanus cajan*

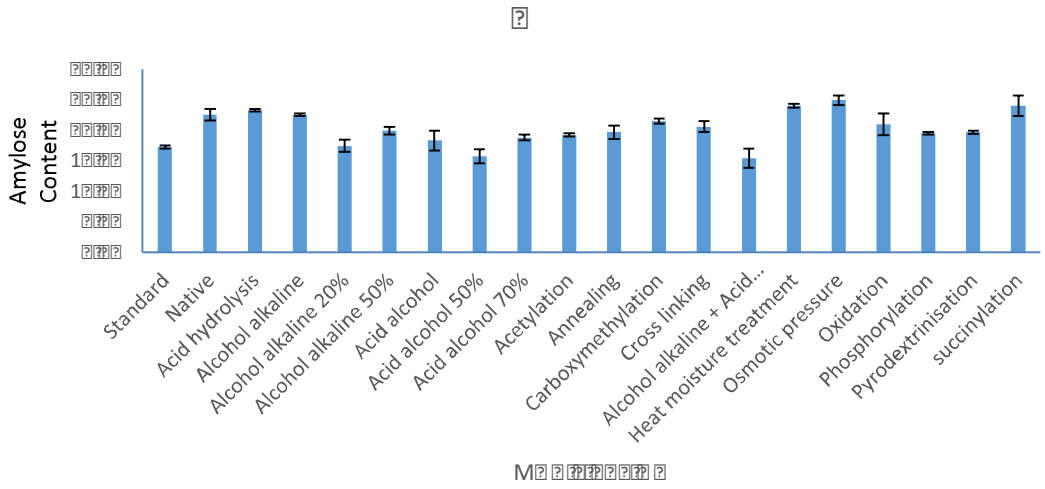


Figure 28: Amylose content result for *Sphenostylis stenocarpa*

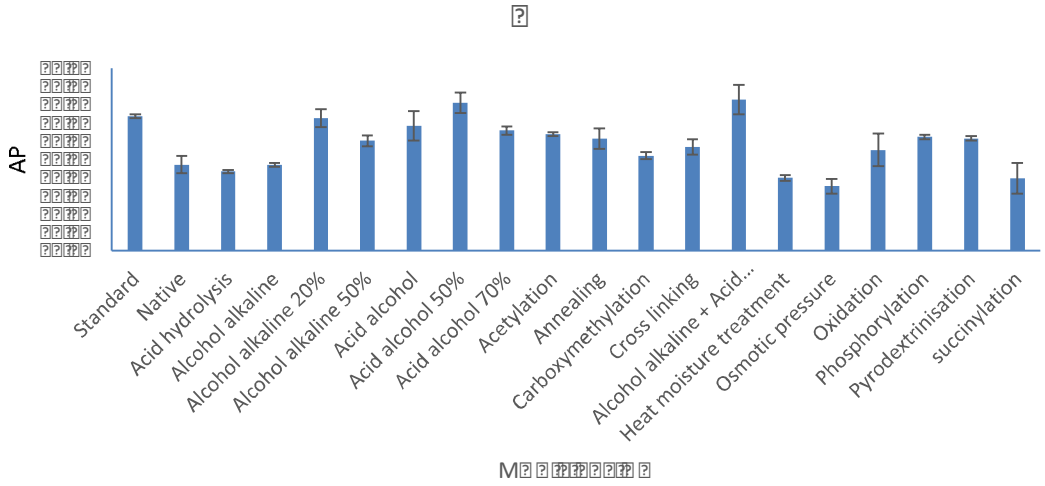


Figure 29: Amylopectin content result for *Sphenostylis stenocarpa*

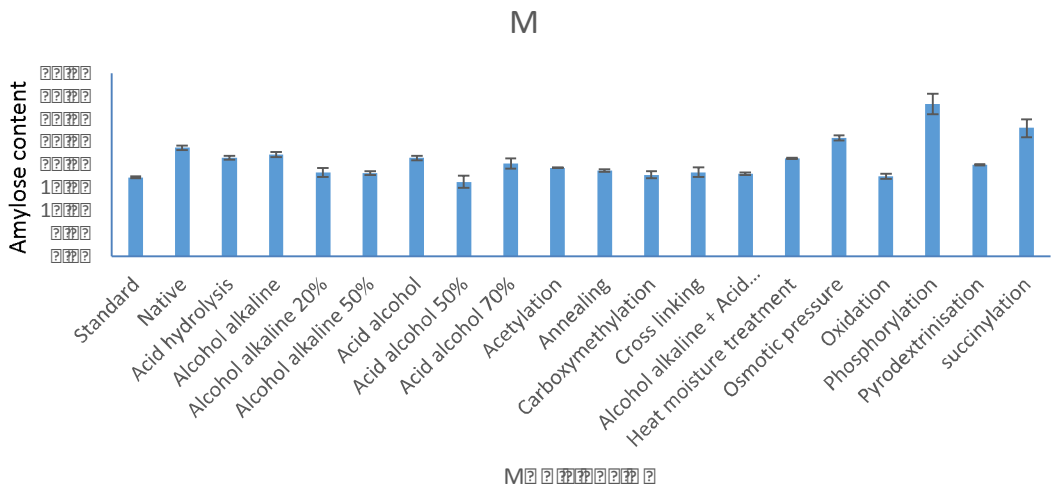


Figure 30: Amylose content result for *Mucuna pruriens var pruriens*

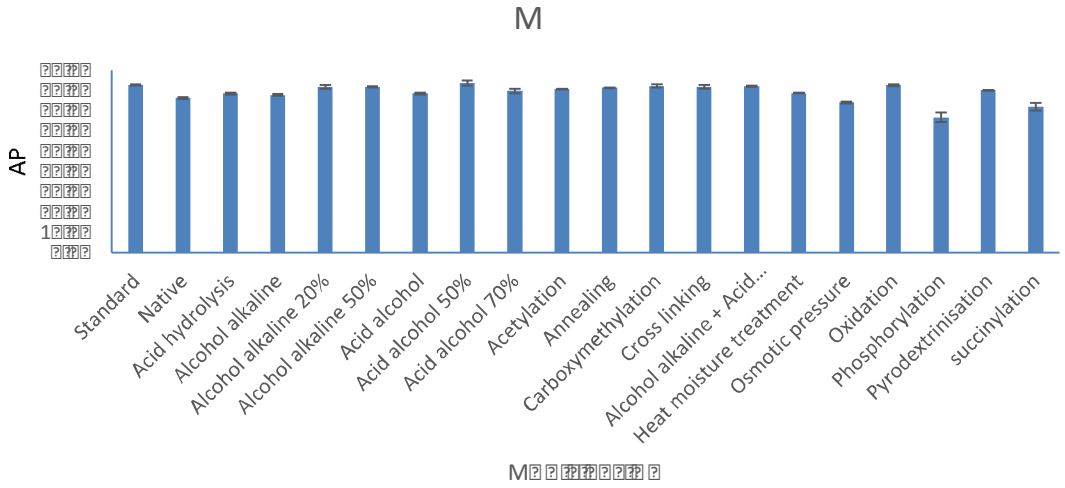


Figure 31: Amylopectin content result for *Mucuna pruriens var pruriens*

3.12 Effect of Modification on the Gelatinisation Temperature of starches.

The result of the experiment on the effect of the modification methods on the gelatinisation temperature of modified starches are shown in figures 32 – 35. The gelatinisation temperature reduced with increasing cold water solubility with the standard, alcohol alkaline treated (including the 20 % and 50 % treated), acid hydrolysed, acid alcohol treated, having low gelatinisation temperature and the native starches had the highest gel temperature (Fig. 32 - 35). There was a significant increase in gelatinisation temperature for most of the modified starches when compared with the standard. This indicates the modifications in most cases gelatinise at room temperature or slightly above.

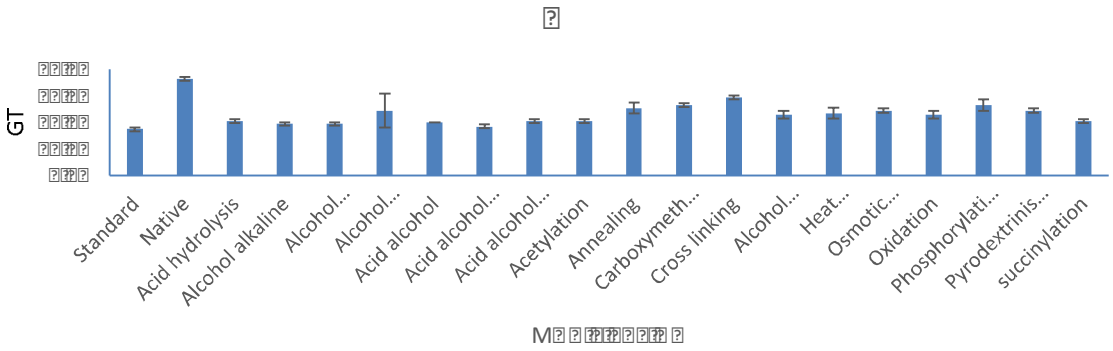


Figure 32: Gelatinisation Temperature for *Cajanus cajan*

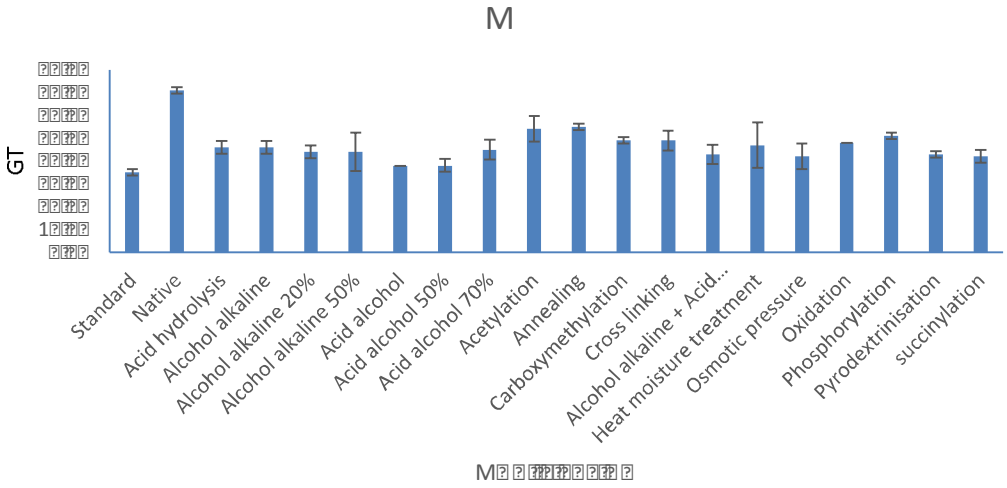


Figure 33: Gelatinisation Temperature for *Mucuna pruriens var pruriens*

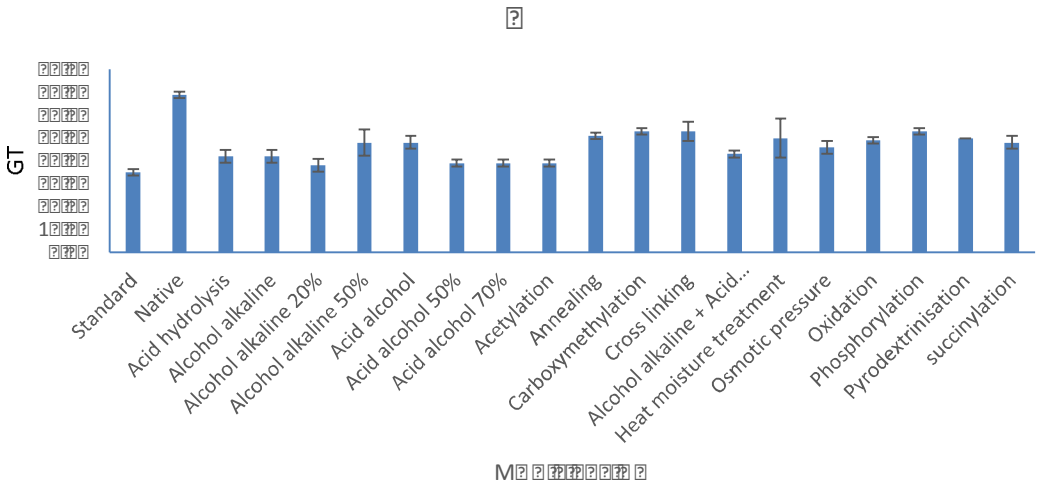


Figure 34: Gelatinisation Temperature for Bambara groundnut (*Vigna subterranea*)

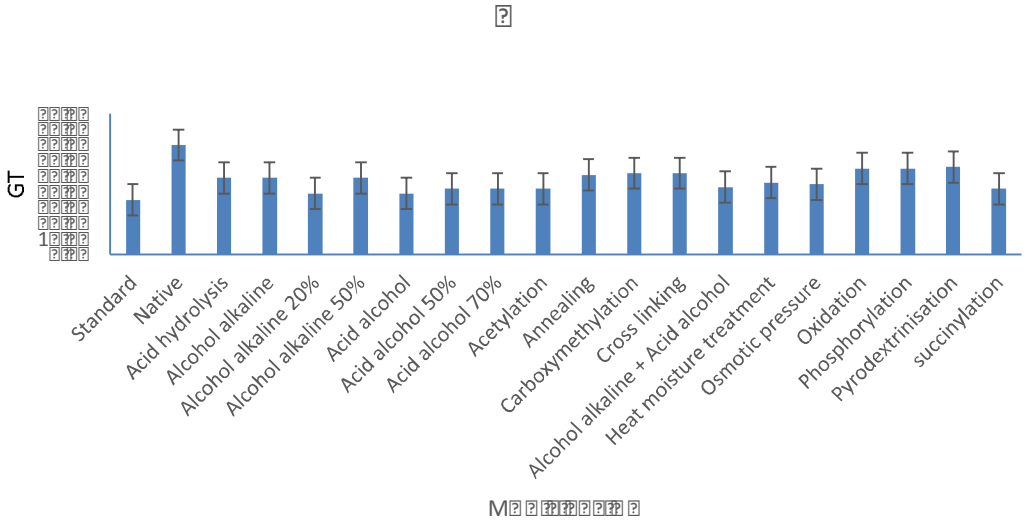


Figure 35: Gelatinisation Temperature for African Yam Bean (*Sphenostylis stenocarpa*)

3.13 Effect of Modification on the Moisture Content of starches.

The result of the experiment on the effect of the modification methods on the moisture content of modified starches are shown in figures 36 – 39. Most of the modified starches had low moisture content which ranged from 1.4 - 13 %, with the exception of those subjected to carboxymethylation, dual modification, and acid-alcohol modified starches (Fig. 36 - 39). The moisture content of the modified starches was significantly higher ($p<0.05$) than that of the standard excluding acetylated, annealed, cross-linked, osmotic pressure treated and succinylated starches.

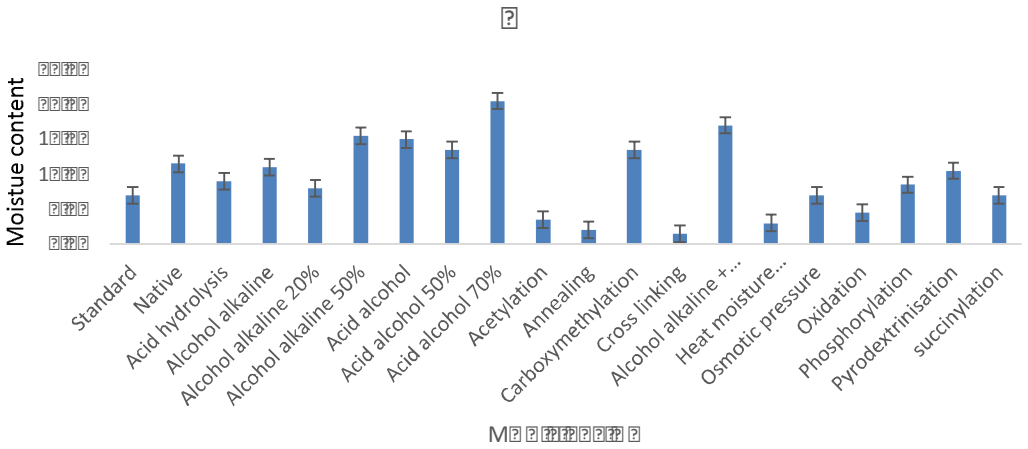


Figure 36: Moisture content for *Sphenostylis stenocarpa*

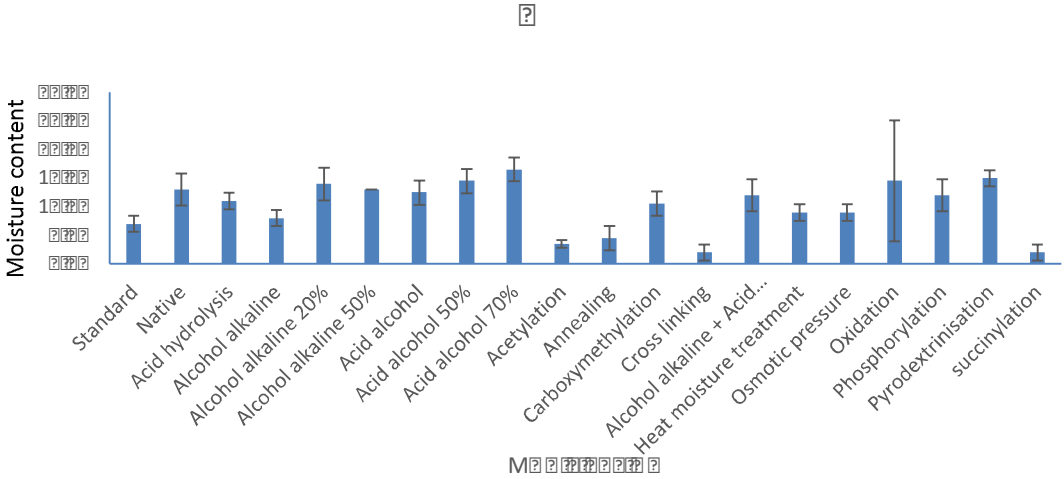


Figure 37: Moisture content for Bambara groundnut (*Vigna subterranea*)

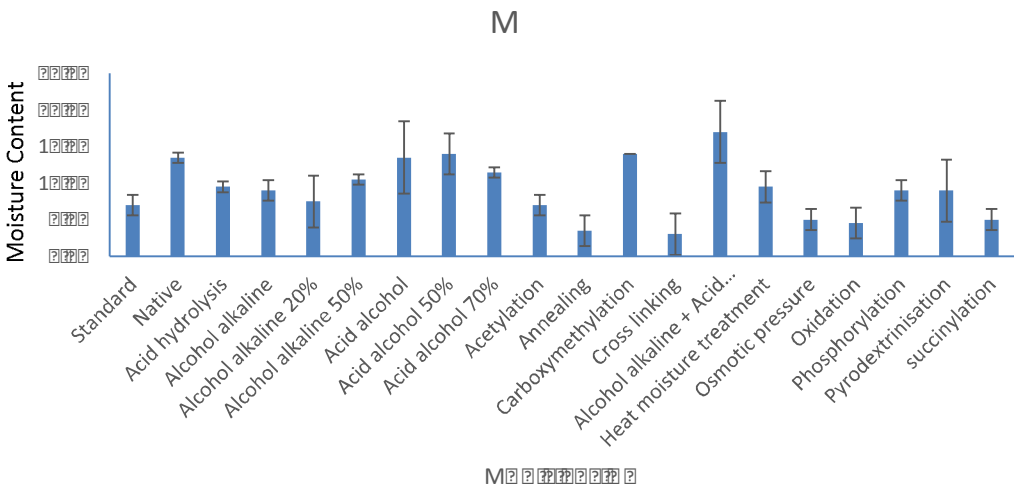


Figure 38: Moisture content for *Mucuna pruriens var pruriens*

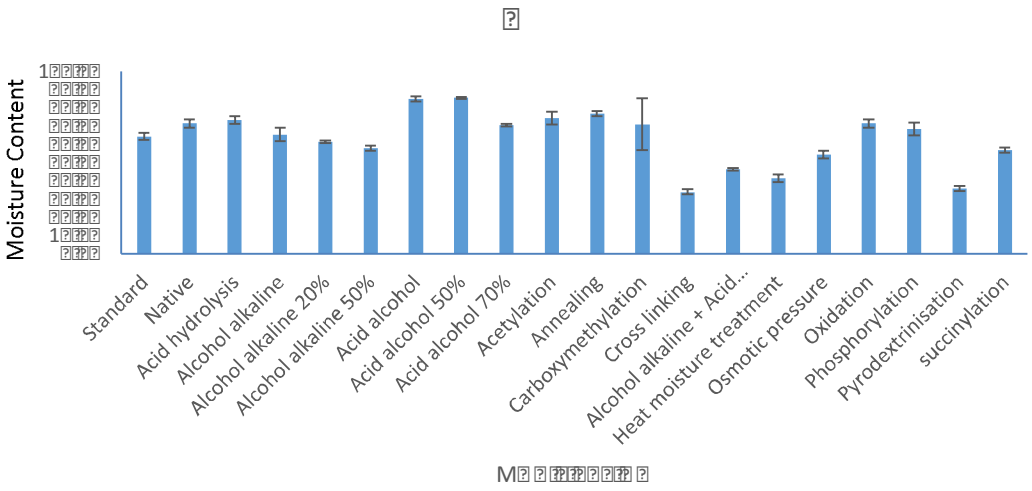


Figure 39: Moisture content for *Cajanus cajan*

3.14 Effect of Modification on the Paste Clarity of starches.

The result of the experiment on the effect of the modification methods on the paste clarity of modified starches are shown in figures 40 – 43 and plate 8 (A – D). Starch paste clarity values are given as the percentage transmittance read directly from a spectrophotometer. Generally, the paste clarity was moderately high for the native legume starches with *Cajanus cajan* having the highest clarity of 71.4 % T (fig. 43; Plate 8) alongside most modified starches with high %T as seen with the acid- alcohol, annealed, acetylated, and oxidized modified starches; while crosslinking, combo (Acid-alcohol + Alcohol alkaline), heat moisture treated and pyrodextrinization modified starches were low (Fig. 40 – 43; Plate 8). There was a significant decrease ($p<0.05$) in most of the modifications when compared with the standard excluding acid-alcohol treated, acetylated, annealed and oxidized starches.

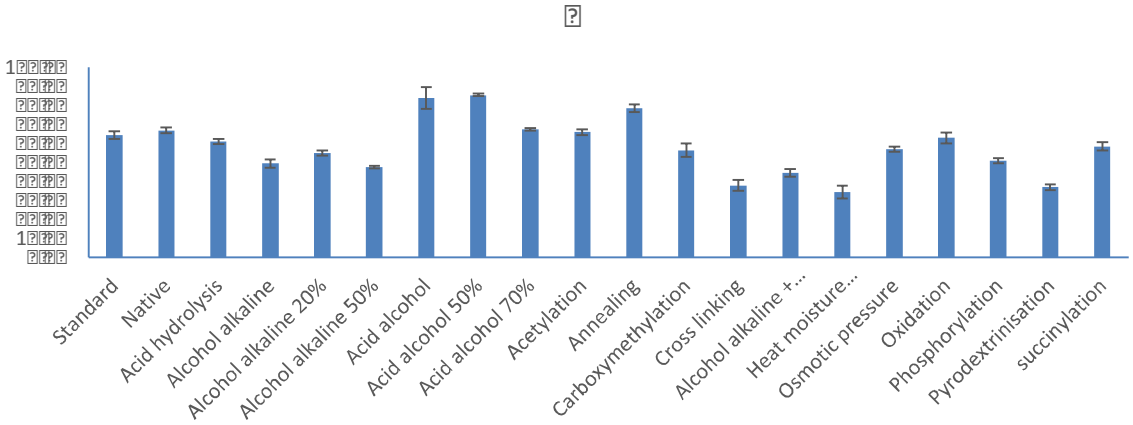


Figure 40: Paste Clarity for African Yam Bean (*Sphenostylis stenocarpa*)

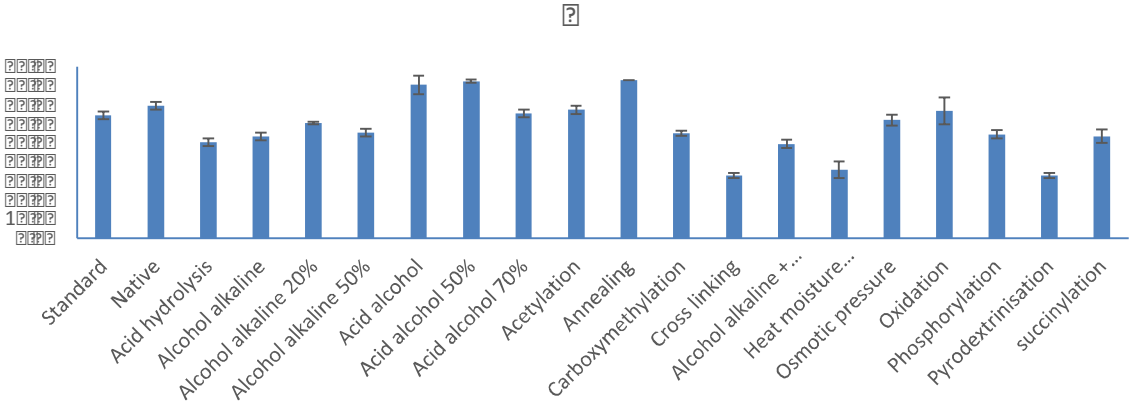


Figure 41: Paste Clarity for Bambara Groundnut (*Vigna subterranea*)

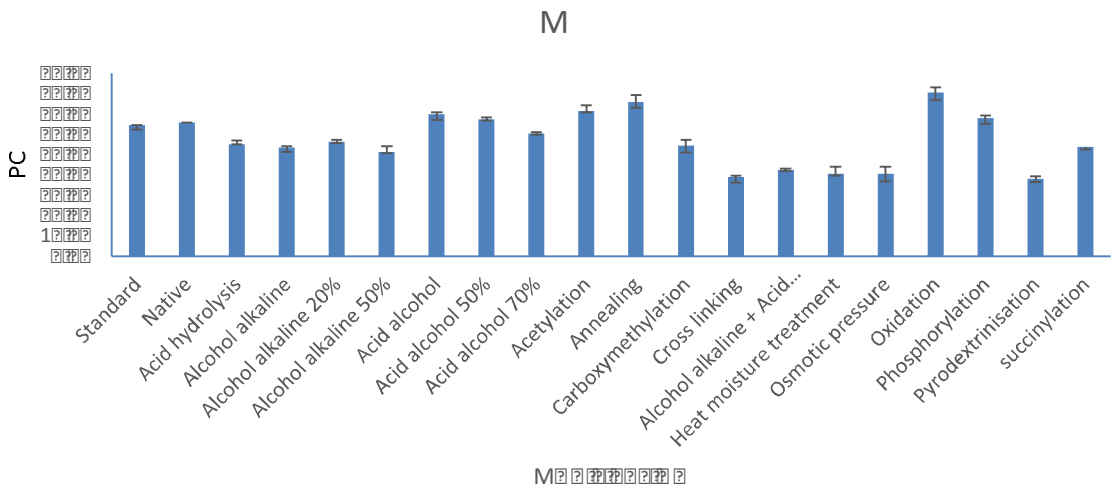


Figure 42: Paste Clarity for *Mucuna pruriens var pruriens*

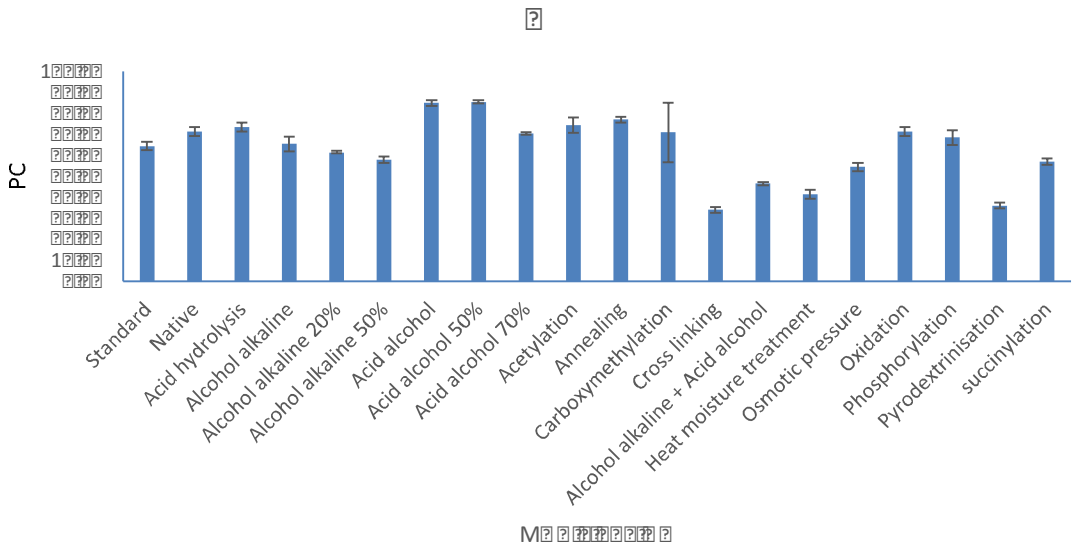


Figure 43: Paste Clarity for *Cajanus cajan* (Pigeon Pea)

(A)

(B)

(C)

(D)

Plate 12: Test tubes showing the visual clarity of

(A) Standard, native, acetylated and osmotic pressure treated starches;

(B) Alcohol-alkaline, acid-hydrolysed, phosphorylation, cross-linked and combo starches;

(C) Acid-alcohol, annealed, oxidized and acetylated starches and

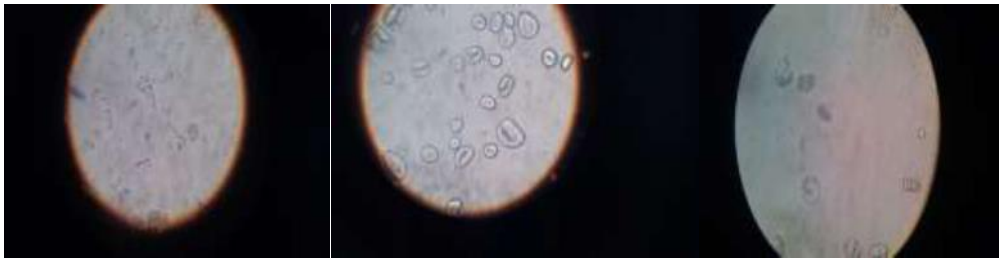
(D) Pyrodextrinized, succinylated, heat moisture and carbpxymethylated starches dissolved in water.

3.15 Morphology of the Native and Modified Starch Granules

The starch granules ranges from small to medium sized with varying shapes mostly ellipsoidal and oval shaped. The granule images were captured at 400 x resolution at 32 ^{o}C (Plate 9). The native starch granules were not damaged while modified starch granules partially lost birefringence and had a rough surface. Most modified starch granules were swollen, causing irregular shapes, and granule aggregates were present.

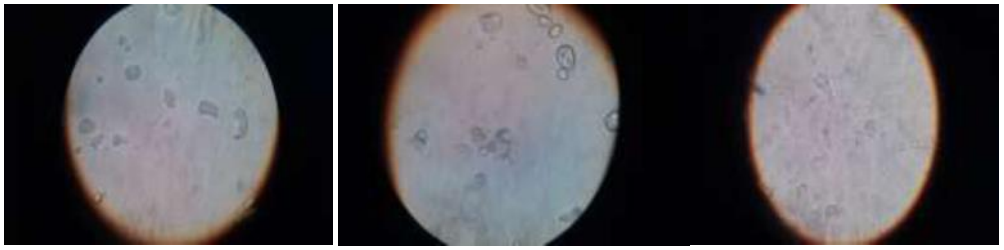
(A)

Control **S-Native** **C-Native**



(B)

M-Native **V- Native** **S-Acid hydrolysed (AH)**



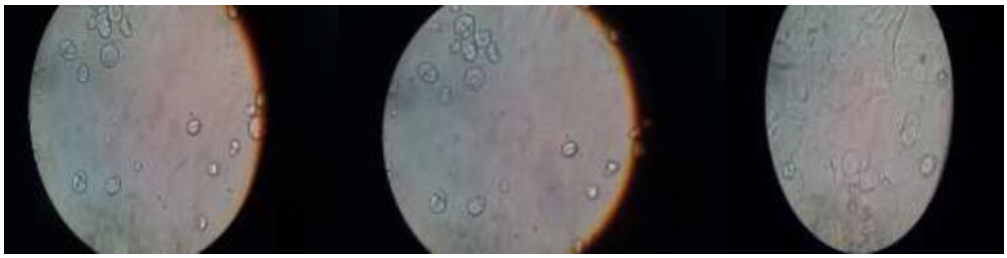
(C)

C-AH **V-AH** **M- AH**

Plate 13 (A): Images showing standard and native starch granules

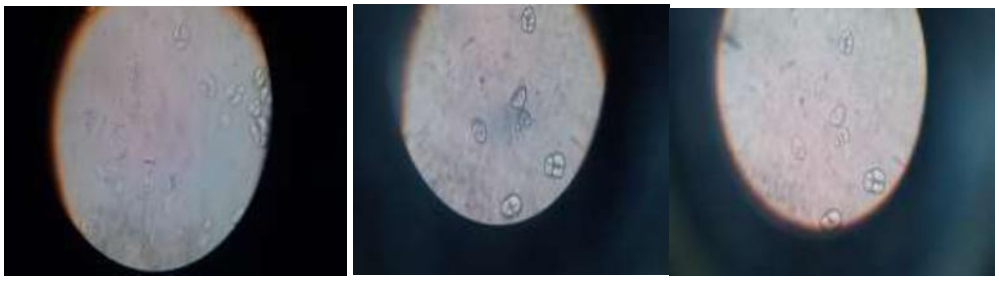
(B): Images showing native and acid-hydrolysed starch granules

(C): Images showing acid-hydrolysed starch granules



(A)

S- Acetylation (Acet) C-Acet M-Acet



(B)

V- Acet V- Ac/Al (Acid Alcohol) C-Ac/Al

(C)

M- Ac/Al S-Ac/Al S-Ac/Al 70 %

(D)

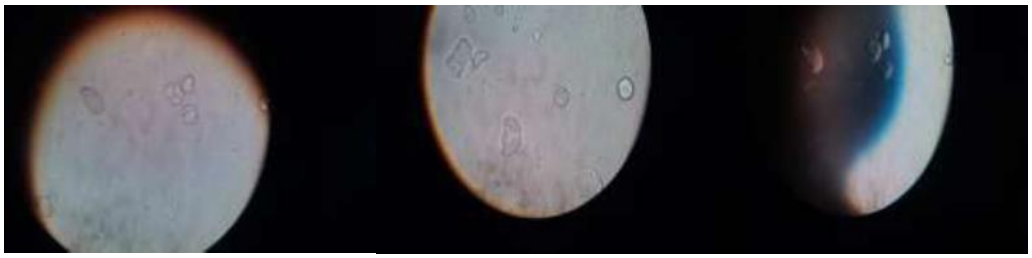
M- Ac/ Al 70 % C- Ac/ Al 70 % V- Ac/ Al 70 %

Plate 14 (A): Images showing acetylated starch granules

(B): Images showing acetylated and acid-alcohol starch granules

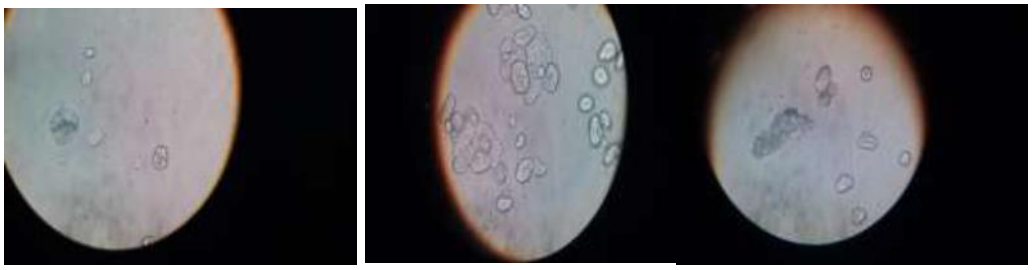
(C): Images showing acid-alcohol starch granules

(D): Images showing acid-alcohol 70 % starch granules



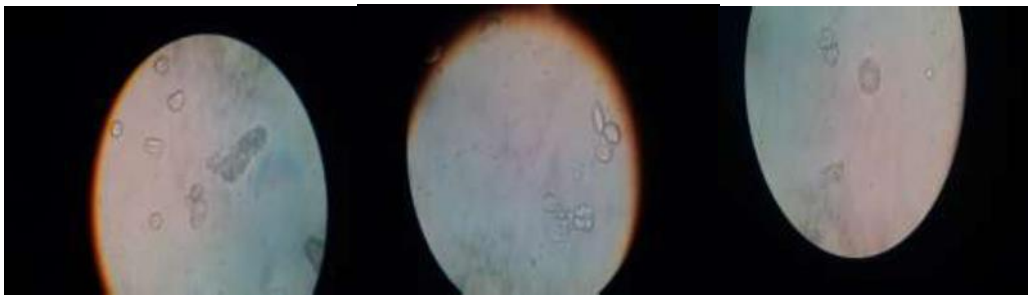
(A)

V- Ac/ Al 50 % C- Ac/ Al 50 % S-Ac/ Al 50 %



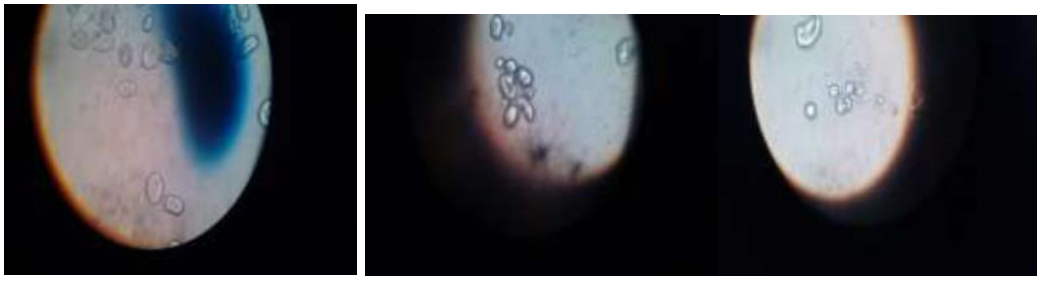
(B)

M- Ac/ Al 50 % M- A/A (Alcohol-alkaline) M -A/A 20 %



(C)

M-A/A 50 % C-A/A 20 % C-A/A 50 %



(D)

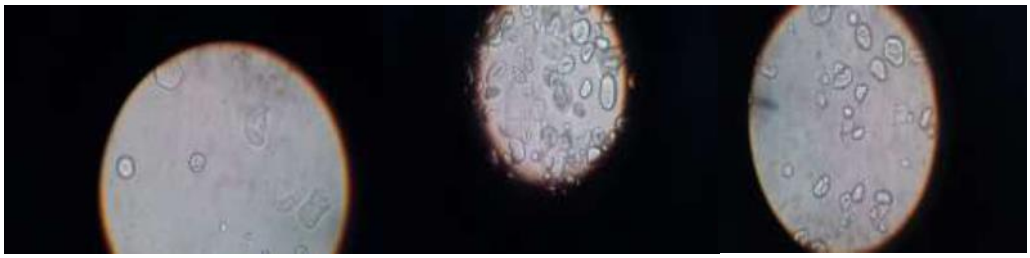
C-A/A V-A/A V-A/A 20 %

Plate 15 (A): Images showing acid-alcohol 50 % starch granules

(B): Images showing acid-alcohol 50 % and alcohol-alkaline starch granules

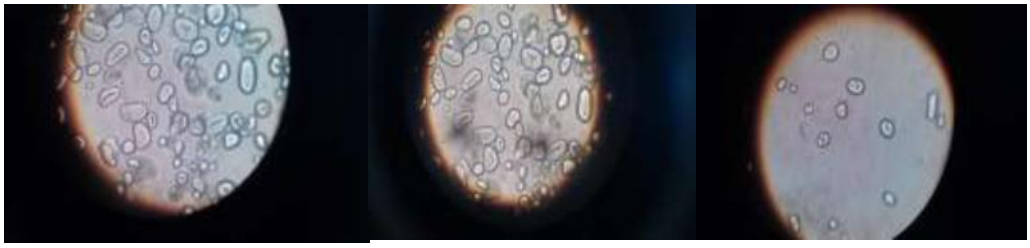
(C): Images showing alcohol-alkaline 20 % and 50 % starch granules

(D): Images showing alcohol-alkaline 20 % starch granules



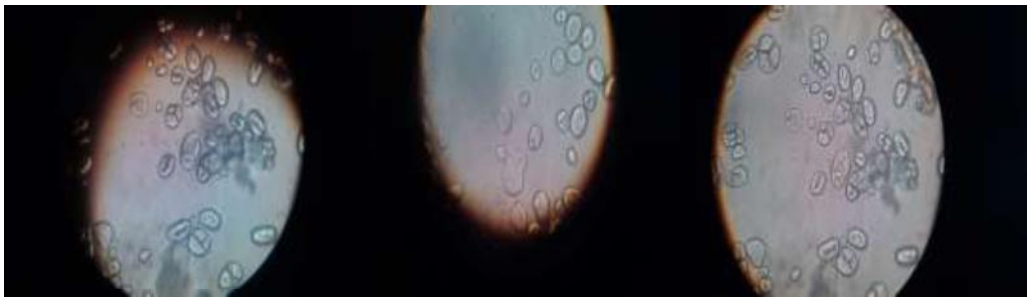
(A)

V-A/A 50 % V – Heat moisture treatment (HMT) C- HMT



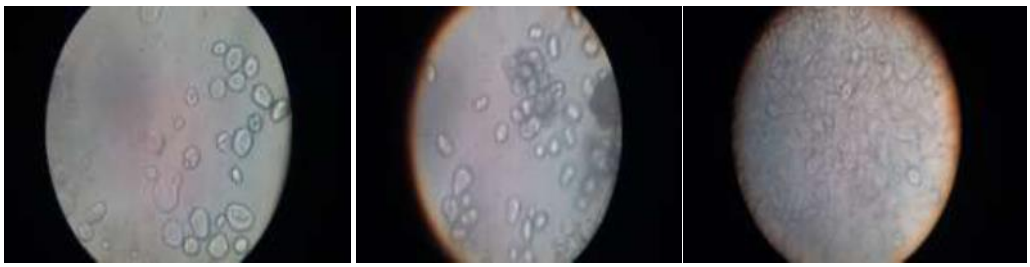
(B)

M- HMT S- HMT S- Osmotic pressure (OP)



(C)

M- OP C- OP V- OP



(D)

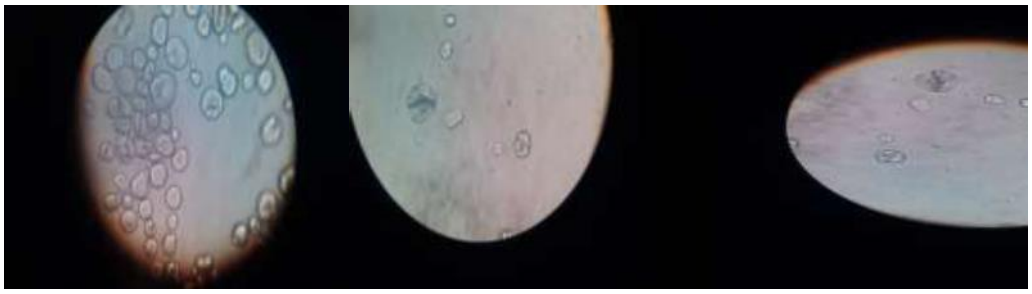
O- Oxidized F- Oxidized M- Oxidized

Plate 16 (A): Images showing alcohol-alkaline 50 % and heat moisture starch granules

(B): Images showing heat moisture and osmotic pressure treated starch granules

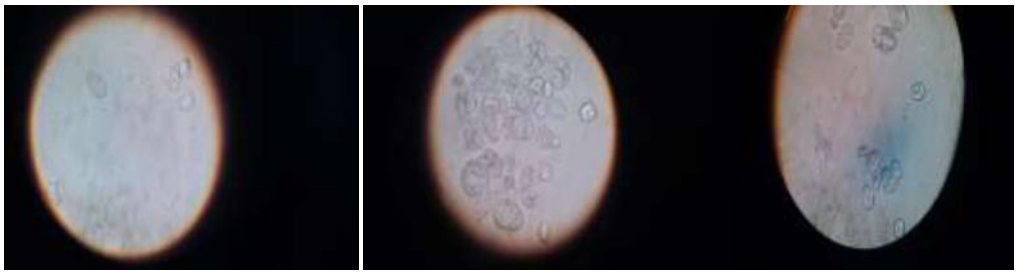
(C): Images showing osmotic pressure treated starch granules

(D): Images showing oxidized starch granules



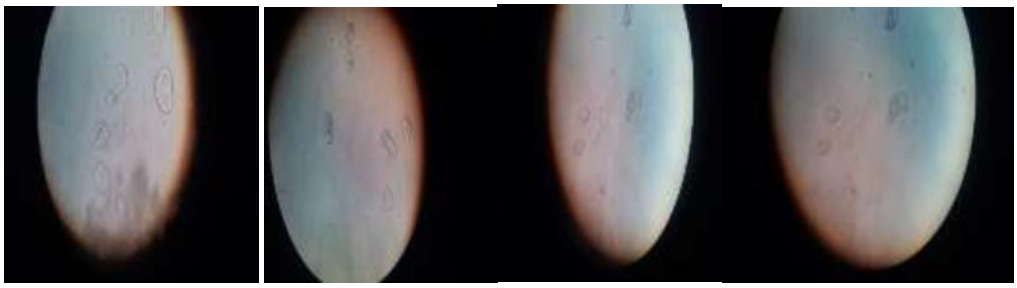
(A)

S- Oxidized **M- Pyrodextrinization** **V- Pyrodextrinization**



(B)

V- Succinylation (succ) **S- Succ** **C- Succ**



(C)

S- Combo **M- Combo** **C-Combo** **V-Combo**

Plate 17 (A): Images showing oxidized and pyrodextrinized starch granules

(B): Images showing succinylated starch granules

(C): Images showing combo starch granules

CHAPTER FOUR

DISCUSSION

The aim of the experiment reported in this thesis was to determine the effect of different modification methods on the cold water solubility of starch isolated from different legumes. The native starch isolated from some of the legumes, namely *Cajanus cajan, Mucuna pruriens var pruriens, Sphenostylis stenocarpa, and Vigna subterranea* were not as brightly coloured as the commercially produced standard starch sample (control). The starch obtained was slightly off-white in colour and had no smell. The colour and intensity of the starch complex depends on the chain length of the amylose (Bailey and Whelan, 1961). One of the major problems encountered in the isolation process was a deposit of a coloured layer on top of the white starch layer after sedimentation of the starch slurry. The coloured layer may be due to the insoluble flocculent protein and fine fibre which changes according to the colour of the seed. Similar difficulties were reported by Waczkowski *et al.* (1989) and Qi and Phillips (2004). To effectively remove the coloured layer, a spatula was used with a light scraping motion to scrape off most of the layer. However, the amount removed was relatively small and this did not cause a significant loss of the starch layer. Some reagents used for some chemical modifications stained the starches leading to a faded white starch colour as observed in the treatments involving concentrated acids, increased molarity alkaline treatments, and absolute alcohol treatments in some cases. The browning of phosphorylated starches is most likely to be as a result of incubating in an oil bath which may have caused a slight burning of the starch granules. The oxidized starches had the whitest appearance. This could be due to the hypochlorite treatment involved in the modification, since hypochlorite is a bleaching agent. It is worthy of note that some of the faded appearance of some of the starches could be due to the focusing of the camera lens.

Vigna subterranea, Cajanus cajan, Sphenostylis stenocarpa and *Mucuna pruriens var pruriens* gave starch yields of 46.05 %, 41.2 %, 48.6 % and 40.6 % respectively. The yield of *Vigna subterranea* was slightly higher than that reported by Piyarat (2008) who reported a yield of 45.57 %. The yield of 40.6 % reported in this experiment for *Mucuna pruriens var pruriens* falls within the range of 36.8 % to 46.0 % reported by Lawal and Adebowale (2005). The percentage yield of the *Cajanus cajan* (41.2 %) was higher than that reported by Lawal (2008) where he reported a 22.5 % yield. The values confirm that these legumes contain significant amounts of starch. However, the differences in yield reported could be

attributed to the geographical origins of the legumes, the varying species of legumes, the period of harvest and the loss of starch experienced during isolation. As expected, the modification steps led to losses of starch. Thus, the amount of starch recovered after modification ranged from 72 % to 99 %. This is in agreement with the findings of Sodhi and Singh (2003) and Rachtanapun, *et al.* (2012). The highest recovery was recorded with 20 % alcohol-alkaline treatment, while the least recovery was observed with pyrodextrinisation modification.

The cold water solubility (CWS) of the starches varied with the legumes. The acid-alcohol, alcohol alkaline and acid hydrolysis gave the best CWS, while heat moisture treatment and carboxymethylation gave the least solubility. The CWS varied with the concentration of ethanol and NaOH in the reaction mixture. Ethanol inhibits the swelling of the starch particles thereby maintaining the integrity of the starch particle. This undue inhibition of the swelling of starch granule delays the dissociation of the starch double helix structure (Xin *et al.*, 2012; Wang and Les, 2012).

Considering the significant improvement in solubility of the alcohol-alkaline and acid-alcohol treatments when compared to the other modifications, some modifications were made on these treatment methods where for the alcohol alkaline treatment, first, the alcohol and alkaline were reduced to 20 % ethanol and 1.5 M NaOH respectively and for the second, the alcohol and alkaline were increased to 20 % ethanol and 4 M NaOH respectively. The both variations on the modification methods improved the solubility of the legume starches when compared to the first treatment (40 % ethanol and 3 M NaOH). For the acid-alcohol treatment, the modifications with an aim of achieving a more soluble yield involved first, reducing the acid concentration from a 36 % to a 20 % concentration while reducing the alcohol concentration from a 70 % to a 50 % concentration (Figure 5 - 8). Finally, the modification was altered again by increasing the concentration of the acid to 45 % and the alcohol maintained at 70 %. These variations to the initial modification method gave solubility higher than that originally reported by Chen and Jane (1994). Reducing the ethanol concentration (20 % treatment) alongside the alkaline (1.5 M NaOH) and acid (1.5 M HCl), gave the highest solubility when compared to the 50 % treated and normal treatment as reported by Chen and Jane (1994). Thus, this implies our method as a better modification for the production of cold water soluble starch.

Also, the results obtained after varying the acid-alcohol modification method originally reported by Lin *et al.* (2003) indicated an improvement in solubility and some functional properties when compared to the original method of modification.

The increased solubility for most of the modification may be due to increased lower molecular weight fraction containing hydroxyl groups after modification (Sandhu and Singh, 2007) especially for the alcohol-alkaline, acid- alcohol, acid hydrolysed modified starches with high cold water solubility.

The swelling power increased with increase in solubility for some of the modifications with some exceptions. Phosphorylation, alcohol alkaline, succinylation and acetylation gave high swelling power, This characteristic may explain the use of these starches in food industry as thickeners because high swelling power results in increased digestibility and ability to use starch in solution suggesting improved dietary properties (Koh and Long, 2012). This makes it possible for starch to be used in a range of dietary applications (Nuwamanya *et al.*, 2011). Thus, this technique can enhance the use of the native and modified legume starches in other industries. Introduction of some functional groups like the acetyl, succinyl and phosphoryl groups reduces the bond strength between starch molecules causing an increase in the swelling power and solubility of the modified starch. Hydrogen bonding is restricted in the starches as a result of inter- and intra-molecular electrostatic repulsion in the starch molecule (Ashogbon and Akintayo, 2014).

The other modifications alongside the standard had an average swelling power of 4 - 4.5. The reduction in the swelling power after some modifications may be attributed to structural disintegration within the granules of the starch during the modification process (Xiao *et al.,* 2012) or the amount of lipid and protein in the starches may affect their swelling behaviour. Protein and starch interact due to the attraction of their opposite charges and form complexes during gelatinisation and therefore inhibits swelling.

Modifications involving treatments with a strong alkali like NaOH yielded moderate to high swelling power possibly as a result of the sodium hydroxide generating negatively-charged alkali starch. The alkali starch combined with the strong hydrated sodium ions. Therefore a lot of water was brought into the starch macromolecules that caused severe swelling of starch and break up the binding force between the amorphous region of macromolecules of starch (Ruan *et al*., 2009). In the case of acid hydrolysis and pyrodextrinization which had the lowest SP, this may be due to the reduction in the

number of binding sites on the granules resulting from the destruction of amorphous region of the granules from the acid attack (Lawal, 2004). According to the method of Geng *et al*. (2010), after neutralization, the starch molecules and ethanol formed a spiral compound (V-complex). Once the ethanol was evaporated, the cavity was formed in the starch particles, thus the starch is in a metastable form that ensured it had excellent cold water solubility. The swelling power of alcohol alkaline starches was high despite the induction of alcohols also. This is probably due to the use of alkaline solution (NaOH) with varying molarity accordingly that caused high 'swellability' in the starches. Leach *et al*. (1959) reported that swelling behaviour of starches is a property of their amylopectin content. Amylose acts as a diluent and inhibitor of swelling, especially in the presence of lipids which can form insoluble complexes with amylose during swelling. Amylose is often believed to act as a restraint on swelling, and cereal starch granules do not exhibit complete swelling until amylose has leached out from the granules (Bowler, 1980).

The pH of the modified starches ranged from 5.8 - 8.8 except for acid hydrolysis modification which had a highly acidic pH (this was later neutralised with 1 M NaOH). The neutrality of the starches increases its applications both for food and industrial purposes.

Water Absorption Capacity (WAC) improved with increasing solubility. Alcohol-alkaline and acid-alcohol modified starches gave the highest WAC (alongside the standard), with some exceptions as with the swelling power analysis. Acid hydrolysis and pyrodextrinisation gave relatively low absorption values. While highly absorbing starches have broad applications for instance in laundry starches, starches with low WAC can be useful also in food industry for example in bread making where a low absorbing starch is required to prevent dry dough formation (Nuwamanya *et al.,* 2011). The amorphous regions of semi-crystalline starch granules are the area most susceptible to the initial water absorption and plasticization. Before hydration the amorphous region is more glassy and static (Ashogbon and Akintayo, 2014). It is the hydration of the starch granules that increases the mobility of the amorphous regions thereby absorbing water. These modifications enhance the mobility and absorption properties of the granules as seen with the alcohol-alkaline treatment, dual modification, acid-alcohol 70 % treatment and the control which was an already modified cold water soluble starch on sale. When starch is heated in the presence of excess water, the granules undergo an order-disorder phase

transition, known as gelatinisation over a temperature range characteristic of the starch source (Ratnayake and Jackson, 2008).

In general, the gelatinisation temperature is defined as the temperature where 98 % of the starch granules undergo birefringence loss when microscopically viewed with a microscope (French, 1984). The gelatinisation temperatures of all starches were less than 60 ^{0}C as shown in table 7 with the standard, alcohol-alkaline, acid-alcohol modified starches having the least gelatinisation temperature of 35 ^{0}C- 39 ^{0}C and the native, pyrodextrinized, oxidized, carboxymethylated starches having the highest gelatinisation temperature of 51 ^{0}C - 73 ^{0}C.

From the study, it can be suggested that the lower the amylose content, the higher the swelling power and the higher the solubility in most cases like the alcohol-alkaline 20 % and 50 % treatments, dual modification of alcohol- alkaline and acid-alcohol treatment and acetylation. There were exceptions especially with acid modified starches: acid hydrolysed, acid-alcohol, pyrodextrinized and succinylated starches. This could be as a result of the reduction in the number of binding sites on the granules resulting from the destruction of amorphous region of the granules from the acid attack (Lawal, 2004). The amylose content of *Vigna subterranea, Cajanus cajan, Sphenostylis stenocarpa* and *Mucuna pruriens* starches were 21.52 %, 20.04 %, 22.58 %, and 23.75 %, respectively. With the standard having 17.29 % amylose content, the modified starches had amylose contents ranging from 13.7 % (alcohol-alkaline 50 % treatment) to 28 % (succinylation). Most of the modified starches had lower amylose contents when compared to their native starches. Low amylose starches have more applications, as in paper making (Eliasson, 2004), food applications, the production of laundry starches, while starches containing large amounts of amylose have applications in the food industry for producing foods like noodles and crackers that require some form of crispiness. They are also used in adhesive production as they form rigid gels and stronger, tougher films.

The modifications altered in the amylose content of the different starches with some having high amylose content while others had low contents as in the case of *Vigna subterranea* alcohol-alkaline treatment having 25.28 % amylose content when compared to the 22 % the other three had. This could be as a result of an interplay of many factors: lipid– amylose complexes; arrangement of amylose chains within the amorphous domain;

interactions between starch chains and the amylose to amylopectin ratio (Ashogbon and Akintayo, 2014).

The gelatinisation temperature of starches decreased with an increase in cold water solubility, this could be as a result of the starch gelatinisation stage. The starch polymeric material was dispersed by swelling and gelatinisation in water resulting in significant hydrolytic cleavage of 1, 4-glycosidic bonds in the starch (Koh and Long, 2012). The result is comparable to that reported by Zuo *et al.* (2012) where he studied the effects of acid hydrolysis temperature on the properties of starch. Applications can be found in the laundry industries where easily gelatinised granular cold water soluble starches can be produced.

The amount of moisture present in the starch was also evaluated. Pyrodextrinization, heat moisture treated, cross-linked and acid hydrolysis gave low moisture contents, while acid alcohol gave the highest moisture contents. The low moisture contents in some of the modifications may be a result of the attack and destruction of the amorphous regions of the granules by acidic and crosslinking reagents thereby reducing the moisture normally present in the granules. This is an advantage as moisture enhances microbial growth and thus reduces the shelf life of products. The decrease in moisture contents acts as an advantage in industrial applications, like in paper making, and in the food industry for instance, in the production of crispy snacks where low moisture is required to prolong the shelf life. The moisture content of starches is closely related to drying conditions applied and particularly to the length of the drying period used following the isolation process.

Generally, the paste clarity was moderately high for the native legume starches with *Cajanus cajan* having the highest clarity of 71.4 % T alongside most modified starches with high % T as seen with the acid- alcohol, annealed, acetylated, and oxidized modified starches. In contrast, crosslinking, combo (acid-alcohol + alcohol alkaline), heat moisture treated and pyrodextrinization modified starches had low paste clarity. The highest clarity of some of the starches may be due to its lowest impurity content as some legumes reduces colour on the starches resulting in low clarity starches. This property may be useful for application in food and textile industries where high clarity is required (Jyothi *et al*., 2005). The low clarity observed for some of the starch can be attributed to the high amounts of interfering substances with transmission of the light by the modified starch gel (Nuwamanya *et al*., 2011).

In general, the more amount of interfering substances and contaminants there are the less clarity as was observed by Jyothi *et al*. (2005). Clarity characteristics of starches are also dependent on the botanical source of the starch and the overall purity of the isolated starch. Increased clarity is required in laundry and paper industries but opaque starches have applications in food industries, such as in the preparation of gravies. Oxidized, acid-alcohol, acetylated, annealed, control, alcohol-alkaline and slightly, carboxymethylated treated starches had high clarity ranging from 85.7 % (Acid-alcohol) to 54 % (carboxymethylated). Crosslinked, pyrodextrinized and heat moisture treated starches had low clarity with crosslinked starches giving the least of 33 % - 38 %; this is consistent with results obtained by Kaur *et al.* (2006) who reported that cross- linked starches showed lower paste clarity than their counterpart native starches. These decreased paste clarity of cross-linked starches were attributed to their being more compact when compared to native starches. Furthermore, it has been indicated that the reduced swelling of cross-linked starches might be partly responsible for their decreased paste clarity (Kaur *et al.*, 2006).

The majority of starch granules were characterized by polyhedral and irregular shapes and only a small minority had spherical shapes. The starch granules ranged from small to medium sized, with varying shapes mostly ellipsoidal and oval shaped. The granule images were captured at 400 x resolution at 32 ^{o}C. The Native Starch (NS) granules were not damaged, while modified starch granules partially lost birefringence and had a rough surface. Most modified starch granules were swollen, causing irregular shapes, and granule aggregates were present. The individuality of starches is best seen in the differences in the morphology of their granules. The morphology of starch granules depends on the biochemistry of the chloroplast or amyloplast, as well as the physiology of the plant (Bodenhuizen, 1969). The granule size of some of the starches were small probably due to the fact that a light microscope was used as against a scanning electron microscope (Bandhari and Singhal, 2002) though small sized starches are more reactive than starches with larger granule size (Trubiano, 1987). Generally, small and medium sized starch granules have been reported to have varied utilization in the food and pharmaceutical industries (Omojola *et al*, 2010) which gives credence to the industrial potentials of the starch.

To further improve the properties and uses of starches, chemical dual modification and other types of dual modifications have been introduced in order to optimize the functionality of modified starches. Generally, dual modifications involve using a

combination of chemical and physical or chemical and enzymatic methods, but specifically dual chemical modifications involve using two types of chemical modification (e.g., acetylation/oxidation, cross-linking/ acetylation, or cross-linking/ hydroxypropylation). Chemical dual modifications are widely used in the food industry as emulsifiers, binders and thickeners. Furthermore, they are also used in the non-food industry as heavy metal adsorbents. In the study, dual modification was applied on the modified starches with the highest cold water solubility and moderate functional properties. This include starch subjected to alcohol-alkaline + acid-alcohol modifications, whose solubility ranged between 59 % and 72 %.

The swelling power and water absorption capacity of the dual modified starches were relatively high. The pH was around neutrality. The amylose content and gelatinisation temperature were also low, but the moisture content was mostly above the value of 14 % reported by Austin (1984) as the maximum allowable limit for moisture in starch. However, such starch with high moisture content can have applications in ready to use food products like jellies and pastries.

4.2 Conclusion

The study demonstrated that all the underutilized legumes could be good sources of starch. The shortcomings the native starches may have had in terms of solubility and functionality were enhanced through a variety of physical and chemical modification treatment. This implies that it is possible to increase the utilisation of these legumes as a possible source of starch which could be used in a variety of industries.

4.3 Suggestion for further studies

The study concentrated on starch isolated from four underutilized legumes, namely *Cajanus cajan, Mucuna pruriens var pruriens, Sphenostylis stenocarpa* and *Vigna subterranea.* Starches from single varieties of the four legumes were isolated, modified and analysed. However, there is need for different species or cultivars of these legumes to be evaluated with a view to finding out how they differ in terms of starch yield and how their starches differ not only in terms of functionality, but also how they are affected by the various modifications.

Some of the legume starches were slightly stained by the seed coat and this affected the paste clarity of the starches even after modification; a dual modification which involves bleaching an already modified starch can be applied to improve paste clarity.

The criteria used in the selection of analyses reported in this study were oriented towards the practical application of the cold water soluble starches for industrial applications. In the context of characterisation, some more functional properties can be analysed in further studies for instance, nuclear magnetic resonance to elucidate crystalline structure of the starch, viscosity and size exclusion chromatography to study the molecular weight distribution and to quantify the relative proportions of amylose and amylopectin. These analyses may provide further insight into the characteristics of the various starches and enhance their industrial potentials.

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APPENDICES

APPENDIX I

PREPARATION OF MATERIALS AND REAGENTS

1. **Preparation of Legumes**:

   The legumes were de-stoned and milled using a mill with a 60 µm pore size into fine flour before proceeding for starch isolation which entailed soaking the flour in distilled water and sieving using muslin cloth after which it was washed and left to decant, washed continuously till neutral.

2. **Preparation of 3 % NaOH:** The solution was prepared by dissolving 3 g of sodium hydroxide pellets in distilled water in a 1000 ml volumetric flask and diluted to the 1000 ml mark. Same method applied for the 10 % NaOH solution.

3. **Preparation of 1 M NaOH:** The solution was prepared by dissolving 40 g (Molecular weight) of sodium hydroxide pellets in distilled water in a 1000 ml volumetric flask and diluted to the 1000 ml mark.

4. **Preparation of 1 N NaOH:** Same as the 1 M NaOH since normality is the multiple of the molarity of a solution which is dependent on the chemical formula of the solution being used = $Na^+ + OH^-$ therefore, 1 M NaOH = 1 N NaOH.

5. **Preparation of 6 % HCl**: A volume of 6 ml of concentrated HCl solution was added to distilled water in a half filled 1000 ml volumetric flask. The content of the flask was then swirled with the volume diluted to the 1000 ml mark with distilled water.

6. **Preparation of 1 M HCl**: A volume of 86 ml of concentrated HCl solution was added to distilled water in a half filled 1000 ml volumetric flask. The content of the flask was then swirled with the volume diluted to the 1000 ml mark with distilled water

Concentrated HCl= 37.5 %; Density of concentrated HCl= 1.189 g/ml; atomic mass of HCl= 36.5

= ((37.5/ 100 %) (1000 ml) (1.189 g / ml)) ÷ 36.5 = 12.2 M

Concentrated HCl = 12.2 M. so to prepare a 1 L solution of 1 M HCl

= (12.2) x = 1(1)

x= 1/ 12.2

x= 0.082 ml/ ml of distilled water

= 82 ml HCl / L.

7. **Preparation of 1 N H_2SO_4**: a volume of 27.5 ml of concentrated H_2SO_4 solution was added to distilled water in a half filled 1000 ml volumetric flask. The content of the flask was then swirled with the volume diluted to the 1000 ml mark with distilled water

8. **Preparation of 1 N Acetic Acid:** A volume of 60 ml of acetic acid solution was added to distilled water in a half filled 1000 ml volumetric flask. The content of the flask was then swirled with the volume diluted to the 1000 ml mark with distilled water.

9. **Preparation of 50 % methanol and ethanol solutions:** 50 ml of absolute methanol/ ethanol was put in a beaker and made up to 100 ml with distilled water.

10. **Preparation of 40 % Mono Chloro Acetic Acid (MCA):** 40 g MCA was dissolved in 100 ml of distilled water.

11. **Preparation of 5 % Succinic Anhydride solution:** 5 g Succinic anhydride was weighed out and dissolved in 100 ml of distilled water.

APPENDIX II

CALCULATIONS

1. Moisture content (MC)

$$\% \text{ MC} = \frac{Weight\ of\ dried\ starch\ X\ 100}{Weight\ of\ fresh\ starch}$$

2. Yield of Bambara groundnut starch (Ybs)

$$\% \text{ Ybs} = \frac{Weight\ of\ isolated\ starch\ X\ 100}{Weight\ of\ initial\ dried\ starch}$$

APPENDIX III

Table 7: Results showing the Cold Water Solubility of The Native and Modified Starches

Modification	CWS 1	CWS 2	CWS 3	AVERAGE CWS
Ctrl	82.8	78.4	80	80.4
C native	23.2	20.4	19.6	21.1
M native	16.8	11.2	4	10.7
V native	23.6	14	20	19.2
S native	4	16	2.8	7.6
C Acid hydrolysis	59.6	75.2	69.2	68.0
M Acid hydrolysis	62.4	64	58.4	61.6
V Acid hydrolysis	50.4	61.6	51.2	54.4
S Acid hydrolysis	51.2	65.2	62	59.5
C A/A				
M A/A	64.8	68.4	71.2	68.1
V A/A	52	54.8	56.4	54.4
S A/A	51.2	53.2	58	54.1
	62	72	56.8	63.6
C A/A 20%				
M A/A 20%	72	74.8	71.6	72.8
V A/A 20%	63.2	64	62	63.1
S A/A 20%	80.4	75.2	71.6	75.7
	66.8	60.8	69.2	65.6
C A/A 50%				
M A/A 50%	72	70	75.2	72.4
V A/A 50%	64.8	68	63.6	65.5
S A/A 50%	84	79.2	72.8	78.7
	70.8	68.8	72.4	70.7
C AC/AL				
M AC/AL	50.4	59.2	55.2	54.9
V AC/AL	51.2	49.6	58.4	53.1
S AC/AL	51.6	54	56.4	54.0

	63.2	65.2	68	65.5
$C_{AC/AL\ 50\%}$				
$M_{AC/AL\ 50\%}$	68.4	59.6	56.4	61.5
$V_{AC/AL\ 50\%}$	62.8	60.4	67.2	63.5
$S_{AC/AL\ 50\%}$	82	86.8	74	80.9
	58	55.2	67.6	60.3
$C_{AC/AL\ 70\%}$				
$M_{AC/AL\ 70\%}$	60	62.8	65.6	62.8
$V_{AC/AL\ 70\%}$	66.4	68.8	72.4	69.2
$S_{AC/AL\ 70\%}$	58.4	68.8	72.4	66.5
	69.2	70.8	70	70.0
$C_{Acetylation}$				
$M_{Acetylation}$	40.4	48.4	54.4	47.7
$V_{Acetylation}$	49.6	51.6	52	51.1
$S_{Acetylation}$	40	50	47.6	45.9
	34.4	44	49.2	42.5
$C_{Annealing}$				
$M_{Annealing}$	42.4	45.2	48.4	45.3
$V_{Annealing}$	49.2	59.2	52	53.5
$S_{Annealing}$	52.8	53.6	57.6	54.7
	43.6	51.2	46.8	47.2
$C_{Carboxymethylation}$				
$M_{Carboxymethylation}$	52.4	44.4	48	48.3
$V_{Carboxymethylation}$	34.8	31.2	36.4	34.1
$S_{Carboxymethylation}$	28.8	40.4	34	34.4
	48.4	45.2	50	47.9
$C_{Crosslinking}$				
$M_{Crosslinking}$	58.4	47.6	52.8	52.9
$V_{Crosslinking}$	42.4	48	51.2	47.2
$S_{Crosslinking}$	51.2	49.2	52.8	51.1
	39.2	41.6	46.4	42.4
C_{Combo}				
M_{Combo}	58.8	54.8	64.4	59.3

V_{Combo}	62.4	59.6	57.6	59.9
S_{Combo}	70.4	72.4	75.6	72.8
	54	56.8	52.4	54.4
$C_{Heat\ Moisture\ Treatment}$				
$M_{Heat\ Moisture\ Treatment}$	26	37.2	41.6	34.9
$V_{Heat\ Moisture\ Treatment}$	48.8	38.8	46.8	44.8
$S_{Heat\ Moisture\ Treatment}$	33.6	38.4	41.6	37.9
	34.4	29.6	36.8	33.6
$C_{Osmotic\ Pressure}$				
$M_{Osmotic\ Pressure}$	49.6	57.6	61.2	56.1
$V_{Osmotic\ Pressure}$	53.6	50	55.6	53.1
$S_{Osmotic\ Pressure}$	59.2	54.8	41.6	51.9
	43.6	45.2	51.2	46.7
$C_{Oxidation}$				
$M_{Oxidation}$	56.8	66.4	61.6	61.6
$V_{Oxidation}$	57.6	56.4	64.4	59.5
$S_{Oxidation}$	52.8	62	56.4	57.1
	43.2	47.6	50.8	47.2
$C_{Phosphorylation}$				
$M_{Phosphorylation}$	55.6	56.8	52.4	54.9
$V_{Phosphorylation}$	56	59.2	55.2	56.8
$S_{Phosphorylation}$	41.6	44.8	53.2	46.5
	46.8	43.6	53.6	48.0
$C_{Pyrodextrinization}$				
$M_{Pyrodextrinization}$	47.2	49.2	52	49.5
$V_{Pyrodextrinization}$	48.4	52.8	54	51.7
$S_{Pyrodextrinization}$	42.4	47.6	59.2	49.7
	53.2	51.2	52	52.1

Table 8: Results showing the swelling power of native and modified starches

	MODIFICATION	Difference 1	Difference 2	SP 1	SP 2	Average
1	Ctrl	0.492	0.436	4.92	4.36	4.64
	C_{native}	0.488	0.444	4.88	4.44	4.66
	M_{native}	0.461	0.385	4.61	3.85	4.23
	V_{native}	0.362	0.432	3.62	4.32	3.97
	S_{native}	0.49	0.45	4.9	4.5	4.7
2	$C_{Acid\ hydrolysis}$	0.343	0.291	3.43	2.91	3.17
	$M_{Acid\ hydrolysis}$	0.305	0.316	3.05	3.16	3.105
	$V_{Acid\ hydrolysis}$	0.286	0.292	2.86	2.92	2.89
	$S_{Acid\ hydrolysis}$	0.261	0.284	2.61	2.84	2.725
3	$C_{A/A}$	0.399	0.408	3.99	4.08	4.035
	$M_{A/A}$	0.373	0.419	3.73	4.19	3.96
	$V_{A/A}$	0.593	0.422	5.93	4.22	5.075
	$S_{A/A}$	0.442	0.466	4.42	4.66	4.54
4	$C_{A/A\ 20\%}$	0.48	0.471	4.8	4.71	4.755
	$M_{A/A\ 20\%}$	0.451	0.438	4.51	4.38	4.445
	$V_{A/A\ 20\%}$	0.675	0.559	6.75	5.59	6.17
	$S_{A/A\ 20\%}$	0.67	0.514	6.7	5.14	5.92
5	$C_{A/A\ 50\%}$	0.374	0.446	3.74	4.46	4.1
	$M_{A/A\ 50\%}$	0.402	0.376	4.02	3.76	3.89
	$V_{A/A\ 50\%}$	0.452	0.506	4.52	5.06	4.79
	$S_{A/A\ 50\%}$	0.479	0.791	4.79	7.91	6.35
				0	0	0
6	$C_{AC/AL}$	0.342	0.196	3.42	1.96	2.69
	$M_{AC/AL}$	0.166	0.422	1.66	4.22	2.94
	$V_{AC/AL}$	0.37	0.411	3.7	4.11	3.905
	$S_{AC/AL}$	0.437	0.405	4.37	4.05	4.21
				0	0	0
7	$C_{AC/AL\ 50\%}$	0.363	0.394	3.63	3.94	3.785
	$M_{AC/AL\ 50\%}$	0.447	0.41	4.47	4.1	4.285

	$V_{AC/AL\ 50\%}$	0.315	0.346	3.15	3.46	3.305
	$S_{AC/AL\ 50\%}$	0.391	0.434	3.91	4.34	4.125
				0	0	0
8	$C_{AC/AL\ 70\%}$	0.284	0.312	2.84	3.12	2.98
	$M_{AC/AL\ 70\%}$	0.417	0.386	4.17	3.86	4.015
	$V_{AC/AL\ 70\%}$	0.388	0.407	3.88	4.07	3.975
	$S_{AC/AL\ 70\%}$	0.327	0.346	3.27	3.46	3.365
9	$C_{Acetylation}$	0.476	0.505	4.76	5.05	4.905
	$M_{Acetylation}$	0.58	0.492	5.8	4.92	5.36
	$V_{Acetylation}$	0.535	0.514	5.35	5.14	5.245
	$S_{Acetylation}$	0.526	0.463	5.26	4.63	4.945
				0	0	0
10	$C_{Annealing}$	0.496	0.477	4.96	4.77	4.865
	$M_{Annealing}$	0.34	0.46	3.4	4.6	4
	$V_{Annealing}$	0.347	0.429	3.47	4.29	3.88
	$S_{Annealing}$	0.459	0.48	4.59	4.8	4.695
11	$C_{Carboxymethylation}$	0.457	0.518	4.57	5.18	4.875
	$M_{Carboxymethylation}$	0.481	0.54	4.81	5.4	5.105
	$V_{Carboxymethylation}$	0.484	0.435	4.84	4.35	4.595
	$S_{Carboxymethylation}$	0.492	0.522	4.92	5.22	5.07
12	$C_{Crosslinking}$	0.415	0.419	4.15	4.19	4.17
	$M_{Crosslinking}$	0.449	0.42	4.49	4.2	4.345
	$V_{Crosslinking}$	0.405	0.412	4.05	4.12	4.085
	$S_{Crosslinking}$	0.427	0.432	4.27	4.32	4.295
13	C_{Combo}	0.444	0.472	4.44	4.72	4.58
	M_{Combo}	0.439	0.418	4.39	4.18	4.285
	V_{Combo}	0.486	0.461	4.86	4.61	4.735
	S_{Combo}	0.64	0.453	6.4	4.53	5.465
14	$C_{Heat\ Moisture\ Treatment}$	0.403	0.45	4.03	4.5	4.265
	$M_{Heat\ Moisture\ Treatment}$	0.43	0.4	4.3	4	4.15
	$V_{Heat\ Moisture\ Treatment}$	0.407	0.415	4.07	4.15	4.11

	S Heat Moisture Treatment	0.428	0.451	4.28	4.51	4.395
15	C Osmotic Pressure	0.34	0.418	3.4	4.18	3.79
	M Osmotic Pressure	0.423	0.224	4.23	2.24	3.235
	V Osmotic Pressure	0.492	0.445	4.92	4.45	4.685
	S Osmotic Pressure	0.519	0.468	5.19	4.68	4.935
16	C Oxidation	0.496	0.512	4.96	5.12	5.04
	M Oxidation	0.436	0.451	4.36	4.51	4.435
	V Oxidation	0.421	0.402	4.21	4.02	4.115
	S Oxidation	0.45	0.479	4.5	4.79	4.645
17	C Phosphorylation	0.541	0.494	5.41	4.94	5.175
	M Phosphorylation	1.314	0.666	13.14	6.66	9.9
	V Phosphorylation	0.878	0.479	8.78	4.79	6.785
	S Phosphorylation	0.542	0.587	5.42	5.87	5.645
18	C Pyrodextrinization	0.327	0.306	3.27	3.06	3.165
	M Pyrodextrinization	0.263	0.312	2.63	3.12	2.875
	V Pyrodextrinization	0.327	0.344	3.27	3.44	3.355
	S Pyrodextrinization	0.416	0.35	4.16	3.5	3.83
19	C Succinylation	0.43	0.52	4.3	5.2	4.75
	M Succinylation	0.39	0.447	3.9	4.47	4.185
	V Succinylation	0.497	0.456	4.97	4.56	4.765
	S Succinylation	0.748	0.482	7.48	4.82	6.15

Table 9: Results showing the pH of native and modified starches

		PH 1	PH 2	AVERAGE
NATIVE				
1	Ctrl	7.3	7.6	7.45
	C_{native}	6.9	7.6	7.25
	M_{native}	6.2	6.8	6.5
	V_{native}	6.9	5.8	6.35
	S_{native}	5.1	6.4	5.75
MODIFICATION				
2	$C_{Acid\ hydrolysis}$	3.2	4	3.6
	$M_{Acid\ hydrolysis}$	3.1	4.2	3.65
	$V_{Acid\ hydrolysis}$	3.4	4.6	4
	$S_{Acid\ hydrolysis}$	3.1	3.8	3.45
3	$C_{A/A}$	8.4	7.8	8.1
	$M_{A/A}$	7.5	7.2	7.35
	$V_{A/A}$	7.3	7	7.15
	$S_{A/A}$	7.4	6.8	7.1
4	$C_{A/A\ 20\%}$	7.2	7	7.1
	$M_{A/A\ 20\%}$	7	6.6	6.8
	$V_{A/A\ 20\%}$	7.4	6.2	6.8
	$S_{A/A\ 20\%}$	6.4	7.6	7
5	$C_{A/A\ 50\%}$	6.4	7.6	7
	$M_{A/A\ 50\%}$	6.2	8	7.1
	$V_{A/A\ 50\%}$	7.6	7	7.3
	$S_{A/A\ 50\%}$	8.4	9	8.7
6	$C_{AC/AL}$	5.3	6.4	5.85
	$M_{AC/AL}$	4.1	5.6	4.85
	$V_{AC/AL}$	3.5	5.2	4.35
	$S_{AC/AL}$	4.2	5.8	5

7	$C_{AC/AL\ 50\%}$	8	8.4	8.2
	$M_{AC/AL\ 50\%}$	7.4	7.2	7.3
	$V_{AC/AL\ 50\%}$	5.2	6.4	5.8
	$S_{AC/AL\ 50\%}$	6	5.8	5.9
8	$C_{AC/AL\ 70\%}$	6.4	6	6.2
	$M_{AC/AL\ 70\%}$	7.4	6	6.7
	$V_{AC/AL\ 70\%}$	5.2	5.6	5.4
	$S_{AC/AL\ 70\%}$	6.8	6.4	6.6
9	$C_{Acetylation}$	6.2	6.8	6.5
	$M_{Acetylation}$	5.9	7.2	6.55
	$V_{Acetylation}$	5.7	6.2	5.95
	$S_{Acetylation}$	5.5	6.8	6.15
10	$C_{Annealing}$	6.8	7.8	7.3
	$M_{Annealing}$	6.2	7	6.6
	$V_{Annealing}$	7	7.2	7.1
	$S_{Annealing}$	7.3	7.8	7.55
11	$C_{Carboxymethylation}$	3.2	4.8	4
	$M_{Carboxymethylation}$	5.8	5	5.4
	$V_{Carboxymethylation}$	4	5.4	4.7
	$S_{Carboxymethylation}$	4	5.8	4.9
12	$C_{Crosslinking}$	7.2	7	7.1
	$M_{Crosslinking}$	8.5	9	8.75
	$V_{Crosslinking}$	7.4	6.8	7.1
	$S_{Crosslinking}$	9	8.6	8.8
13	C_{Combo}	7.4	7	7.2
	M_{Combo}	6.8	7.2	7
	V_{Combo}	6.2	6.6	6.4
	S_{Combo}	7.6	7.2	7.4
14	$C_{Heat\ Moisture\ Treatment}$	6.5	7	6.75

	M Heat Moisture Treatment	6.1	6.8	6.45
	V Heat Moisture Treatment	7.1	6.8	6.95
	S Heat Moisture Treatment	5.5	6.6	6.05
15	C Osmotic Pressure	7.1	7.2	7.15
	M Osmotic Pressure	6.3	6.8	6.55
	V Osmotic Pressure	7.1	6.8	6.95
	S Osmotic Pressure	7.5	7.2	7.35
16	C Oxidation	6.8	6.2	6.5
	M Oxidation	5.8	6.2	6
	V Oxidation	7.3	7	7.15
	S Oxidation	6.6	7.4	7
17	C Phosphorylation	8	7.8	7.9
	M Phosphorylation	7.4	7.2	7.3
	V Phosphorylation	6.9	7.2	7.05
	S Phosphorylation	7.5	8.2	7.85
18	C Pyrodextrinization	4.5	6.4	4.5
	M Pyrodextrinization	3.8	5.2	3.8
	V Pyrodextrinization	3.5	4.6	3.5
	S Pyrodextrinization	3.9	4.4	3.9
19	C Succinylation	8.6	7.8	8.2
	M Succinylation	6.5	7.2	6.85
	V Succinylation	6.1	7	6.55
	S Succinylation	6.6	6.8	6.7

Table 10: Results showing the Water Absorption Capacity of the native and modified starches

		DIFFERENCE 1	WAC 1 (g)	DIFFERENCE 2	WAC 2 (g)	AVERAGE
NATIVE						
1	Ctrl	2.835	1	3.749	1	3.29
	C_{native}	1.97	1	2.125	1	2.05
	M_{native}	2.318	1	1.711	1	2.01
	V_{native}	2.386	1	2.17	1	2.28
	S_{native}	1.464	1	2.024	1	1.74
	MODIFICATION					
2	$C_{Acid\ hydrolysis}$	1.154	1	0.875	1	1.01
	$M_{Acid\ hydrolysis}$	1.243	1	0.592	1	0.92
	$V_{Acid\ hydrolysis}$	1.19	1	0.838	1	1.01
	$S_{Acid\ hydrolysis}$	1.3	1	0.696	1	1.00
3	$C_{A/A}$	2.228	1	2.101	1	2.16
	$M_{A/A}$	2.576	1	1.884	1	2.23
	$V_{A/A}$	2.056	1	1.659	1	1.86
	$S_{A/A}$	2.089	1	2.213	1	2.15
4	$C_{A/A\ 20\%}$	7.944	1	3.031	1	5.49
	$M_{A/A\ 20\%}$	2.584	1	2.737	1	2.66
	$V_{A/A\ 20\%}$	2.811	1	3.108	1	2.96
	$S_{A/A\ 20\%}$	2.34	1	2.894	1	2.62
5	$C_{A/A\ 50\%}$	3.296	1	3.741	1	3.52
	$M_{A/A\ 50\%}$	3.456	1	3.872	1	3.66
	$V_{A/A\ 50\%}$	3.442	1	3.1	1	3.27
	$S_{A/A\ 50\%}$	2.9	1	3.26	1	3.08
6	$C_{AC/AL}$	2.206	1	1.984	1	2.10
	$M_{AC/AL}$	2.104	1	2.313	1	2.21

	$V_{AC/AL}$	1.936	1	2.471	1	2.20
	$S_{AC/AL}$	2.152	1	2.084	1	2.12
7	$C_{AC/AL\ 50\%}$	2.296	1	1.76	1	2.03
	$M_{AC/AL\ 50\%}$	2.38	1	2.436	1	2.41
	$V_{AC/AL\ 50\%}$	2.568	1	2.219	1	2.39
	$S_{AC/AL\ 50\%}$	2.276	1	2.392	1	2.33
8	$C_{AC/AL\ 70\%}$	2.434	1	2.756	1	2.60
	$M_{AC/AL\ 70\%}$	2.336	1	2.684	1	2.51
	$V_{AC/AL\ 70\%}$	2.506	1	2.76	1	2.63
	$S_{AC/AL\ 70\%}$	1.816	1	2.612	1	2.21
9	$C_{Acetylation}$	2.678	1	2.164	1	2.42
	$M_{Acetylation}$	1.781	1	2.047	1	1.91
	$V_{Acetylation}$	1.877	1	1.56	1	1.72
	$S_{Acetylation}$	1.349	1	1.476	1	1.41
10	$C_{Annealing}$	2.16	1	2.232	1	2.20
	$M_{Annealing}$	1.949	1	2.041	1	2.00
	$V_{Annealing}$	1.932	1	2.12	1	2.03
	$S_{Annealing}$	2.202	1	2.456	1	2.33
11	$C_{Carboxymethylation}$	1.051	1	1.422	1	1.24
	$M_{Carboxymethylation}$	1.557	1	1.369	1	1.46
	$V_{Carboxymethylation}$	1.365	1	1.483	1	1.42
	$S_{Carboxymethylation}$	1.167	1	1.378	1	1.27
12	$C_{Crosslinking}$	1.107	1	1.318	1	1.21
	$M_{Crosslinking}$	1.575	1	1.407	1	1.49
	$V_{Crosslinking}$	1.535	1	2.318	1	1.93
	$S_{Crosslinking}$	1.558	1	1.184	1	1.37
13	C_{Combo}	3.324	1	3.536	1	3.43
	M_{Combo}	3.156	1	3.112	1	3.13
	V_{Combo}	3.642	1	3.424	1	3.53

	S_{Combo}	3.386	1	2.871	1	3.13
14	$C_{Heat\ Moisture\ Treatment}$	1.814	1	1.751	1	1.78
	$M_{Heat\ Moisture\ Treatment}$	2.063	1	1.835	1	1.95
	$V_{Heat\ Moisture\ Treatment}$	2.096	1	1.947	1	2.02
	$S_{Heat\ Moisture\ Treatment}$	1.896	1	1.288	1	1.59
15	$C_{Osmotic\ Pressure}$	1.131	1	1.215	1	1.17
	$M_{Osmotic\ Pressure}$	1.285	1	1.13	1	1.21
	$V_{Osmotic\ Pressure}$	0.972	1	0.923	1	0.95
	$S_{Osmotic\ Pressure}$	1.231	1	0.837	1	1.03
16	$C_{Oxidation}$	2.111	1	1.169	1	1.64
	$M_{Oxidation}$	1.151	1	1.294	1	1.22
	$V_{Oxidation}$	1.237	1	1.078	1	1.16
	$S_{Oxidation}$	0.844	1	1.386	1	1.12
17	$C_{Phosphorylation}$	1.918	1	1.426	1	1.67
	$M_{Phosphorylation}$	0.987	1	3.533	1	2.26
	$V_{Phosphorylation}$	1.664	1	1.368	1	1.52
	$S_{Phosphorylation}$	1.275	1	1.415	1	1.35
18	$C_{Pyrodextrinization}$	0.964	1	1.14	1	1.05
	$M_{Pyrodextrinization}$	1.279	1	0.795	1	1.04
	$V_{Pyrodextrinization}$	1.008	1	0.751	1	0.88
	$S_{Pyrodextrinization}$	0.838	1	0.641	1	0.74
19	$C_{Succinylation}$	1.334	1	1.086	1	1.21
	$M_{Succinylation}$	1.864	1	1.378	1	1.62
	$V_{Succinylation}$	0.937	1	1.149	1	1.04
	$S_{Succinylation}$	1.445	1	1.364	1	1.40

Table 11: Results showing the amylose (AM) and amylopectin (AP) content of native and modified starches

	MODIFICATION	1	2	AVERAGE	AC %	AP
NATIVE						
1	Ctrl	0.285	0.28	0.283	17.29	82.71
	C_{native}	0.318	0.337	0.328	20.04	79.96
	M_{native}	0.394	0.382	0.388	23.75	76.25
	V_{native}	0.355	0.349	0.352	21.54	78.46
	S_{native}	0.38	0.358	0.369	22.58	77.42
	MODIFICATION					
2	$C_{Acid\ hydrolysis}$	0.395	0.372	0.384	23.47	76.53
	$M_{Acid\ hydrolysis}$	0.348	0.357	0.353	21.57	78.43
	$V_{Acid\ hydrolysis}$	0.317	0.32	0.319	19.49	80.51
	$S_{Acid\ hydrolysis}$	0.379	0.383	0.381	23.32	76.68
3	$C_{A/A}$	0.368	0.374	0.371	22.71	77.29
	$M_{A/A}$	0.37	0.357	0.364	22.25	77.75
	$V_{A/A}$	0.401	0.425	0.413	25.28	74.72
	$S_{A/A}$	0.372	0.367	0.370	22.61	77.39
4	$C_{A/A\ 20\%}$	0.236	0.242	0.239	14.63	85.37
	$M_{A/A\ 20\%}$	0.311	0.289	0.300	18.36	81.64
	$V_{A/A\ 20\%}$	0.258	0.283	0.271	16.55	83.45
	$S_{A/A\ 20\%}$	0.297	0.274	0.286	17.47	82.53
5	$C_{A/A\ 50\%}$	0.22	0.228	0.224	13.71	86.29
	$M_{A/A\ 50\%}$	0.293	0.302	0.298	18.21	81.79
	$V_{A/A\ 50\%}$	0.247	0.258	0.253	15.45	84.55
	$S_{A/A\ 50\%}$	0.333	0.319	0.326	19.95	80.05
6	$C_{AC/AL}$	0.255	0.249	0.252	15.42	84.58
	$M_{AC/AL}$	0.357	0.346	0.352	21.51	78.49
	$V_{AC/AL}$	0.278	0.283	0.281	17.17	82.83
	$S_{AC/AL}$	0.318	0.281	0.300	18.33	81.67

7	$C_{AC/AL\ 50\%}$	0.242	0.25	0.246	15.06	84.94
	$M_{AC/AL\ 50\%}$	0.282	0.251	0.267	16.31	83.69
	$V_{AC/AL\ 50\%}$	0.221	0.262	0.242	14.78	85.22
	$S_{AC/AL\ 50\%}$	0.271	0.245	0.258	15.79	84.21
8	$C_{AC/AL\ 70\%}$	0.283	0.271	0.277	16.95	83.05
	$M_{AC/AL\ 70\%}$	0.344	0.318	0.331	20.26	79.74
	$V_{AC/AL\ 70\%}$	0.266	0.292	0.279	17.07	82.93
	$S_{AC/AL\ 70\%}$	0.313	0.303	0.308	18.85	81.15
9	$C_{Acetylation}$	0.291	0.308	0.300	18.33	81.67
	$M_{Acetylation}$	0.317	0.315	0.316	19.34	80.66
	$V_{Acetylation}$	0.343	0.326	0.335	20.47	79.53
	$S_{Acetylation}$	0.317	0.312	0.315	19.25	80.75
10	$C_{Annealing}$	0.282	0.274	0.278	17.01	82.99
	$M_{Annealing}$	0.303	0.31	0.307	18.76	81.24
	$V_{Annealing}$	0.321	0.33	0.326	19.92	80.08
	$S_{Annealing}$	0.335	0.309	0.322	19.71	80.29
11	$C_{Carboxymethylation}$	0.336	0.312	0.324	19.83	80.17
	$M_{Carboxymethylation}$	0.282	0.3	0.291	17.81	82.19
	$V_{Carboxymethylation}$	0.461	0.417	0.439	26.87	73.13
	$S_{Carboxymethylation}$	0.357	0.348	0.353	21.57	78.43
12	$C_{Crosslinking}$	0.317	0.325	0.321	19.65	80.35
	$M_{Crosslinking}$	0.288	0.312	0.300	18.36	81.64
	$V_{Crosslinking}$	0.351	0.321	0.336	20.56	79.44
	$S_{Crosslinking}$	0.347	0.327	0.337	20.62	79.38
13	C_{Combo}	0.277	0.252	0.265	16.19	83.81
	M_{Combo}	0.291	0.298	0.295	18.02	81.98
	V_{Combo}	0.302	0.285	0.294	17.96	82.04
	S_{Combo}	0.271	0.234	0.253	15.45	84.55
14	$C_{Heat\ Moisture\ Treatment}$	0.323	0.297	0.310	18.97	81.03

	M Heat Moisture Treatment	0.348	0.352	0.350	21.42	78.58
	V Heat Moisture Treatment	0.315	0.319	0.317	19.40	80.60
	S Heat Moisture Treatment	0.389	0.396	0.393	24.02	75.98
15	C Osmotic Pressure	0.352	0.321	0.337	20.59	79.41
	M Osmotic Pressure	0.429	0.417	0.423	25.89	74.11
	V Osmotic Pressure	0.408	0.412	0.410	25.09	74.91
	S Osmotic Pressure	0.398	0.417	0.408	24.94	75.06
16	C Oxidation	0.271	0.297	0.284	17.38	82.62
	M Oxidation	0.279	0.292	0.286	17.47	82.53
	V Oxidation	0.333	0.321	0.327	20.01	79.99
	S Oxidation	0.322	0.363	0.343	20.96	79.04
17	C Phosphorylation	0.317	0.304	0.311	19.00	81.00
	M Phosphorylation	0.571	0.519	0.545	33.35	66.65
	V Phosphorylation	0.353	0.378	0.366	22.37	77.63
	S Phosphorylation	0.322	0.316	0.319	19.52	80.48
18	C Pyrodextrinization	0.351	0.335	0.343	20.99	79.01
	M Pyrodextrinization	0.325	0.328	0.327	19.98	80.02
	V Pyrodextrinization	0.378	0.387	0.383	23.41	76.59
	S Pyrodextrinization	0.325	0.319	0.322	19.71	80.29
19	C Succinylation	0.305	0.294	0.300	18.33	81.67
	M Succinylation	0.481	0.436	0.459	28.06	71.94
	V Succinylation	0.302	0.288	0.295	18.05	81.95
	S Succinylation	0.374	0.413	0.394	24.08	75.92

Table 12: Results showing the gelatinisation temperature for the native and modified starches

		GT 1	GT 2	AVERAGE
NATIVE				
1	Ctrl	34	36	35
	C_{native}	74	72	73
	M_{native}	70	72	71
	V_{native}	68	70	69
	S_{native}	70	70	70
MODIFICATION				
2	$C_{Acid\ hydrolysis}$	40	42	41
	$M_{Acid\ hydrolysis}$	48	44	46
	$V_{Acid\ hydrolysis}$	40	44	42
	$S_{Acid\ hydrolysis}$	50	48	49
3	$C_{A/A}$	40	38	39
	$M_{A/A}$	46	42	44
	$V_{A/A}$	36	40	38
	$S_{A/A}$	38	40	39
4	$C_{A/A\ 20\%}$	40	58	49
	$M_{A/A\ 20\%}$	38	50	44
	$V_{A/A\ 20\%}$	44	52	48
	$S_{A/A\ 20\%}$	42	56	49
5	$C_{A/A\ 50\%}$	40	40	40
	$M_{A/A\ 50\%}$	38	38	38
	$V_{A/A\ 50\%}$	50	46	48
	$S_{A/A\ 50\%}$	38	40	39
6	$C_{AC/AL}$	36	338	187
	$M_{AC/AL}$	36	40	38
	$V_{AC/AL}$	38	40	39
	$S_{AC/AL}$	44	40	42

7	$C_{AC/AL\ 50\%}$	42	40	41
	$M_{AC/AL\ 50\%}$	48	42	45
	$V_{AC/AL\ 50\%}$	38	40	39
	$S_{AC/AL\ 50\%}$	44	40	42
8	$C_{AC/AL\ 70\%}$	42	40	41
	$M_{AC/AL\ 70\%}$	58	50	54
	$V_{AC/AL\ 70\%}$	40	38	39
	$S_{AC/AL\ 70\%}$	40	44	42
9	$C_{Acetylation}$	48	54	51
	$M_{Acetylation}$	54	56	55
	$V_{Acetylation}$	50	52	51
	$S_{Acetylation}$	50	52	51
10	$C_{Annealing}$	52	54	53
	$M_{Annealing}$	48	50	49
	$V_{Annealing}$	54	52	53
	$S_{Annealing}$	50	54	52
11	$C_{Carboxymethylation}$	60	58	59
	$M_{Carboxymethylation}$	46	52	49
	$V_{Carboxymethylation}$	50	56	53
	$S_{Carboxymethylation}$	52	52	52
12	$C_{Crosslinking}$	44	48	46
	$M_{Crosslinking}$	40	46	43
	$V_{Crosslinking}$	44	42	43
	$S_{Crosslinking}$	44	42	43
13	C_{Combo}	50	44	47
	M_{Combo}	40	54	47
	V_{Combo}	56	44	50
	S_{Combo}	42	50	46
14	$C_{Heat\ Moisture\ Treatment}$	50	48	49

	M Heat Moisture Treatment	38	46	42
	V Heat Moisture Treatment	44	48	46
	S Heat Moisture Treatment	42	48	45
15	C Osmotic Pressure	44	48	46
	M Osmotic Pressure	48	48	48
	V Osmotic Pressure	50	48	49
	S Osmotic Pressure	56	54	55
16	C Oxidation	50	56	53
	M Oxidation	50	52	51
	V Oxidation	54	52	53
	S Oxidation	52	58	55
17	C Phosphorylation	48	50	49
	M Phosphorylation	42	44	43
	V Phosphorylation	50	50	50
	S Phosphorylation	58	54	56
18	C Pyrodextrinization	48	54	49
	M Pyrodextrinization	56	50	53
	V Pyrodextrinization	50	58	54
	S Pyrodextrinization	60	56	58
19	C Succinylation	42	40	41
	M Succinylation	40	44	42
	V Succinylation	46	50	48
	S Succinylation	38	46	42

Table 13: Results showing the moisture content of native and modified starches

	MODIFICATION	A	% A	B	% B	INITIAL WEIGHT (g)	AVERAGE %
NATIVE							
1	Ctrl	0.47	6	0.462	7.6	0.5	6.8
	C_{native}	0.462	7.6	0.45	10	0.5	8.8
	M_{native}	0.433	13.4	0.431	13.8	0.5	13.6
	V_{native}	0.447	10.6	0.423	15.4	0.5	13
	S_{native}	0.443	11.4	0.438	12.4	0.5	11.9
	MODIFICATION						
2	$C_{Acid\ hydrolysis}$	0.459	8.2	0.463	7.4	0.5	7.8
	$M_{Acid\ hydrolysis}$	0.457	8.6	0.452	9.6	0.5	9.1
	$V_{Acid\ hydrolysis}$	0.44	12	0.452	9.6	0.5	10.8
	$S_{Acid\ hydrolysis}$	0.448	10.4	0.458	8.4	0.5	9.4
3	$C_{A/A}$	0.445	11	0.451	9.8	0.5	10.4
	$M_{A/A}$	0.461	7.8	0.448	10.4	0.5	9.1
	$V_{A/A}$	0.465	7	0.453	9.4	0.5	8.2
	$S_{A/A}$	0.442	11.6	0.449	10.2	0.5	10.9
4	$C_{A/A\ 20\%}$	0.482	3.6	0.468	6.4	0.5	5
	$M_{A/A\ 20\%}$	0.451	9.8	0.474	5.2	0.5	7.5
	$V_{A/A\ 20\%}$	0.421	15.8	0.44	12	0.5	13.9
	$S_{A/A\ 20\%}$	0.467	6.6	0.455	9	0.5	7.8
5	$C_{A/A\ 50\%}$	0.46	8	0.468	6.4	0.5	7.2
	$M_{A/A\ 50\%}$	0.447	10.6	0.452	9.6	0.5	10.1
	$V_{A/A\ 50\%}$	0.436	12.8	0.461	7.8	0.5	10.3
	$S_{A/A\ 50\%}$	0.409	18.2	0.435	13	0.5	15.6
6	$C_{AC/AL}$	0.415	17	0.428	14.4	0.5	15.7
	$M_{AC/AL}$	0.448	10.4	0.417	16.6	0.5	13.5
	$V_{AC/AL}$	0.43	14	0.444	11.2	0.5	12.6

	$S_{AC/AL}$	0.432	13.6	0.421	15.8	0.5	14.7
7	$C_{AC/AL\ 50\%}$	0.439	12.2	0.457	8.6	0.5	10.4
	$M_{AC/AL\ 50\%}$	0.419	16.2	0.442	11.6	0.5	13.9
	$V_{AC/AL\ 50\%}$	0.42	16	0.436	12.8	0.5	14.4
	$S_{AC/AL\ 50\%}$	0.441	11.8	0.427	14.6	0.5	13.2
8	$C_{AC/AL\ 70\%}$	0.445	11	0.437	12.6	0.5	11.8
	$M_{AC/AL\ 70\%}$	0.446	10.8	0.438	12.4	0.5	11.6
	$V_{AC/AL\ 70\%}$	0.408	18.4	0.426	14.8	0.5	16.6
	$S_{AC/AL\ 70\%}$	0.43	14	0.366	26.8	0.5	20.4
9	$C_{Acetylation}$	0.472	5.6	0.488	2.4	0.5	4
	$M_{Acetylation}$	0.461	7.8	0.469	6.2	0.5	7
	$V_{Acetylation}$	0.484	3.2	0.48	4	0.5	3.6
	$S_{Acetylation}$	0.492	1.6	0.477	4.6	0.5	3.1
10	$C_{Annealing}$						
	$M_{Annealing}$	0.492	1.6	0.476	4.8	0.5	3.2
	$V_{Annealing}$	0.485	3	0.471	5.8	0.5	4.4
	$S_{Annealing}$	0.489	2.2	0.491	1.8	0.5	2
11	$C_{Carboxymethylation}$	0.361	27.8	0.422	15.6	0.5	21.7
	$M_{Carboxymethylation}$	0.431	13.8	0.428	14.4	0.5	14.1
	$V_{Carboxymethylation}$	0.453	9.4	0.439	12.2	0.5	10.8
	$S_{Carboxymethylation}$	0.425	15	0.441	11.8	0.5	13.4
12	$C_{Crosslinking}$	0.497	0.6	0.474	5.2	0.5	2.9
	$M_{Crosslinking}$	0.495	1	0.477	4.6	0.5	2.8
	$V_{Crosslinking}$	0.495	1	0.485	3	0.5	2
	$S_{Crosslinking}$	0.499	0.2	0.487	2.6	0.5	1.4
13	C_{Combo}	0.424	15.2	0.438	12.4	0.5	13.8
	M_{Combo}	0.431	13.8	0.399	20.2	0.5	17
	V_{Combo}	0.449	10.2	0.429	14.2	0.5	12.2
	S_{Combo}	0.408	18.4	0.419	16.2	0.5	17.3

14	C $_{\text{Heat Moisture Treatment}}$	0.493	1.4	0.486	2.8	0.5	2.1
	M $_{\text{Heat Moisture Treatment}}$	0.462	7.6	0.445	11	0.5	9.3
	V $_{\text{Heat Moisture Treatment}}$	0.451	9.8	0.458	8.4	0.5	9.1
	S $_{\text{Heat Moisture Treatment}}$	0.48	4	0.491	1.8	0.5	2.9
15	C $_{\text{Osmotic Pressure}}$	0.478	4.4	0.463	7.4	0.5	5.9
	M $_{\text{Osmotic Pressure}}$	0.47	6	0.481	3.8	0.5	4.9
	V $_{\text{Osmotic Pressure}}$	0.449	10.2	0.46	8	0.5	9.1
	S $_{\text{Osmotic Pressure}}$	0.471	5.8	0.458	8.4	0.5	7.1
16	C $_{\text{Oxidation}}$	0.481	3.8	0.476	4.8	0.5	4.3
	M $_{\text{Oxidation}}$	0.471	5.8	0.484	3.2	0.5	4.5
	V $_{\text{Oxidation}}$	0.463	7.4	0.389	22.2	0.5	14.8
	S $_{\text{Oxidation}}$	0.475	5	0.482	3.6	0.5	4.3
17	C $_{\text{Phosphorylation}}$	0.461	7.8	0.473	5.4	0.5	6.6
	M $_{\text{Phosphorylation}}$	0.45	10	0.461	7.8	0.5	8.9
	V $_{\text{Phosphorylation}}$	0.452	9.6	0.43	14	0.5	11.8
	S $_{\text{Phosphorylation}}$	0.464	7.2	0.45	10	0.5	8.6
18	C $_{\text{Pyrodextrinization}}$	0.415	17	0.459	8.2	0.5	12.6
	M $_{\text{Pyrodextrinization}}$	0.472	5.6	0.44	12	0.5	8.8
	V $_{\text{Pyrodextrinization}}$	0.432	13.6	0.421	15.8	0.5	14.7
	S $_{\text{Pyrodextrinization}}$	0.446	10.8	0.451	9.8	0.5	10.3
19	C $_{\text{Succinylation}}$	0.461	7.8	0.473	5.4	0.5	6.6
	M $_{\text{Succinylation}}$	0.468	6.4	0.48	4	0.5	5.2
	V $_{\text{Succinylation}}$	0.495	1	0.483	3.4	0.5	2.2
	S $_{\text{Succinylation}}$	0.47	6	0.458	8.4	0.5	7.2

Table 14: Results for the Paste clarity of native and modified starches

	MODIFICATION	ABSORBANCE (650 nm)	% TRANSMITTANCE	ABSORBANCE (650 nm)	% TRANSMITTANCE	AVERAGE
1	Ctrl	0.18	66	0.201	63.1	64.55
	C_{native}	0.134	73.4	0.158	69.5	71.45
	M_{native}	0.182	65.8	0.179	66.2	66
	V_{native}	0.151	70.6	0.167	68.1	69.35
	S_{native}	0.182	65.8	0.17	67.6	66.7
2	$C_{Acid\ hydrolysis}$	0.126	74.8	0.142	72.1	73.45
	$M_{Acid\ hydrolysis}$	0.262	54.7	0.257	55.3	55
	$V_{Acid\ hydrolysis}$	0.307	49.3	0.283	52.1	50.7
	$S_{Acid\ hydrolysis}$	0.219	60.4	0.209	61.8	61.1
3	$C_{A/A}$	0.199	63.2	0.167	68	65.6
	$M_{A/A}$	0.261	54.8	0.286	51.7	53.25
	$V_{A/A}$	0.256	55.4	0.281	52.1	53.75
	$S_{A/A}$	0.296	50.6	0.315	48.4	49.5
4	$C_{A/A\ 20\%}$	0.208	61.9	0.217	60.7	61.3
	$M_{A/A\ 20\%}$	0.251	56.1	0.246	56.8	56.45
	$V_{A/A\ 20\%}$	0.212	61.4	0.223	59.8	60.6
	$S_{A/A\ 20\%}$	0.248	56.5	0.266	54.2	55.35
5	$C_{A/A\ 50\%}$	0.227	59.3	0.245	56.9	58.1
	$M_{A/A\ 50\%}$	0.283	52.1	0.292	51	51.55
	$V_{A/A\ 50\%}$	0.271	53.6	0.246	56.8	55.2
	$S_{A/A\ 50\%}$	0.321	47.8	0.328	47	47.4
6	$C_{AC/AL}$	0.078	83.5	0.063	86.5	85
	$M_{AC/AL}$	0.165	68.4	0.143	71.9	70.15
	$V_{AC/AL}$	0.075	84.1	0.112	77.2	80.65
	$S_{AC/AL}$	0.058	87.5	0.097	80	83.75
7	$C_{AC/AL\ 50\%}$	0.064	86.3	0.07	85.1	85.7

	M AC/AL 50%	0.169	67.8	0.175	66.8	67.3
	V AC/AL 50%	0.081	83	0.084	82.4	82.7
	S AC/AL 50%	0.067	85.7	0.072	84.7	85.2
8	C AC/AL 70%	0.152	70.4	0.147	71.3	70.85
	M AC/AL 70%	0.224	59.7	0.215	61	60.35
	V AC/AL 70%	0.171	67.4	0.192	64.3	65.85
	S AC/AL 70%	0.164	68.5	0.172	67.3	67.9
9	C Acetylation	0.142	72.1	0.113	77	74.55
	M Acetylation	0.148	71.1	0.143	71.9	71.5
	V Acetylation	0.183	65.6	0.161	69	67.3
	S Acetylation	0.187	65	0.175	66.8	65.9
10	C Annealing	0.121	75.6	0.108	77.9	76.75
	M Annealing	0.107	78.2	0.13	74.1	76.15
	V Annealing	0.079	83.4	0.082	82.8	83.1
	S Annealing	0.099	79.6	0.117	77.3	78.45
11	C Carboxymethylation	0.212	61.3	0.93	80.7	71
	M Carboxymethylation	0.282	52.2	0.244	57	54.6
	V Carboxymethylation	0.245	55.9	0.264	54.4	55.15
	S Carboxymethylation	0.232	58.6	0.267	54.1	56.35
12	C Crosslinking	0.459	34.8	0.477	33.3	34.05
	M Crosslinking	0.431	37.1	0.39	40.7	38.9
	V Crosslinking	0.498	31.8	0.462	34.5	33.15
	S Crosslinking	0.393	40.4	0.441	36.2	38.3
13	C Combo	0.328	47	0.341	45.6	46.3
	M Combo	0.371	42.5	0.363	43.3	42.9
	V Combo	0.291	51.2	0.317	48.2	49.7
	S Combo	0.369	42.8	0.341	45.6	44.2
14	C Heat Moisture Treatment	0.403	39.5	0.367	43	41.25
	M Heat Moisture Treatment	0.388	40.9	0.392	40.5	40.7

	V Heat Moisture Treatment	0.477	33.3	0.412	38.7	36
	S Heat Moisture Treatment	0.497	31.8	0.435	36.7	34.25
15	C Osmotic Pressure	0.253	55.8	0.279	52.6	54.2
	M Osmotic Pressure	0.362	43.4	0.418	38.2	40.8
	V Osmotic Pressure	0.192	64.2	0.221	60.1	62.15
	S Osmotic Pressure	0.262	57.7	0.251	56.1	56.9
16	C Oxidation	0.139	72.6	0.153	70.3	71.45
	M Oxidation	0.085	82.8	0.106	78.3	80.55
	V Oxidation	0.207	62.1	0.142	72.1	67.1
	S Oxidation	0.186	65.2	0.215	60.9	63.05
17	C Phosphorylation	0.183	65.6	0.146	71.4	68.5
	M Phosphorylation	0.182	65.8	0.154	70.1	67.95
	V Phosphorylation	0.275	53.1	0.253	55.8	54.45
	S Phosphorylation	0.298	50.4	0.282	52.2	51.3
18	C Pyrodextrinization	0.454	35.1	0.427	37.4	36.25
	M Pyrodextrinization	0.43	37.1	0.411	38.8	37.95
	V Pyrodextrinization	0.496	31.9	0.473	33.7	32.8
	S Pyrodextrinization	0.415	38.4	0.446	35.8	37.1
19	C Succinylation	0.235	58.2	0.255	55.6	56.9
	M Succinylation	0.278	52.7	0.261	54.8	53.75
	V Succinylation	0.294	50.8	0.248	56.5	53.65
	S Succinylation	0.241	57.4	0.218	60.5	58.95