DEVELOPMENT AND PHYSICO-CHEMICAL CHARACTERIZATION OF GRANULAR COLD WATER-SOLUBLE SAGO (*Metroxylon sagu* Rottb.) STARCH

by

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LIST OF SYMBOLS / ABBREVIATIONS

Symbol/Abbreviation	Caption
HPSEC	High performance size exclusion chromatography
SEM	Scanning electron microscopy
LM	Light microscopy
DSC	Differential scanning calorimetry
MDSC	Modulated differential scanning calorimetry
RVA	Rapid visco analyzer
RVU	Rapid visco unit
DP	Degree of polymerization
M_w	Weight-average molecular weight
M_n	Number-average molecular weight
P	Polydispersity index
T_o	Onset temperature
T_p	Peak temperature
T_g	Glass transition temperature
ΔH	Gelatinisation enthalpy
G’	Storage modulus
G”	Loss modulus
Tan δ	Loss factor
η’	Dynamic viscosity
[η]	Intrinsic viscosity
RVP	Relative vapour pressure
LCDA	Land, Custody and Development Authority

CRAUN	Crop Research and Application Unit
NSS	Native sago starch
NCS	Native corn starch
CSS	Control sago starch only treated with ethanol
30SS	Sago starch treated with 30 g of 3 M NaOH
45SS	Sago starch treated with 45 g of 3 M NaOH
60SS	Sago starch treated with 60 g of 3 M NaOH
75SS	Sago starch treated with 75 g of 3 M NaOH
75CS	Corn starch treated with 75 g of 3 M NaOH
NaOH	Sodium hydroxide
EtOH	Ethanol
GCWS	Granular cold water-soluble
CWS	Cold water-solubility
DMSO	Dimethylsulfoxide

LIST OF APPENDICES

LIST OF PUBLICATIONS AND SEMINARS

1. Bhupinder, K., Karim, A.A., Norziah, M.H. and Seow, C.C. (2002). Preparation of granular cold water-soluble sago starch: A preliminary study. In *Proceedings of the International Symposium on Sago (Sago 2001) held on October 15-17, 2001, at the Tsukuba International Congress Center Japan* (Kainuma, K., Okazaki, M., Toyoda, Y. and Cecil, J.E., eds.), p. 355-357. Universal Academy Press,Inc., Tokyo, Japan.

2. Bhupinder, K., Karim,A.A. and Seow, C.C. (2001). Production of granular cold water-soluble sago starch using Response Surface Methodology. Book of Abstracts. *JSPS Seminar III: Development of Sago Industry BioThailand 2001: From Research to Market, Bangkok, Thailand, 2001*. Poster presentation.

PERKEMBANGAN DAN PENCIRIAN FIZIKO-KIMIA KANJI SAGU (*Metroxylon sagu* Rottb.) GRANULAR LARUT-AIR SEJUK

ABSTRAK

Analisis permukaan sambutan (RSM) telah dilakukan untuk mengkaji kesan interaksi antara alkohol, alkali dan suhu terhadap kebolehlarutan air sejuk (CWS), kejernihan dan viskositi kanji sagu. Kadar kebolehlarutan air dan viskositi menunjukkan hasil yang tidak memenuhi ujian kekurangan penyesuaian secara signifikan, maka tidak mampu untuk menunjukkan sifat sebenar sampel. Pemerhatian terhadap sampel terubah-suai dengan menggunakan mikroskop elektron penskanan (SEM) mendapati sampel telah mengalami gelatinisasi awal. Kemungkinan gelatinisasi sejuk boleh berlaku jika sampel terubah-suai tidak diberikan masa yang secukupnya untuk dineutralkan. Bahagian kedua penyelidikan ini adalah kajian terhadap kesan kepekatan NaOH pada suhu dan kepekatan etanol malar. Pada amnya, CWS kanji terubah-suai meningkat dengan peningkatan kepekatan NaOH, di mana CWS yang paling tinggi adalah 91.4% pada kepekatan efektif NaOH 0.82 M. Pada keadaan yang sama, kanji jagung telah memberikan CWS sebanyak 15.4%. Corak difraksi X-ray berubah daripada C kepada V bagi kanji dengan CWS yang tinggi (63.8% dan 91.4%). Ketidakhadiran palang Maltese telah diperhatikan bagi sampel kanji terubah-suai yang mempunyai CWS 63.8% dan 91.4% dengan menggunakan mikroskop cahaya (LM). Granul-granul ini adalah lebih besar daripada kanji asal dan mempunyai bentuk yang berlekuk bila diperhatikan dengan SEM. Kuasa pembengkakan kanji terubah-suai telah meningkat dengan peningkatan CWS dan suhu. Kandungan amilosa dalam kanji terubah-suai juga meningkat secara signifikan

dengan peningkatan CWS untuk kanji sagu. Nilai kepekatan intrinsik dan kromatogram HPSEC menunjukkan degradasi telah berlaku dimana terdapat peralihan dalam kurva amilopektin untuk kanji terubah-suai. Kebolehadaman kanji terubah-suai menunjukkan hasil yang kurang signifikan berbanding dengan kanji asal tetapi meningkat dengan suhu inkubasi walaupun secara perlahan. Kejernihan kanji terubah-suai juga menunjukkan penurunan secara signifikan berbanding dengan kanji asal dan terus menurun dengan masa penstoran pada suhu 4 ^{o}C. Ini menunjukkan peningkatan kecenderungan kanji terubah-suai untuk meretrogradasi. Ini telah disokong dengan hasil kajian sifat pempesan dan kestabilan beku-nyahbeku. Viskositi puncak kanji terubah-suai adalah rendah secara signifikan berbanding dengan kanji asal dan pemerhatian ini juga adalah bersamaan dengan kajian aliran. Kanji yang terubah-suai dengan CWS air sejuk tertinggi mempunyai kebolehan yang sama dari segi keadaan viskoelastik kanji sagu kawalan. Kanji terubah-suai mempunyai keupayaan penyerapan air yang lebih rendah berkemungkinan kerana penurunan tapak berpolar. Dengan peningkatan kepekatan NaOH, entalpi gelatinisasi menurun dan tidak dapat dikesan unuk kanji sagu dan jagung terubah-suai dengan 0.69 M dan 0.82 M NaOH.

DEVELOPMENT AND PHYSICO-CHEMICAL CHARACTERIZATION OF GRANULAR COLD WATER-SOLUBLE SAGO (*Metroxylon sagu* Rottb.) STARCH

ABSTRACT

A Response Surface analysis was carried out to study the interaction of alcohol and alkali with temperature to the response on the cold water-solubility (CWS), clarity and viscosity of sago starch. As the cold water-solubility and viscosity presented a significant lack of fit, the models would not be able to adequately represent the behaviour of the sample. On viewing the treated samples with scanning electron micrograph (SEM) it was found that some of the samples had been pre-gelatinized. The possibility of cold gelatinisation occurring was identified if the treated samples were not given enough time to neutralise. The second part of the research studied the effect of NaOH concentration at constant temperature and ethanol concentration. Generally the CWS of all the samples increased with an increase in the concentration of NaOH, the highest being at 91.4% with the effective concentration of 0.82 M. Treated at the same conditions, corn starch, which was used in comparison gave a CWS of 15.4%. The X-ray diffraction pattern changed from a C to V for treated starches of high CWS (63.8% and 91.4%). The absence of the Maltese cross was noticed for sago samples with 63.8% and 91.4% CWS when viewed using light microscope (LM). These granules were also larger than the native starches and had an indented appearance as seen with the SEM. The swelling power of the treated samples increased with their CWS and with an increase in temperature. The amylose content of the treated starch increased significantly with the increase in

CWS for sago starches. The values of intrinsic viscosity and HPSEC chromatograms show that some degradation did take place as there is a shift in the amylopectin curve for the treated starches observed in the chromatogram. The digestibility of the treated starches was significantly less than that of the cooked native starches but it increased with the increase in incubation time albeit a little slow. The clarity of the treated starches was significantly lower than native starches and it further decreased with storage time at 4 ^{o}C. This showed a higher tendency for the treated starches to retrograde. The results of pasting properties and freeze-thaw stability studies concurred with this finding. The peak viscosity of the treated starches was significantly lower than that of the native starch and this was also observed in the study in the flow behaviour of the starches. Treated sago starch with highest CWS, was able to achieve the same viscoelastic conditions as the control sago starch. The treated starches had lower water adsorption ability probably due to a decrease in the number of polar sites. With the increase in the concentration of NaOH the gelatinisation enthalpy decreased and was not visible for sago and corn starch treated with an effective concentration of 0.69 M and 0.82 M of NaOH.

CHAPTER 1 – INTRODUCTION

1.1 Background

Starch plays an important role as an essential source of carbohydrate in the human diet as it provides between 70-80% of the calories consumed (Thomas and Atwell, 1999). Starch is used extensively in the food industry. It is used as a thickening and binding agent and in the production of puddings, soups, sauces, salad dressings, diet food preparations for infants, pastry filling and mayonnaise. Starch is also an important raw material for the production of glucose syrup, glucose and other fine chemicals such as gluconic acid and monosodium glutamate (Belitz *et al.*, 2004). Besides the food industry, starch is also used in the paper industry for surface sizing and coating, as adhesive for gummed paper, gummed tape and wallpaper, in the textile industry, pharmaceutical industry and also as animal feed. Starch can be found in cereals (corn, wheat, rice), tuber (potato, tapioca) and also stem (sago) and currently there has been interest in legume starches as well.

Sago palm, *Metroxylon sagu* is one of the oldest species of palms that has been exploited for its stem starch. Tropical Asia has been home to the mostly 2,500,000 ha of sago palm in the world (Oates and Hicks, 2002). In Malaysia, cultivation of sago palm is now concentrated in Sarawak. Sago palm has a natural adaptation to peat soils of low nutritional value and high acidity and therefore the soil needs no reclamation and is considered by farmers as a minimal risk crop as it is least affected by drought, pest and disease infestations and flooding (Zulpilip *et al.*, 1991). Having recognized its great commercial potential, the Land, Custody and Development Authority (LCDA) in Sarawak established PELITA Estate in 1987 to plant sago commercially. In addition, a Crop Research and Application Unit

(CRAUN) set up in 1994, carries out research and development work on the sago palm (Jong, 1995; Hassan, 2002).

Based on a report by LCDA (Jackson, 2007), Sarawak general manager, Abdullah Chek Sahamat said the current output of sago flour is 47,000 metric tonnes per year and the state's revenue is expected to increase from RM36 million per year to RM2.5 billion per year by the year 2015. As there is a strong and growing demand for sago starch in Malaysia mainly in the manufacture of monosodium glutamate, glucose and paper products, besides yeast, small foods, textiles and laundry industries (Tan *et al.*, 2002) it is timely that more diversified research on the use of sago starch be undertaken.

To expand the usefulness of starch, a variety of techniques have been used to modify the characteristics of these starches. Food processors have an adaptable tool in modified starches to meet specific requirements of a variety of food systems (Wurzburg and Szymanski, 1970). Native starch has a complex semi-crystalline structure which requires energy to gelatinize it. Physical modification alone or together with chemical reaction has been applied to change the granular structure and convert native starch into cold water-soluble starch (Jane, 1992). This type of starch modification is relatively new and has been used for microwave-cooked and instant foods such as puddings, instant fillings, sauces and dry mixtures that can be reconstituted with cold or ambient temperature liquids. Cold-water-soluble granular starch for gelled food compositions using corn starch was produced by Eastman and Moore (1984). Here granular corn starch, slurried in selected aqueous alcohols were subjected to conditions of high temperature and pressure. Rajagopalan and Seib (1992a) further prepared granular cold-water-soluble starches by heating a starch slurry in a mixture of water–polyhydric alcohol at atmospheric pressure using wheat,

corn, potato, tapioca and mung bean starches. In 1991, Jane and Seib had patented a method for preparing granular cold water swelling/soluble starches by alcoholic-alkali treatments. This process can be used on waxy, high amylose, tuber and normal starches. Alcoholic-alkaline treatment of starches to produce granular cold-water-soluble corn, waxy corn and high amylase corn starches was carried out by Chen and Jane (1994a). Subsequently, granular cold-water-soluble banana starch was prepared and studied by Bello-Perez *et al.* (2000) using the alcoholic-alkaline method. The most recent study on granular cold water soluble corn and potato starch was carried out by Singh and Singh in 2003.

Food being an ever evolving entity, it forever craves new innovations. The work on granular cold-water soluble sago starch was thus undertaken in view of the need to be self-sufficient, our country needs to fully-utilise its raw materials. The worldwide surge in commodity prices has rekindled a resurgence of interest in underutilized sources of plant starch. Among the not so actively researched sources is sago palm. This is compounded by the global surge in demand for food. With this in mind the current research was undertaken where the potential use of this granular cold water-soluble starch can be seen in instant foods as the trend in consumer consumption continues to grow in this direction.

1.2 Specific Objectives

The aim of this research was to create a novelty starch using sago starch as the raw material and further enhancing the usage of sago starch. As research on cold water-soluble starches is relatively new and literature on it is scarce, the present study of producing granular cold water-soluble sago starch was undertaken. There is at present no known work on the study of the reaction of alcohol and alkali on the

sago starch granules. Therefore, the development of granular cold water-soluble sago starch was aimed at developing a background understanding on the interaction of alcohol and alkali on the internal structure of the sago starch granule. As sago starch gelatinises at a lower temperature compared to other starches, it will be an interesting undertaking to see if it suffers from cold gelatinization in the presence of an alkali. The challenge would be to have the granular structure intact. Any change in internal structure will bring about a whole dimension of new possibilities in terms of physico-chemical behaviours. Cold water-solubility of sago starch was induced at varied parameters to have a better understanding on the behaviour of the granules at different levels of modification. Having produced cold water-soluble starch, the characteristics of this starch was studied in comparison to native sago starch and corn starch as corn starch is the most widely used and researched starch.

Therefore the specific objectives of this research were:

i) To study the interaction between concentration of sodium hyroxide (NaOH), concentration of alcohol and temperature on the cold water-solubility, clarity and viscosity of sago starches using RSM.

ii) To produce cold water-soluble sago starch with varying degrees of cold water-solubility by varying the concentration of NaOH.

iii) To study the characteristics of the cold water-soluble sago starches produced.

iv) To develop an understanding on the interaction of alcohol and alkali and its resultant effect on the internal structure of the sago starch granule that brings about the changes in the physico-chemical properties in comparison to native sago starch and to identify the

physico-chemical properties that will act as the basis for further development of an instant cooking product using granular cold water-soluble sago starch.

CHAPTER 2 – LITERATURE REVIEW

2.1 The Sago Palm

2.1.1 Origin

Sago palm is a hapaxanthy (once-flowering), monocotyledonous plant belonging to the family *Palmae* Jussieu, subfamily *Calamoideae* Griffith and genus *Metroxylon* Rottbuell (Uhl & Dransfield, 1987; Sukri,1992). *Metroxylon*, derived from the Greek language means "pith" for 'metra' and "xylem" for 'xylon' (Flach, 1977; Singhal *et al.*,2008).

The *Metroxylon*, a Malesian domesticate originating from Maluku-New Guinea (Tan, 1983) has been the most widely known and exploited palm for consumption, found growing from the Santa Cruz islands in the east to South Thailand in the west, from the Kai-Aru islands in the south to Mindanao in the north (Avé,1977). The *Metroxylon sagu* Rottb. has been the most important of several palm species for starch production in the Malay Archipelago (Abd-Aziz, 2002)

Sago palm grows and thrives well in peat swamp rainforest as it is tolerant of low pH, high Al, Fe and Mn in the soil, soil salinity and heavily impermeable clay. Thus it's advantage in being able to thrive in under-utilized land resources in tropical countries (Ng, 2007).

The vegetative phase in the sago palm lasts 7-15 years during which time starch accumulates in the pith core of the sago palm stem (Cecil *et al*., 1982; Kraalingen, 1986). Pei-Lang *et al.* (2006) found the starch content to increase from the mature vegetative growth to the flowering stage (10 to 13 years of age from planting) and decrease from the fruiting stage (14 years of age from planting) onwards. Maximum starch content occurs just before the flowering stage (Singhal *et*

al., 2008). The palm begins to use the starch it stored earlier at flower initiation. Therefore the palm should be harvested just before flower initiation if a high yield of starch per unit area is wanted. This is the normal practice where sago is cultivated. However, in wild stands where the palms are plentiful, the palm is harvested just before the fruit forms so as to get the highest starch yield per palm (Oates and Hicks, 2002). The timing for the felling of the palms can still be disputed as the general belief in Indonesia and Sarawak is that the harvesting of the sago palm is best done after the flowering but before the fruiting stage (Tan, 1982).

2.1.2 Extraction of sago starch

The domestic method is usually practised by the individual farmer. The sago palm is felled in the forest with an axe and then split lengthwise. The exposed pith is then rasped into pulp using a chopper or a small hoe. This allows easy removal of the starch. A worn-out wood-working axe or a stone carved specifically as a pith-chopper is used (Rhoads, 1977). Starch is then washed out using bowls and cloth.

A tool for extracting sago was developed in Malaysia at the end of the 19th century which was made up of a flat wooden board studded with nails. This tool produced fine pulp which in return gave a higher yield of starch. It was also easy to use. Sago palms were cut into shorter lengths and transported via rivers to purpose-built extraction platforms which were erected over rivers. The pulp was trampled on a woven rattan mat. The milk passing through the mat was filtered using a coarsely woven cloth. A disused dugout canoe was used to let the starch to settle out. The starch obtained was sun-dried in large shallow circular baskets (Cecil, 2002).

Currently, the most technologically advanced extraction processors in the world are found in Malaysia. There are nine fully mechanized factories operating in

Sarawak (Manan *et al.*, 2003). The logs are split lengthwise into 8 segments and fed into slicers that separate the pith from the bark. The pith is then chopped into chips which are then disintegrated with a pin mill. Water is added and the slurry obtained is made to pass through a series of centrifugal sieves to separate the coarse fibre, which is then sent to a screw press to extract out the water that still contains a large amount of starch. The starch water is returned to the process to lower the starch losses. Starch obtained is often bleached with an acceptable food additive. It is further purified in a nozzle separator. Dewatering of the starch is carried out using a basket centrifuge or a rotary vacuum filter. It is dried in hot air using a flash drier to a product that has a moisture content of between 12 to 14% (Oates and Hicks, 2002).

2.1.3 Utilisation of sago starch

Sago starch has been an important source of dietary fibre in the Asia –Pacific region and the main carbohydrate source in Malaysia (Douglas and William, 1984). In Sarawak, extraction of 175 kg of sago starch per palm, which is equivalent to 25 tons of sago starch/ha is possible in well managed farms. Sago starch is produced at a rate of 300 million tones per year here (Pushpamalar *et al*., 2006).

The rapidly increasing awareness of the importance of sago palm has brought about a considerable amount of research on sago starch. Every part of the palm has found a use in the human world as shown in Table 2.1.

Table 2.1 Utilisation of sago starch

Sago palm part	Usage/Utilisation
Refined sago starch	An ingredient of noodles, vermicelli, Kuah-Tiau, biscuits and many other foods Used industrially in products such as monosodium glutamate, glucose, caramel, fructose, syrups, derivatised into oxidised starches and used as coatings in the paper industry, dialdehyde starches and used in the paper industry and also as ethers and esters and used in the pharmaceutical industries.
Sago fiber	Provides bulk for rumen fermentation and used as animal feed.
Sago pitch	Used as an animal feedstuff and in the livestock industry
Sago fronds	Used in the pulp and paper industries and also as thatching

(Abd-Aziz, 2002, Singhal *et al*., 2008)

Sago starch has been found to easily gelatinize as its gelatinization occurs at a low temperature, it has a high viscosity and is easily moulded besides having a low syneresis. All these properties make it a possible ingredient in food cooking and processing (Takahashi, 1986). It has been used in the making of jellies, puddings and sweet desserts with sago pearls and also as a thickener in the making of soups and baby food (Zulpilip *et al*., 1991; Takahashi, 1986).

The potential of sago starch in the production of biodegradable fillers in the plastic industry has been studied by Griffin (1977). This has been followed by more work on it by Pranamuda *et al.* (1998) and Ishiaku *et al.* (2002).

Table 2.2 shows some of the characteristics of sago starch as compared to some other starches.

Table 2.2: Characteristics of the starch granule from various sources

Starch	Diameter microns (μm)	Morphology	Gelatinisation temp. ^{o}C	Pasting temp.^{o}C (a)	Amylose content	Cooked properties
Maize (b)	5-30	Round Polygonal	62-72	80	25	Opaque gel
Waxy maize	5-30	Round Polygonal	63-72	74	< 1	Clear cohesive
Tapioca	4-35	Oval Truncated 'kettle drum'	62-73	63	17	Clear cohesive, tendency to gel
Potato	5-100	Oval Spherical	59-68	64	20	Clear cohesive, tendency to gel
Wheat	1-45	Round Lenticular	58-64	77	25	Opaque gel
Rice	3-8	Polygonal Spherical Compound	68-78	81	19	Opaque gel
Sago	15-65	Oval Truncated	69-74	74	26	Opaque gel
High Amylose Maize	5-30	Polygonal Irregular Elongated	63-92(c)	> 90	50-90	Very opaque, very strong gel

(a) Measured for 5% starch suspension.
(b) Maize is also often referred to as 'corn',' dent corn' or 'regular maize'.
(c) High amylose maize starches are not completely gelatinised in boiling water.
(Murphy, 2000)

2.2 Starch

In the process of photosynthesis, leaves trap light energy and through a series of physico-chemical processes involving carbon dioxide and water produce sugar molecules such as glucose. As glucose is too mobile for long term storage it is

immobilised by forming a polymer whereby glucose chains are linked together by the condensation of water (Murphy, 2000). The synthesis of starch occurs in the amyloplast of the plant and deposited in the form of granules. It acts as a store of carbon and energy for plants. Starch has no structural function in plants but in food it acts as a structuring agent due to transformations during processing (Conde-Petit, 2001). During digestion, the trapped energy is released as the starch is broken down by hydrolysis back to glucose molecules and further back to the original carbon dioxide and water (Murphy, 2000).

Starch contributes about 70-80% of calories consumed by humans. Starch granules can be found in many parts of plants which include pollen, leaves, stems, woody tissues, roots, tubers, bulbs, rhizomes, fruits, flowers and the pericarp, cotyledons, embryo and endosperm of seeds (Shannon and Garwood, 1984). Commercial starches are obtained from corn, waxy corn, high-amylose corn, wheat, various rices, potato, sweet potato and tapioca (Whistler and BeMiller, 1997).

Starch granules are water-insoluble, quasi-crystalline, dense and in the presence of iodine give a characteristic blue stain (French, 1984; Biliaderis, 1998). Starch granules also vary in size and shape as can be seen in Table 2.2. Cereal starches are known to be generally small and polyhydric whereas tuber starches are large and ellipsoidal or spherical. Starch granules can vary in size from less than 1 μm to more than 100 μm. Most starches are known to show a single size distribution (unimodal) with the exception of wheat, barley and rye granules which show two separate distributions (bimodal). For example, wheat starch has large, oval granules of about 35 μm and smaller, spherical granules of about 3 μm in diameter. There are also compound starch granules found in oats and rice, where small, individual granules are bound together in clusters (Biliaderis, 1998; Thomas and Atwell, 1999;

Hoover *et al.*, 2003). Particle size analysis has been done using various methods which includes sieving, sedimentation, electrone sensing using the Coulter Counter, microscopy and laser diffraction technique (Rawle, 2007).

Starch granules are made up of a mixture of two polymers i.e. amylose and amylopectin and also minor constituents such as lipids, proteins, phosphate and ash that can affect the functional properties of starch in various applications (Banks and Greenwood, 1975; French, 1984; Biliaderis, 1998).

2.2.1 Amylose and amylopectin

Starch is made up of two fractions: amylose and amylopectin and the overall behaviour of starch is determined predominantly by the relative amounts of these two fractions. Each fraction has unique properties that attribute differently to the functionality of starch.

Figure 2.1 shows the structure of amylose and amylopectin which is essentially made up of glucose units. Some of the properties of amylose and amylopectin are shown in Table 2.3.

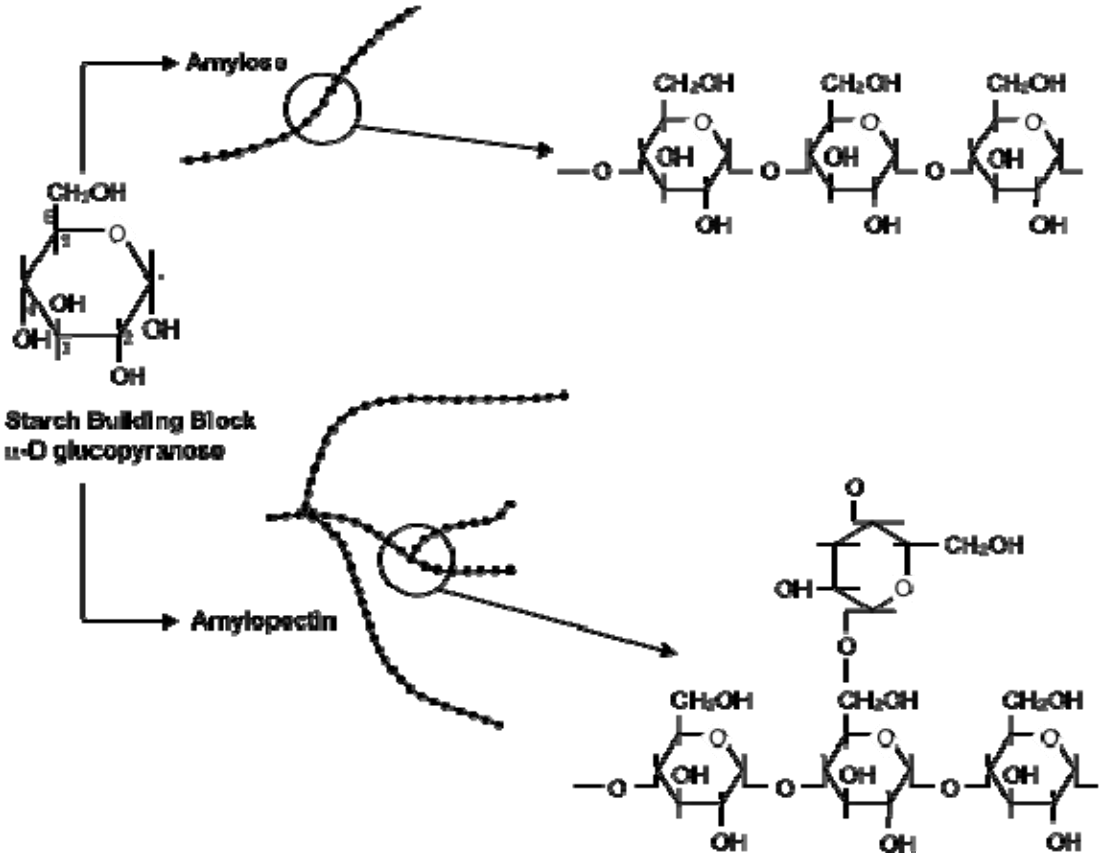

Figure 2.1: Structures of amylose and amylopectin
(Adapted from, Murphy, 2000).

Table 2.3: Properties of amylose and amylopectin

Property	Amylose			Amylopectin	Intermediate fraction
	Whole	Linear	Branched		
Branched linkage (%)	0.2-0.7	0	0.2-1.2	4.0-5.5	2-3.5
Average chain length (CL)	100-550	800	140-250	18-25	30-50
Average degree of polymerization (DP)	700-5000		10^3-10^4	10^4-10^8	10^2-10^4
λ_{max} (nm)	640-660			530-570	570-580
Blue value[b]	1.2-1.6			0-0.2	0.3-0.7
Iodine affinity (g per 100 mg)	19-20.5			0-1.2	2-10
Helix formation[c]	Yes	Yes	Yes	No	No
β-Amylolysis limit	70-95	100	40	55-60	57-75

[a] Data from Hizukuri (1996).
[b] Blue value: absorbance at 680 nm of the iodine complex in controlled conditions.
[c] With 1-butanol.
(Sivak and Preiss, 1998)

Amylose is essentially a linear chain made up of α-(1→4) D-glucopyranosyl units, with about 0.3-0.5% of α-(1→6) branches (Hizukuri *et al*., 1981; Curá *et al*., 1995; Whistler and BeMiller, 1997, Biliaderis, 1998). These branches are either very short or very long and separated by large distances.

There are about 1.8 x 10^9 amylose molecules per average starch granule (Buléon *et al.*, 1998a). Hizukuri *et al*. (1981) discovered that there are about 9 – 20 branch points per molecule and the chain length of the side chains are from 4 to over 100 glucosyl units. The extent of branching increases with the molecular size of amylose and is reflected in the susceptibility of amylose to hydrolysis by β-amylase, which can vary between 73% to 95%, depending on the extraction procedure and origin of amylose (Banks and Greenwood, 1975). Amylose has a molecular weight (M_w) that varies from 2.0 x 10^5 and 1.2 x 10^6, with polydispersity indices (M_w/M_n) between 1.3 and 5.8 (Banks and Greenwood, 1975; Hizukuri *et al.*, 1989; Roger and Colonna, 1993). In normal starch granules amylose is always considered to be amorphous although studies show that high amylose starches have the capability to contain amylose double helices which cause crystalline structures (Banks and Greenwood, 1975; Tester, 1997).

Amylose has a helical shape due to the axial-equatorial position coupling the α-(1→4) D-glucopyranosyl units. The interior of the helix is lipophilic as it contains mostly hydrogen atoms whereas the exterior coil has hydroxyl groups positioned on it (Whistler and BeMiller, 1997). A typical helical conformation is shown in Figure 2.2. This ability to form helical inclusion complexes gives rise to the typical deep blue colour in the presence of polyiodide ions. A variety of polar and non-polar ligands are also induced in the amylose aqueous solutions due to the coil → helix transitions. Although slightly branched, amylose behaves like a linear polymer and forms films and complexes with ligands (Banks and Greenwood, 1975; Biliaderis, 1998).

The nutritional and technological properties such as susceptibility to enzymatic hydrolysis, gelling and pasting behaviour as well as retrogradation is

known to be influenced by the amylose content (Thomas and Atwell, 1999). Amylose is unstable in dilute aqueous solutions but is able to solubilise in 0.5 mol/L KOH, DMSO and formamide. The gels formed are firm and irreversible (Cornell, 2004).

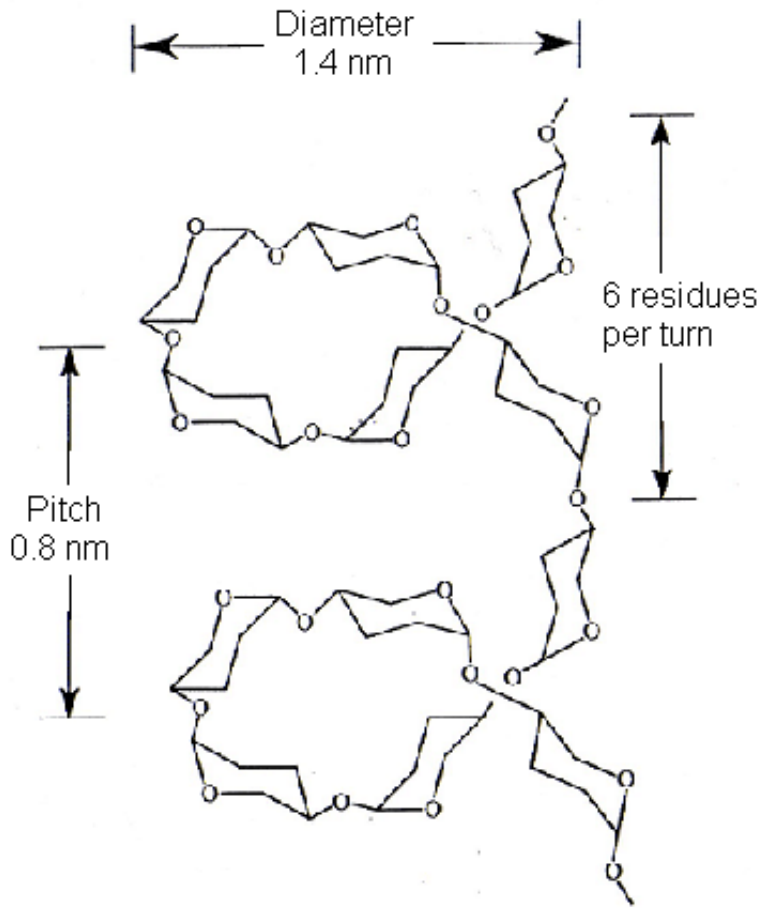

Figure 2.2: Helical conformation of amylose, a left-handed helix containing six anhydroglucose units per turn (Cornell, 2004).

Amylopectin, which is the major component of most starches is made up of a large number of short chains linked together at their reducing end side by a α-(1→6) linkage. Amylopectin has only one free aldehyde (reducing) group in each molecule (Thomas and Atwell, 1999; McWilliams, 2001). The molecular weight of amylopectin is between 10^7-10^9 and is one of the largest naturally occurring polymers (Banks and Greenwood, 1975). The average size of the unit chains is 20-25 and there are several distributions of chains differing in chain length (Hizukuri, 1986). The extensive branching restricts the β-amylolysis to about 55-60% only which is significantly less than that for amylose. The polydispersity indices (M_w/M_n) for amylopectin are about 300 which indicate a wide distribution of molecular sizes (Biliaderis, 1998). Due to the lack of helical configuration within the amylopectin, the iodine test gives only a purplish-red colour when the fraction is amylopectin (Hizukuri, 1996; McWilliams, 2001).

The structure of the amylopectin has evolved from the one initially proposed by Haworth (1937) and Staudinger (1937) to the currently accepted structure proposed by Hizukuri, 1986 and these are shown in Figure 2.3. Initially the structure was analysed by way of methylation and osmotic pressure or viscosity measurement which gave rise to the laminated and comb-like models of Haworth (1937) and Staudinger (1937). In 1940, Meyer *et al*., managed to separate amylose and amylopectin and through chemical, physical and enzymatic analysis, proposed the randomly branched, bush-like structure. Repeated hydrolysis of amylopectin with β-amylase caused a reduction in the yield, thus the deduction of a bush-like structure. However, questions arose regarding the irregular structure of amylopectin which made Whelan (1971) propose a revised structure of amylopectin, thus the depiction of the cluster model.

The cluster structure for amylopectin was further studied by French (1972). The high viscosity of amylopectin and the possibility of building high molecular weight amylopectin (10^7-10^8 g/mol) by increasing the number of clusters was explained by this model. The exterior chains of these molecules are said to be double helices within the clusters that form the crystalline domains that are interspersed with amorphous regions (Kainuma, 1988; Tester and Karkalas, 2002).

Studies by Hizukuri (1986) using enzymes to debranch amylopectin and high performance liquid chromatography (HPLC) to determine the branch size distribution gave rise to the currently accepted amylopectin structure as shown in Figure 2.3. The chains of amylopectin are classified into A, B and C chains. The non-branched chains are the A chains, chains branched at the C-6 positions are the B chains and the single C chain also known as the "backbone" of the amylopectin molecule, is the only chain having one reducing residue. To characterize the mode of branching of the amylopectin it is useful to know the ratio of the A to B chains. (Hizukuri, 1986; Kainuma, 1988; Hizukuri *et al.*, 1997; Tester and Karkalas, 2002). The amount of maltose and maltotriose released from β-amylase limit dextrin by pullulanase is used to determine the ratio of A:B chains (Kainuma, 1988), with studies by Manners (1985) concluding that the ratio lies within the range of 1:1 to 1.5:1. The chain lengths (CL) of A, B_1, B_2, B_3 and B_4 chains are in the range of 12-16, 20-24, 42-48, 69-75 and 101-119 respectively (Hizukuri, 1986; Hizukuri 1988). The most external chains, A and B_1 chains form double helices within the native granules (Tester and Karkalas, 2002).

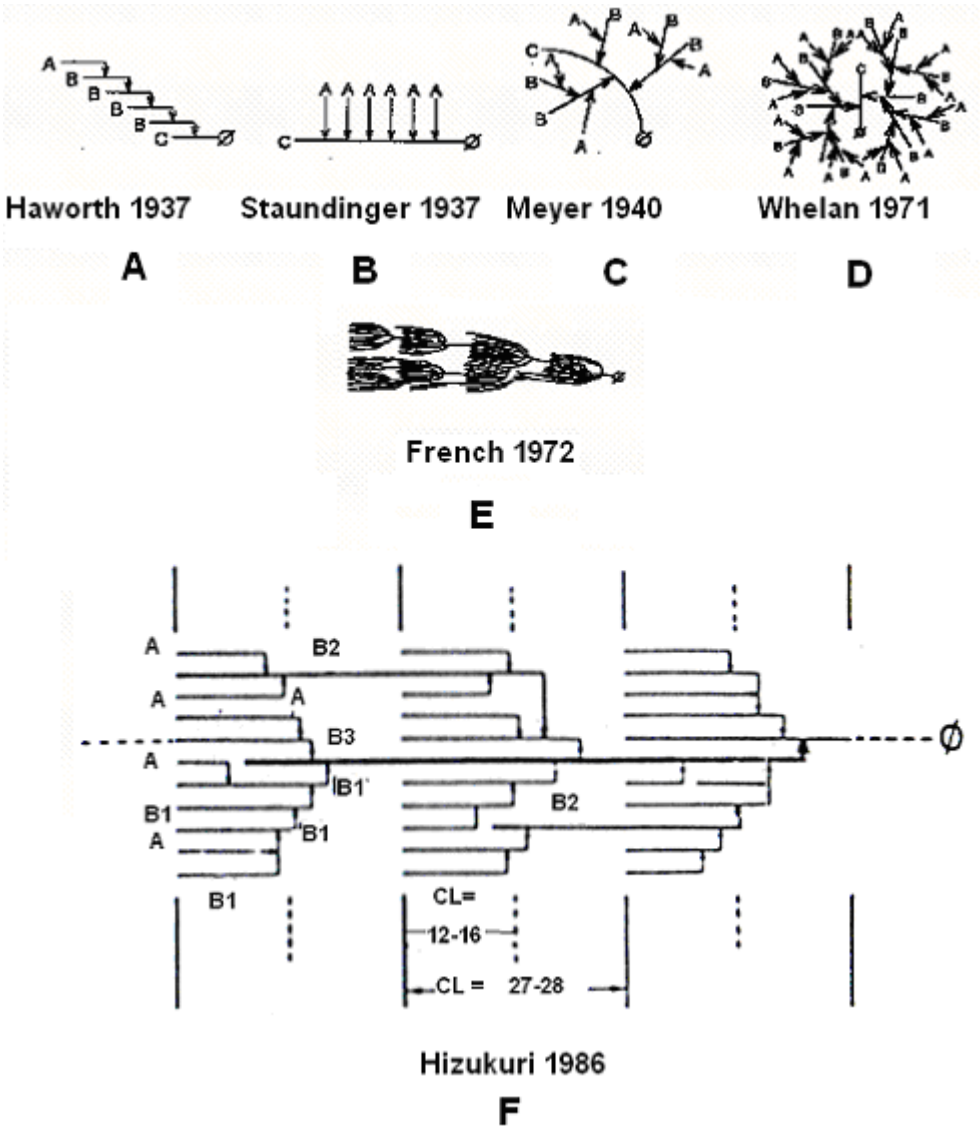

Figure 2.3: The evolvement of the amylopectin structure from the Haworth (A), Staudinger (B), Meyer (C), Whelan (D), French (E) and the currently accepted Hizukuri structure (F). (Hizukuri, 1996).

2.2.2 Structure and organisation

The general formula of starch, $(C_6H_{10}O_5)_n$, came about as a result of some hard and dedicated work of Standinger (1932) and Carothers (1940). Starch granules are made up of concentric layers of amylopectin molecules interrupted by some amylose molecules, arranged in an organized manner within the layers as growth rings and can range in size from 1 200Å and 4 000Å (French, 1984; Jenkins *et al*., 1993).

A model of the starch granule structure is shown in Figure 2.4. Ultrastructural studies have shown that the growth rings are made up of alternating crystalline, which represent the double-stranded helices of short degree of polymerization chains of amylopectin, and amorphous lamellae (Figure 2.4a) (French, 1984). These growth rings are formed due to the recurrence of biosynthesis. The dense layer in the growth rings is made up of about 16 layers of alternating crystalline (5-6 nm) and amorphous (2-5 nm) lamellae (Figure 2.4b) (Cameron and Donald, 1992; Jenkins *et al.*, 1994). Starch granules are partially crystalline with a degree of crystallinity between 20-40% (Hizukuri, 1996). Periodicity has been used to describe the repeated distances of the crystalline and amorphous lamellae (Yamaguchi *et al*., 1979) and using small-angle X-ray and neutron scattering, a periodicity of 9-11 nm has been found for starches from various botanical sources (Oostergetal and Van Bruggen, 1989; Cameron and Donald, 1992; Jenkins *et al*., 1994). Amylopectin double helices are packed in a parallel way in the crystalline lamellae which forms the backbone of the starch granule whereas the amorphous lamella contains the amylopectin branch points as shown in Figure 2.4c (Gallant *et al.*, 1997; Jacobs and Delcour, 1998).

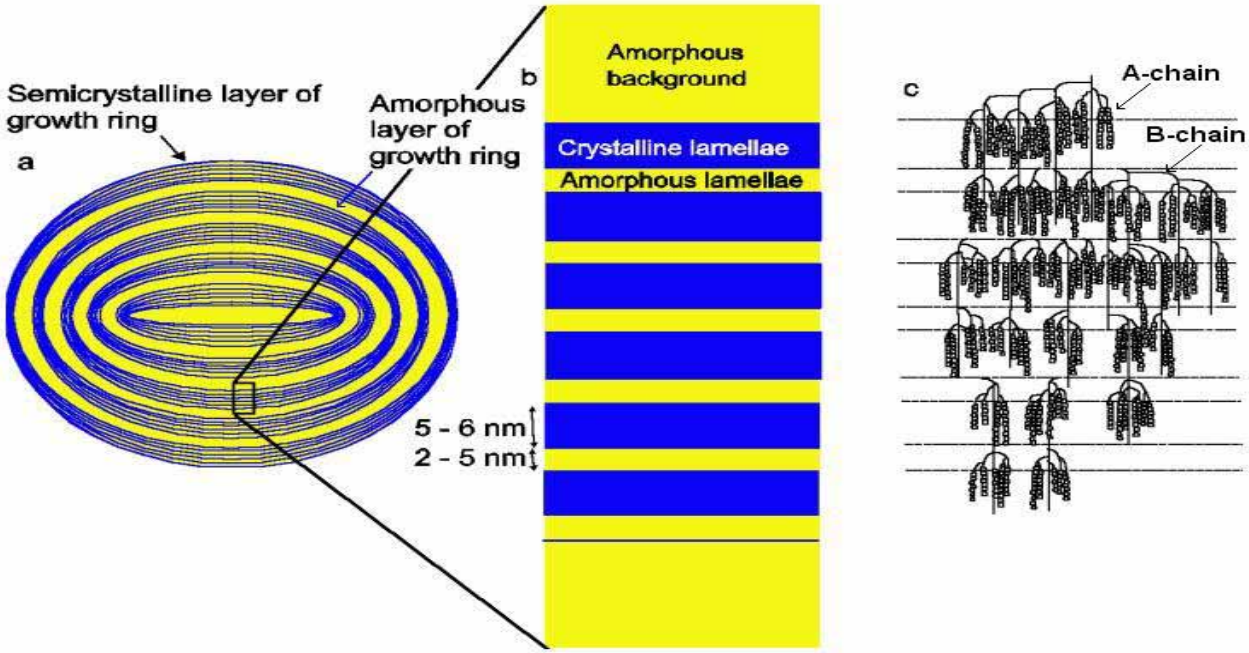

Figure 2.4: A schematic representation of starch granule structure (Jenkins *et al.*, 1994)

Gallant *et al*., (1997) have recently proposed the blocklet concept of the starch granule structure as shown in Figure 2.5. Evidence from scanning electron microscope (SEM), transmission electron microscope (TEM), enzyme degradation studies and atomic force microscope (AFM), has indicated the crystalline and amorphous lamellae of the amylopectin are organized into larger, more or less spherical structures called blocklets with diameters from 20 to 500 nm depending on botanical source and locality in the granule. The presence of blocklet structures have also been confirmed by Baker *et al.* (2001) and Ridout *et al.* (2002). The size of the blocklet and its arrangement has been found to be a factor in starch resistance besides amylose content, location and interaction with amylopectin. As the amylopectin content is higher than amylose in native starches and the double helical order in the granule significantly higher than the level of granule crystallinity, it can be inferred that amylopectin can be found in both the crystalline and semi-crystalline regions (Gidley and Bociek, 1985; Gallant *et al*., 1997). In this type of granule organisation, the amorphous region can exist in different regions: (1) in each lamella where the branching zones of amylopectin are found; (2) between clusters of side chains within each lamella; (3) around each blocklet of side-chain clusters and (4) in radially arranged channels in granules making the exit of amylose during gelatinisation possible (Biliaderis, 1998).

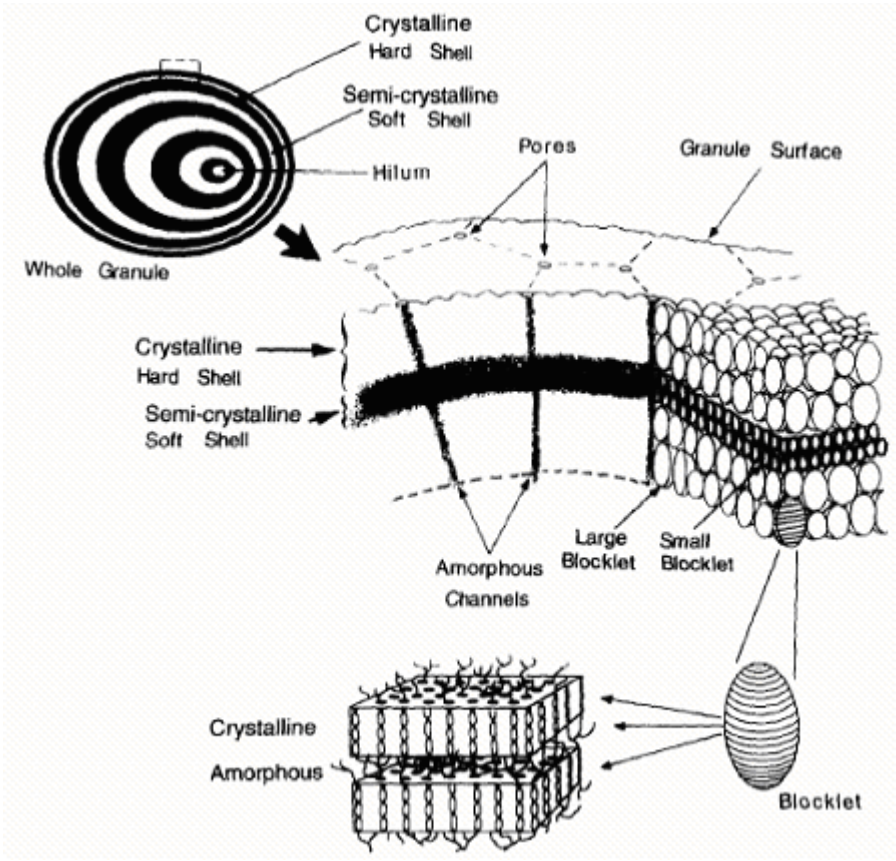

Figure 2.5: Starch granule structure as proposed by Gallant *et al*. (1997)

Further research by Tang *et al.* (2006), on the starch structure has brought about a new perspective on the position of the blocklets in the starch structure as shown in Figure 2.6. The blocklet is a semi-crystalline structure consisting of several amylopectin molecules. It has been deduced that the reducing end of the amylopectin molecule in the blocklets is inclined towards the hilum of the granules. The semi-crystalline blocklets that form the basic units in the construction of the starch granules can be divided into two types, "normal" and "defect". The hard shell is made up of the normal blocklet and the soft shell of the defect blocklets. A normal blocklet constitutes the crystalline and amorphous lamellae that are found in the amylopectin molecules. A defective blocklet is produced when lower branching

molecules such as amylose and intermediate materials that cannot crystallize are installed in the blocklet ultrastructure. These blocklets may be arranged in two ways as heterogeneous shells and homogeneous shells. In a heterogeneous shell, the normal blocklets are interspersed among the defective blocklets in the soft shell, while the defective blocklets are interspersed among the normal blocklets in the hard shell. In the homogeneous shell, the normal blocklets and defective blocklets are continual with no disruption.

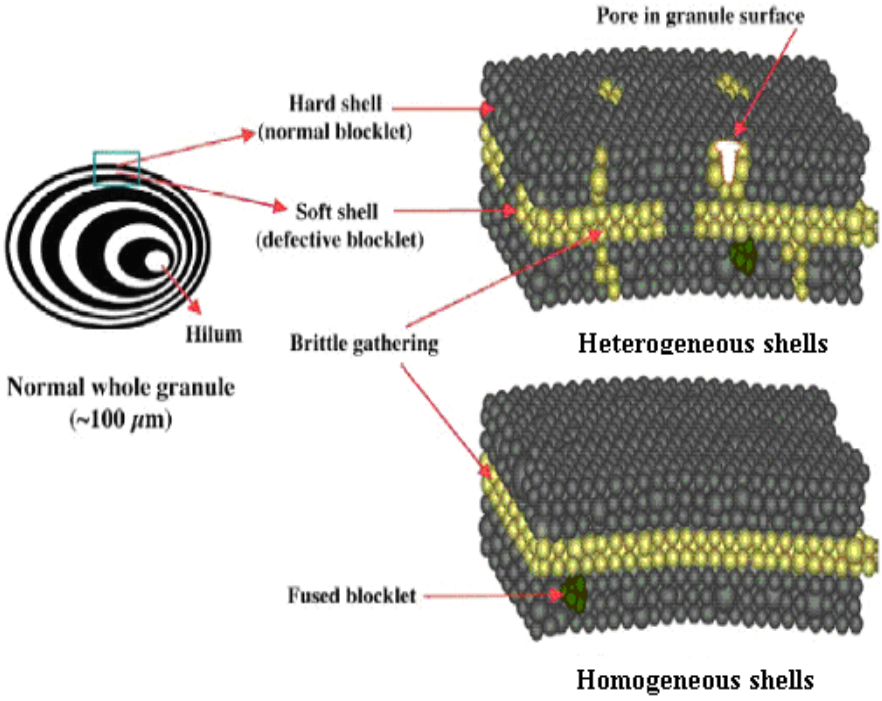

Figure 2.6: Starch granule structure (Tang *et al.*, 2006)