PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF ENZYME MODIFIED TAPIOCA STARCHES

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PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF ENZYME MODIFIED TAPIOCA STARCHES

by

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Abbreviations	Caption
HPSEC	High performance size exclusion chromatography
SEM	Scanning electron microscopy
NMR	Nuclear magnetic resonance
DSC	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_c	Conclusion temperature
T_c-T_o	Melting temperature range
ΔH	Enthalpy
G’	Storage modulus
G’’	Loss modulus
$\|\eta^*\|$	Complex viscosity

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SIFAT-SIFAT FIZIKOKIMIA DAN BERFUNGSI KANJI UBI KAYU TERUBAHSUAI SECARA ENZIMATIK.

ABSTRAK

Dalam kajian ini, proses penyahcabangan telah dijalankan ke atas kanji ubi kayu iaitu ubi kayu komersil (CT) dan variati Sri Kanji Satu (SK1) dengan menggunakan 10%, 20% dan 30% enzim pullulanase (v/wt kanji,db), pada suhu 58 ˚C selama 48 j. Sampel dikeluarkan pada pelbagai julat masa (0, 4, 8, 20, 24, 48 j) untuk analisis bagi peratusan hidrolisis dan purata darjah pempolimeran (DP). Daripada kedua-dua kanji, penyahcabangan menggunakan 30% pullulanase memberikan peratusan hidrolisis yang paling tinggi dan purata DP yang paling rendah menunjukkan lebih banyak penyahcabangan berlaku daripada tindakan pullulanase. Oleh sebab itu, fraksi kanji terubahsuai enzim (EMS) yang disediakan daripada 30% enzim telah dikaji secara lebih mendalam terhadap sifat-sifat fizikokimia dan berfungsi. Sifat-sifat fizikokimia, termasuk sifat pempesan dan termal telah dijalankan. Kromatogram yang diperolehi daripada 'high performance size exclusion chromatography (HPSEC)' menunjukkan puncak tambahan dalam fraksi EMS, iaitu kehadiran fraksi berberat molekul rendah. Daripada mikroskop penskanan electron (SEM), selepas proses penayhcabangan oleh pullulanase, retakan yang ketara telah diperhatikan pada granul kanji akibat daripada tindakan enzim. Didapati tiada perubahan pada corak pembelauan sinar-X untuk sampel fraksi EMS berbanding dengan kanji natif. Dengan peningkatan masa penyahcabangan, fraksi EMS menunjukkan

peningkatan ‘trend’ pada kuasa pembengkakan tetapi penurunan ‘trend’ pada keterlarutan. Pengukuran kalorimetri penskanan pembezaan (DSC) menunjukkan penyahcabangan enzimatik pada kanji ubi kayu mengakibatkan suhu puncak gelatinisasi (T_p) yang lebih tinggi berbanding dengan kanji natif. Sifat pempesan yang perolehi daripada ‘rapid visco analyzer (RVA)’ menunjukkan semua fraksi EMS memberi kelikatan pempesan dan nilai ‘setback’ (retrogradasi) yang lebih rendah berbanding dengan kanji natif. Selepas proses penyahcabangan, saiz partikel granul EMS 48j daripada SK1 menjadi lebih kecil secara signifikan. Ujian lubrikasi menunjukkan fraksi EMS mempunyai sifat-sifat pengganti lemak dengan membentuk pes yang licin, berkrim dan ‘opaque’. Keupayaan fraksi EMS untuk bertindak sebagai pengganti lemak juga dikaji dengan applikasinya ke dalam formulasi mayonis kurang lemak. EMS 20j daripada CT dan SK1 telah disediakan dalam keadaan sluri dan ditambah ke dalam enam formulasi mayonis pada tahap penggantian 25%, 40% dan 55% daripada minyak soya untuk menghasilkan mayonis kurang lemak (RF) dan dikenali sebagai ECT 25, 40 and 55 dan juga ESK1 25, 40 dan 55. Sampel-sampel ini dibandingkan dengan satu mayonis kawalan (CM) yang tanpa penggantian EMS dan dua mayonis komersil, iaitu mayonis penuh lemak (CFF) dan mayonis kurang lemak (CRF). Daripada kajian reologikal, ujian osilatori menunjukkan sifat-sifat gel lemah dan viskoelastik dengan ‘storage modulus (G’)’ lebih tinggi daripada ‘loss modulus (G’’)’ dalam semua sampel mayonis. Walaubagaimanapun, mayonis RF dengan penggantian EMS menunjukkan nilai G’ yang lebih tinggi berbanding dengan mayonis CM, CFF dan CRF. Nilai G’

didapati paling tinggi pada mayonis ECT 55 dan ESK1 55 berbanding dengan semua sampel mayonis yang lain. ‘Absolute complex viscosity (|η*|)’ pada mayonis RF meningkat dengan darjah penggantian EMS. Daripada ‘back extrusion test’ dalam analisis tekstur, mayonis RF dengan penggantian EMS menunjukkan nilai kekerasan dan kelekatan yang lebih tinggi berbanding dengan mayonis CM, CFF dan CRF. Mayonis RF pada darjah penggantian 55% memberikan nilai kekerasan dan kelekatan yang paling tinggi berbanding dengan mayonis RF pada darjah penggantian 25 dan 40%. Penilaian sensori pada sampel mayonis RF menunjukkan penerimaan keseluruhan di kalangan ahli panel sehingga tahap penggantian 40% dengan tiada perubahan signifikan berbanding mayonis CFF dan CRF. Amaun kalori pada mayonis RF menurun secara signifikan ($p<0.05$) sebanyak 44% berbanding sampel mayonis CM. Keputusan ini menunjukkan keupayaan potensi EMS untuk bertindak sebagai pengganti lemak dalam produk makanan seperti mayonis.

PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF ENZYME MODIFIED TAPIOCA STARCHES.

ABSTRACT

In the present study, debranching process was carried out on tapioca starches namely commercial tapioca (CT) and Sri Kanji One variety (SK1) using 10%, 20% and 30% of pullulanase enzyme (v/wt starch, db), at 58 ˚C over a 48 hr period. Samples were withdrawn at various time intervals (0, 4, 8, 20, 24 and 48 hr) for analysis of percent hydrolysis and average degree of polymerization (DP). In both starches, debranching using 30% pullulanase gave the highest percent hydrolysis and lowest average DP indicated more debranching occurred through the action of pullulanase. Thus, enzyme modified starch fractions (EMS) prepared from 30% pullulanase enzyme was further investigated for their physicochemical and functional properties. The physicochemical properties, including pasting and thermal properties were studied. Chromatograms obtained from high performance size exclusion chromatography (HPSEC) showed additional peak in EMS fractions which correspond to lower molecular weight fractions. From scanning electron microscopy (SEM), after debranching process by pullulanase, pronounced cracks were observed in starch granules indicative of enzyme attack. X-ray pattern of EMS fractions remained unchanged compared to native starches. Upon debranching time, EMS fractions displayed an increasing trend in swelling power but decreasing trend in solubility. Differential scanning calorimetry (DSC) measurements revealed that enzymatic debranching of

tapioca starches resulted in higher peak temperature of gelatinization (T_p) compared to their native starches. Pasting properties obtained from rapid visco analyzer (RVA) showed that all EMS fractions exhibited lower pasting viscosity and setback values (retrogradation) as compared to their native starches. After debranching process, granular particle size of EMS 48h of SK1 became significantly reduced. Lubricating test showed that EMS fractions showed fat mimetic properties by forming a smooth, creamy and opaque paste. The potential use of EMS fractions as fat mimetic was also evaluated by applying them in reduced fat mayonnaise formulations. EMS 20h from CT and SK1 were prepared in a slurry form and used in the preparation of six formulations of mayonnaise at 25%, 40% and 55% substitution of soybean oil to produce reduced fat (RF) mayonnaise abbreviated as ECT 25, 40 and 55 and also ESK1 25, 40 and 55. These samples were then compared to a control (CM) mayonnaise without substitution of EMS and two other commercially prepared mayonnaise, full fat (CFF) mayonnaise and reduced fat (CRF) mayonnaise. From the rheological studies conducted on these samples, oscillatory tests showed weak gels and viscoelastic properties with storage modulus (G') greater than loss modulus (G'') in all mayonnaise samples. However, RF mayonnaise with EMS substitution gave higher G' values compared to CM, CFF and CRF mayonnaise. G' values were found to be highest in ECT 55 and ESK1 55 mayonnaise compared to all other mayonnaise samples. The absolute complex viscosity ($|\eta^*|$) in RF mayonnaise increased with degree of substitution of EMS. From back extrusion test in texture analysis, RF mayonnaise with substitution of EMS gave higher

firmness and adhesiveness values compared to CM, CFF and CRF mayonnaise. RF mayonnaise at 55% substitution level gave highest firmness and adhesiveness values compared to samples at 25% and 40% substitution level. Sensory evaluation of RF mayonnaise showed overall acceptability by a panel of up to 40% substitution level with no significant difference compared to CFF and CRF mayonnaise. The amount of calories was found to decrease significantly ($p<0.05$) by 44% in RF mayonnaise compared to CM mayonnaise. These results indicate the potential ability of the EMS to behave as fat replacers in food products such as mayonnaise.

CHAPTER ONE

INTRODUCTION

1.1 Background

Starch is an essential source of carbohydrate in human diet. In addition to its nutritive value, starch plays an important role to impart certain functional properties such as viscosity and texture. It has contributed a great economic importance and is isolated on an industrial scale from various sources of starch such as tapioca, corn, potato, rice, wheat and others. Although native starch does have its industrial applications, most of the time the industry require improved functionality from modified starch. This research is focused on the production of enzyme modified starch using pullulanase enzyme. Starch consists of amylose and amylopectin. Amylose is essentially a linear molecule with degree of polymerization (dp) of about 1,500 - 6,000 glucose unit and is connected through α-(1→4) linkages. Amylopectin, the predominant molecule in most normal starches, is a branched polymer which is much larger than amylose. DP of amylopectin is about 300,000-3,000,000 glucose unit and is composed of α-(1→4) linked D-glucose segments connected by α-(1→6) linked branched points. The enzyme, pullulanase attacks α-(1→6) linked branched points to produce more linear fractions (amylose). According to Chiu and Mason (1995), these enzyme modified starch containing more linear fractions have shown to have unique functional properties such as fat replacers.

Starches with high linear fractions (amylose content) are able to bind strongly and orient water in such a way as to provide a sensation similar to the

rheology of fat in the oral cavity. In food product with formulations high in fat or oil content, high linear fractions starches can act as an alternative to replace oil to produce lower calorie food product. Low fat meat patties, frozen desserts, dairy products, sauces and gravies were successfully formulated by using modified food starch together with other hydrocolloids (Giese, 1992; Yackel and Cox, 1992; Zoulias *et al*., 2002). High linear fractions starches are believed to possess the ability to form good edible films which have good barrier against oxygen and fats (Anonymous, 1997; Krochta and Mulder-Johnston, 1997). Fried foods coated with high linear fractions starches absorb less oil and make the fried food become less greasy. Amylose, the linear fraction of starch, is known to form coherent, relatively strong, free-standing films in contrast to amylopectin films which are brittle and non-continuous (Gennadios *et al*., 1997). Edible films and coatings prepared from high linear fractions starches are used in dried fruits, candied fruits and deep fried potato products (Krochta and Mulder-Johnston, 1997).

Fat replacer can be a promising additive in the future. This is because society today is very much concern with healthy food. As we know, obesity is the side effect of excessive intake of fat. Obesity can link to several chronic disease including cardiovascular disease and Type 2 diabetes. Production of high linear fractions tapioca starch can serve as an alternative choice to food developers. Furthermore, high linear fractions tapioca starch produced can boost our country's economy by exporting it to overseas and reduce the import of other fat replacers.

Using tapioca starch to produce fat replacer has several advantages. Food developer is always aiming for improved functionality ingredient but at the same time, the cost of an ingredient still remains as an important factor due to cost constraint. Starch being the cheapest form of polysaccharides has become the choice for food product innovation by the food industry. Even though modified starch is more expensive than native starch but its price is still considered reasonable compared to other hydrocolloids, according to a survey done by Williams and Phillips (2000).

Under European Union labeling regulation, most of the chemically modified food starches that have been approved as a food additive have to be assigned with E-number classification. Modified starches which are categorized as additives are defined as ”substances obtained by one or more chemical treatments of edible starch which may have undergone a physical or enzymatic treatment and may be acid- or alkali-thinned or bleached”. Thermally treated starches, native starches, dextrins, starch modified by acid or alkali treatment, bleached starch and starch treated by enzymes are categorized as food ingredients and have no E-number classification (Murphy, 2000). Thus, enzyme modified starches may appear more natural and appealing to consumers.

Apart from that, tapioca starch has the potential to serve as a fat replacer in food formulation because it forms a bland and clearer gel and is non allergen (gluten free), easy to digest and has ideal viscosity (Anonymous, 2006b). With these advantages, it is believed that high linear fractions tapioca starch has the

potential to compete with other fat replacers in the market not only as a food ingredient but also to serve as alternative yet economic fat replacers.

In addition, through this research, knowledge of the effect of pullulanase enzyme towards the properties of starch can be gained. Most researchers carried out debranching process on solubilised starch either solubilised with DMSO, NaOH or autoclave (Kimura and Robyt, 1996; Wang *et al.*, 1996; Kujawski *et al*., 2003). At present, to our knowledge, there are very few articles reported on debranching raw starch granule. Many previous studies investigate hydrolysis treatments with amyloglucosidase, α- and β-amylase towards raw starch granule to examine how these enzymes affect patterns of hydrolysis (surface erosion, endoerosion, and the erosion at the equatorial groove plane), x-ray diffraction pattern, gelatinization, granular size and degree of hydrolysis (Franco, 1988; Planchot *et al*., 1995; Wang *et al*., 1996; Sarikaya, 2000; Li *et al*., 2004). This study is expected to provide an insight of the action of pullulanase on raw starch granule and may become useful information to other scientific studies in the future.

There is a high demand for tapioca starch in Malaysia (Tengku Ahmad, 1998) as the total imports of tapioca had risen from RM 2.8 million to almost RM 47 million in 1995. Thailand is the world's largest exporter of tapioca starch and its derivatives (Anonymous, 2001). They produced various type of modified tapioca starch including hydrolyzed starch by enzyme, acid thinned starch, pre-gelatinized starch, oxidized starch, dextrinized starch, starch ether, starch ester and cross-linked starch. The Thai government had put a lot of effort on the

research and also policies in order to improve tapioca production, modification, application, and also local and international trading (Ratanawaraha *et al*., 2000). It is hoped that this research on the preparation of modified starch from tapioca starch could increased utilization of local tapioca starch as well as provide value-added food ingredients based on tapioca starch.

1.2 Objectives

The objectives of this project were to produce modified tapioca starch containing higher linear fractions (or amylose content) by using an enzymatic debranching process. The debranching was carried out on two varieties of tapioca starch. Studies on the properties of modified starch would be carried out using instrumental methods and chemical analysis. The debranched tapioca starch was applied as a fat replacer in a food model system such as in mayonnaise formulation.

The specific objectives of the research are:

i) To produce enzyme modified starch with high linear fractions from two tapioca starch varieties using pullulanase enzyme

ii) To study the effects of debranching enzyme on starch granule in terms of physical and chemical properties

iii) To investigate the functional properties of these modified starch fractions in mayonnaise formulations as fat mimetic through rheological, textural and sensorial studies.

CHAPTER TWO

LITERATURE REVIEW

2.1 Starch

Most of the starch utilized world-wide comes from a relatively small number of crops, the most important being maize, potato, wheat and tapioca with smaller amounts from rice, sorghum, sweet potato, arrowroot, sago and mung beans. Within Europe, the main sources of starch are maize, wheat and potato (Batchelor *et al*., 1996). Starch is the primary source of stored energy in cereal grains, tubers and other parts of plant. Starch provides 70-80% of the calories consumed by humans worldwide. In addition to being a major component of the human diet and their nutritive value, starches and modified starches are an excellent raw material for modifying the physical properties of many foods for example as gelling, thickening, adhesion, moisture retention, stabilizing, texturizing, and antistaling applications. Starch and products derived from starch are also important in numerous nonfood applications, such as pharmaceuticals, paper, textile industries, alcohol-based fuels and adhesives (Thomas and Atwell, 1997; Biliaderis, 1998; Tester and Karkalas, 2002).

2.1.1 Overview of tapioca plant (*Manihot esculenta*)

The plant known as tapioca in the East is also known as cassava in Africa and manioc or yucca in South America (Watson, 1979; Tester and Karkalas, 2002). Originally it is native to South America and southern and western Mexico.

From Mid and South America, tapioca spread to other parts of the world. It was widely grown in Africa and Southeast Asia by 1850s (Okigbo, 2006).

There are several advantages regarding tapioca crop. It can adapt to poor soils and resist drought, making it a good famine crop. It is easily propagated by stem cuttings. It gives a relatively high yield and an excellent source of calories. Furthermore, it is relatively inexpensive to produce and (i) requires very little weeding when planted in optimal plant populations; (ii) has no critical planting date, provided there is enough moisture at planting; and (iii) its roots can be left stored in the ground and harvested when required (Okigbo, 2006).

There are bitter and sweet tapioca varieties. Figure 2.1 shows a typical picture of tapioca tuber. Tapioca tubers contain a cyanohydrin (a β-glucoside also known as linamarin or phaseolunatin), which liberates hydrogen cyanide (HCN), benzaldehyde and glucose on hydrolysis in an acid medium. It can be removed after fermentation and by repeated washing of the crushed tubers. Nowadays, the tubers (containing ~ 25% starch) are processed in modern plants. Processing comprises of rasping or hammer milling of the tubers, sieving to remove fibrous and cellular material, repeated washing in centrifuges or hydrocyclones, dewatering and drying. A thoroughly purified starch may contain 0.04% protein, <0.1% lipid, and ~0.1% ash (Tester and Karkalas, 2002).

Tapioca products generally can be divided into three groups including human consumption, livestock feed and industrial products. In Far East, Africa, India and other developing countries tapioca product become their important staple food (Hrishi and Balagopal, 1979; Tester and Karkalas, 2002). Since

1970s, tapioca product as animal feed has became important industry in Tonga, New Caledonia and Western Samoa and research on animal feed even being carried out in Western Samoa (Chandra, 1979; Lambert, 1979).

Industrial products including starch production, substrate for single cell protein and alcohol production, conversion of tapioca starch into alcohol, glucose, dextrin, monosodium glutamate and single cell protein (Philips, 1979; Okigbo, 2006). Tapioca starch is widely used in noodle, bakery products, confectionery, snacks and tapioca pearl which has commercial value. Tapioca starch has remarkable characteristic including white colour, odorless, bland flavour, paste clarity, high viscosity and freeze-thaw stability. Tapioca starch has been modified to improve its functionality such as pre-gelatinized starch, acid-thinned starch, starch ethers, starch ester, and cross-linked starch (Anonymous, 2001). In 1979, the feasibility of using tapioca as a substrate in the production of single cell protein (SCP) has been demonstrated by research carried out at Universities of Guelph, and the International Center for Tropical Agriculture (CIAT). SCP production technique combined with improved cassava production techniques is believed to provide means of increasing both the quality and quantity of animal feed. Another emerging market for tapioca is as substrate for production of ethyl alcohol (ethanol). Brazil is promoting ethanol production in an attempt to become nearly self-sufficient in fuel production. The ethanol can be added to gasoline (up to 20%) to replace imported fuel (Philips, 1979).

Figure 2.1 Tapioca tuber (Alden, 2005).

2.1.2 Tapioca starch in Malaysia and Asia countries

Tapioca is known as 'ubi kayu' in Malaysia. Malaysia harvested 400,000 tons of tapioca in 1999 from an area of 39,000 hectares. Yield of tapioca is 10.26 tons per hectares in 1999. Malaysia exported 52 tons of dried cassava, 18 tons of tapioca starch and tapioca of 1,215 tons in 1998 (FAO database, 2000). In Peninsular Malaysia, production of tapioca is concentrated in the Perak state (Onwueme, 2002). Sole cropping of tapioca is commonly practice in Malaysia but sometimes it is also intercropped with maize or groundnut (Onwueme, 2000). Tapioca is mainly used to manufacture starch and starch products as well as animal feeds (Senik, 1995; Tengku Ahmad, 1998; Onwueme, 2002). Small amount of tapioca tubers are usually processed into snack foods or fermented into a product called 'tapai' (Senik, 1995). This is usually done by the small scale food processing enterprise. Small scale enterprises played an important role in Malaysia economy, especially in generating vacancy, better income distribution and as a training ground for entrepreneurs prior to investment in a larger enterprise (Senik, 1995). Malaysia also imports some tapioca starch and dried

tapioca from Thailand. Tapioca research is done in the Malaysian Agricultural Research and Development (MARDI) (Onwueme, 2002).

Nearly 37% of starch produced in the world is contributed by Asia in 2002. While about 70% of world starch production was derived from maize. Maize accounted for only 45 percent of starch production in Asia. Root and tuber crops supplied more than half of Asia's starch needs, especially tapioca (24.7 percent), sweetpotato (23.5 percent) and potato (6.0 percent) (Fuglie *et al*., 2005). In Asia countries, Thailand is the world largest exporter of tapioca, representing about 80 percent of the total world exports. However, Thailand only ranks the third among the world's producers of tapioca, after Brazil and Nigeria (Anonymous, 2003).

According to Anonymous (2005b) on the global market analysis by FAO, Asia countries will become major destination of international trade in tapioca. China will become leading cassava importer. In 2005, China accounted for 53 percent of the global market and is expected to remain the major destination of trade in tapioca starch and tapioca feed ingredients in 2006. Demand in tapioca starch in Japan, as well as in China, Indonesia and Malaysia, will remain increasing in the coming years.

2.2 Molecular structure of starch granules

Starch is synthesized in many parts of plants, e.g., pollen, leaves, stems, roots, tubers, bulbs, rhizomes, fruits, flowers, and seeds. The biosynthesis of starch occurs within three types of membrane-bound organelles: chloroplasts, chloroamyloplasts, and amyloplasts (Shannon and Garwood, 1984; Buléon *et al*.,

1998a; Tester and Karkalas, 2002). Starch is unique among carbohydrate as it occurs naturally as discrete particles, called granules. Starch granules are relatively dense, are insoluble, and hydrate slightly in room temperature water (Whistler and Bemiller, 1997; Biliaderis, 1998). Characteristics of starch granules are different among the plants botanical origin (Table 2.1) in terms of the size, distribution, shape, and morphology (Banks and Greenwood, 1975; Whistler and Bemiller, 1997; Tester and Karkalas, 2002). From Table 2.1, most of the starches show single size distribution (unimodal), however, barley, rye and wheat exhibit bimodal distributions (spherical B-granules, 2-10 μm, and lenticular A-granules, 10-40 μm). Rice starch and oat starch granules tend to exist in clusters of individual granules which are known as compound granules (Juliano, 1984; Hoover *et al*., 2003; Bao and Bergman, 2004).

Another uniqueness of starch is that most starch granules are composed of a mixture of two polymers: amylose and amylopectin (Banks and Greenwood, 1975; Whistler and Bemiller, 1997; Biliaderis, 1998; Taggart, 2004). These two polymers are built up of the six-carbon sugar D-glucose, often referred to as the "building block" of starch (Fig. 2.2). Despite both polymers composed of D-glucose, the structural differences between these two polymers contribute to large differences in starch properties and functionality. Some important characteristics of amylose and amylopectin are listed in Table 2.2.

Table 2.1 Characteristics of starch granules of diverse botanical origin.

Starch	Type	Character	Shape	Diameter (μm)
Barley	Cereal	Bimodal	Lenticular (A-type) Spherical (B-type)	15-25 2-5
Rye	Cereal	Bimodal	Lenticular (A-type) Sperical (B-type)	10-40 5-10
Wheat	Cereal	Bimodal	Lenticular (A-type) Spherical (B-type)	15-35 2-10
Oat	Cereal	Unimodal	Polyhedral	3-10 (single) 80 (compound)
Rice	Cereal	Unimodal	Polyhedral	3-8 (single) 150 (compound)
Sorghum	Cereal	Unimodal	Spherical	5-20
Triticale	Cereal	Unimodal	Spherical	1-30
Millet	Cereal	Unimodal	Polyhedral	4-12
Amylomaize	Cereal	Unimodal	Irregular	2-30
Maize (waxy and normal)	Cereal	Unimodal	Spherical/ polyhedral	2-30
Pea	Legume	Unimodal	Reniform (single)	5-10
Potato	Tuber	Unimodal	Lenticular	5-100
Tapioca	Tuber	Unimodal	Spherical/ Lenticular	5-35

(Tester and Karkalas, 2002).

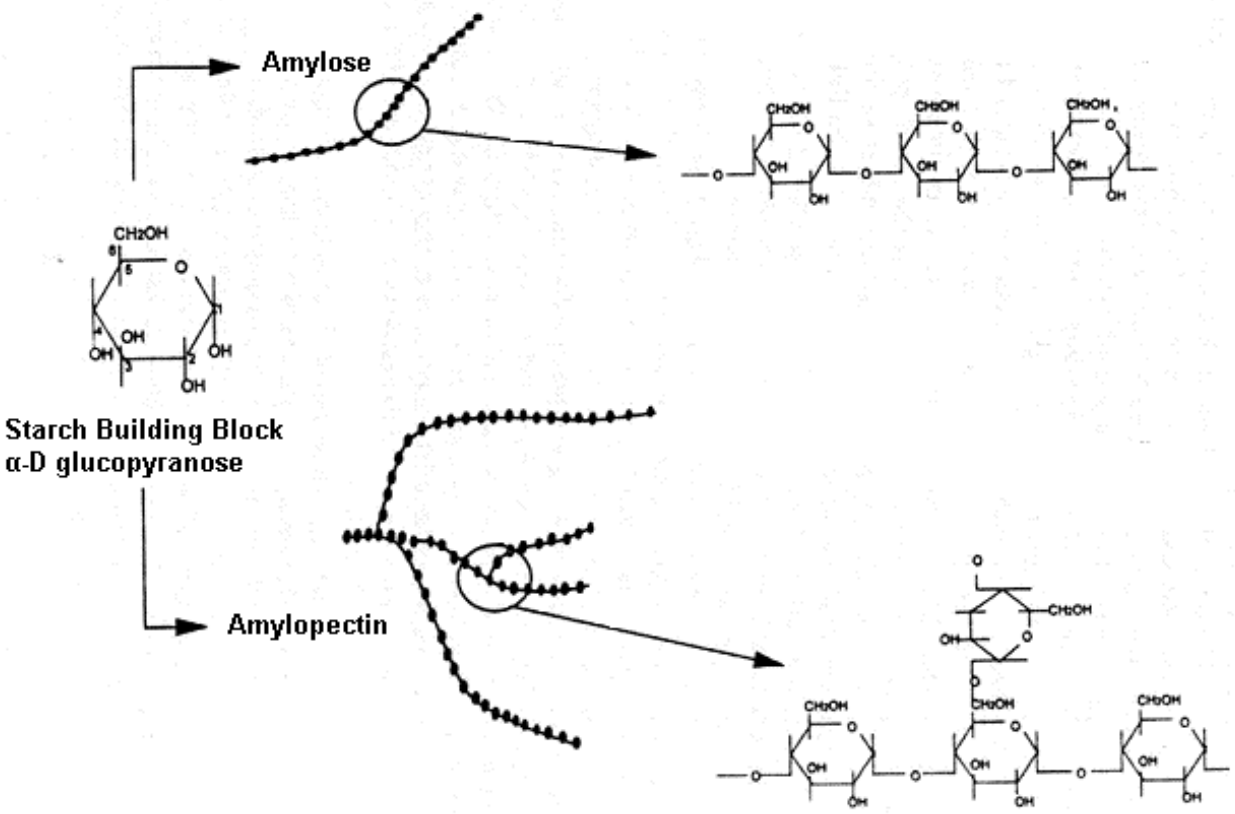

Figure 2.2 Amylose and amylopectin structure.

Table 2.2 Characteristics of amylose and amylopectin.

Characteristic	Amylose	Amylopectin
Shape	Essentially linear	Branched
Linkage	α-(1→4); [some α-(1→6)-]	α-(1→4)and α-(1→6)
Molecular Weight	Typically <0.5 million g/mol	50-500 million g/mol
Films	Strong	Weak
Gel Formation	Firm	Non gelling to soft
Colour with iodine	Blue	Reddish brown

(Thomas and Atwell, 1997).

The ratio of the two components varies according to the botanical origin with “waxy” starches containing less than about 15% amylose, normal starches 20-35% and high amylose starches greater than about 40% (Thomas and Atwell, 1997; Tester and Karkalas, 2002). Besides from that, starches also may contain some minor constituents of proteins, lipids, moisture and ash (Galliard and Bowler, 1987; Thomas and Atwell, 1997; Tester *et al*., 2004).

2.2.1 Amylose

Amylose is essentially linear polymer composed almost entirely of α-(1→4)-linked D-glucose (Fig 2.2). However there is evidence suggested that some branches are present on the amylose polymer (Banks and Greenwood, 1975; Hizukuri *et al*., 1981; Curá *et al.*, 1995). The molecular weight (M_w) is approximately 1 x 10^5 to 1 x 10^6 g/mol (Biliaderis, 1998; Buléon *et al*., 1998a). According to Hizukuri *et al.* (1981) there are 9-20 branch points per molecule and the side chains range in chain length (CL) from 4 to over 100 D-glucose units. It has been calculated that there are about 1.8x10^9 amylose molecules per average starch granule (Buléon *et al*., 1998a). Degree of polymerization (DP) of amylose range from 1,500-6,000 (Zobel, 1988a). The size and structure of amylose and its polydispersity, however, varies according to the plant source, the method of polymer isolation (usually a solvent precipitation method is used), and the method of M_w determination (Thomas and Atwell, 1997; Tester and Karkalas, 2002). Amylose chain contain reducing end which is the highly reactive aldehyde group (Thomas and Atwell, 1997).

There are two typical properties of amylose, namely its behaviour to form complex with complexing agents and also its tendency to aggregate in neutral aqueous solution. In neutral solutions, amylose exists as a random coil, but in the presence of complexing agents, including iodine, fatty acids, fatty acid components of glycerides and some alcohols, it will form a regular helical structure with about six D-glucose per helical turn with the complexing agent occupying a position at the centre of the helix (Banks and Greenwood, 1975; Rappenecker and Zugenmaier, 1981; French, 1984; Fennema, 1985; Tester and Karkalas, 2002). The interior of the helix is hydrophobic with a diameter of 0.6 nm whereas outer diameter of helix is 1.4 nm and a pitch per helical turn is 0.8 nm (Fig. 2.3) (Tester and Karkalas, 2002; Cornell, 2004). Iodine complexation has become an important diagnostic tool for the characterization of starch. Amylose molecule binds approximately 20% (w/w) iodine at 20 ºC under conditions in which amylopectin does not absorb iodine to any appreciable amount (Banks and Greenwood, 1975). The complex formation with fats and food emulsifiers such as mono- and diglycerides can shift starch gelatinization temperatures, alter textural and viscosity profiles of the resultant paste, and limit retrogradation (Thomas and Atwell, 1997). Another interesting attribute of amylose is its ability to form a gel after the starch granule is cooked, i.e., gelatinized and pasted (Table 2.2). Amylose in aqueous solution is unstable in nature. During cooling, amylose chains which are linear have the tendency to reassociate by hydrogen bonding to precipitate (retrograde) and set up to form a gel. High amylose starches

particularly are quick to set into a gel form after cooling (Zobel, 1988a; Thomas and Atwell, 1997; Biliaderis, 1998; Taggart, 2004).

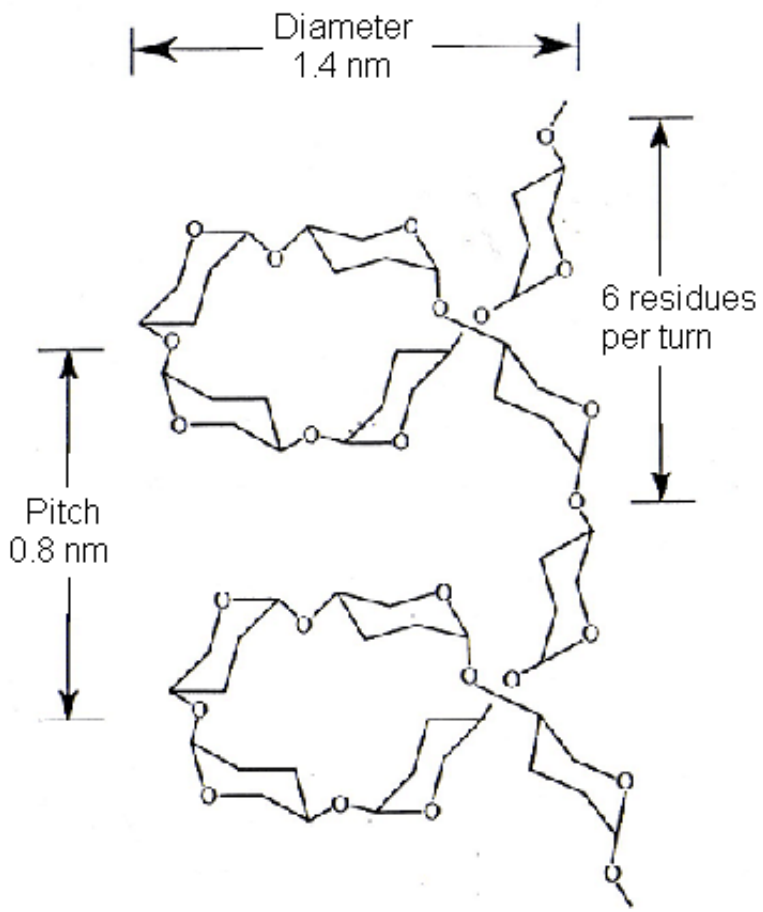

Figure 2.3 Helical conformation of amylose, left handed helix containing six glucose units per turn (Cornell, 2004).

2.2.2 Amylopectin

Amylopectin, the predominant molecule in most normal starches, is a branched polymer which is much larger than amylose. Amylopectin with a M_w of the order of 10^7-10^8 g/mol (Banks and Greenwood, 1975; Morrison and Karkalas, 1990; Biliaderis, 1998) is one of the largest known naturally occurring polymers. It is much more heavily branched, with about 95% α-(1→4) linkages (Fig 2.2). DP of amylopectin varies from $3x10^5$-$3x10^6$ (Zobel, 1988a). The size of this polymer and its polydispersity varies as a function of botanical origin (Thomas and Atwell, 1997; Tester and Karkalas, 2002). It has been estimated that about 4-6% of the linkages within an average amylopectin molecule are α-(1→6) linkages. This may appear to be a small percentage, but it results in more than 20,000 branches in an average molecule, although the branches themselves are not large (Banks and Greenwood, 1975; Thomas and Atwell, 1997). Amylopectin is unable to form stable complexes with iodine because of the short side chains, so only small amounts of iodine are bound (<0.6%); a red brown complex is formed with iodine (λmax ≈ 530-540 nm) (Banks and Greenwood, 1975).

Various amylopectin structures have been proposed since 1930s. The classic structures of the 'laminated' and the 'comb-like' models (Fig. 2.4A and B) of amylopectin were proposed (Haworth *et al*., 1937; Staudinger and Huseman, 1937). The amylopectin structure further evolved to 'bush structure' (Fig. 2.4C) (Meyer *et al*., 1940). However, in 1960s, question arose regarding the Meyer's irregular structure of amylopectin. In answer to these questions, Whelan (1971)

submmitted a revision of the structure (Fig. 2.4D) proposed by Meyer. Later on, 'cluster model' was proposed by other authors as describe below.

French (1972) proposed a 'cluster model' for amylopectin molecule. This model also explained the high viscosity of amylopectin and the possibility of building the high molecular weight amylopectin (10^7-10^8 g/mol) with the short side chains of DP 20-25. According to the cluster model, it is easy to increase the molecular weight by simply increasing the number of clusters (Fig. 2.4E). He also suggested that the exterior chains of amylopectin molecules as double helices forming crystalline region, which interspersed with amorphous regions.

In 1974, Robin and co-workers used gel chromatography and enzymatic treatment on native and lintnerized potato starch which had lead to a new model of amylopectin (Fig. 2.5) containing clusters of highly ordered side chains with DP of 15. They indicated the crystalline region of amylopectin consisting of closely packed chains of about 15 glucose units with a dimension of 60 Å. This crystalline region appeared to be acid resistant. Amylopectin chains were said to be responsible for the crystallinity of starch whereas there was no evidence of amylose participation in the crystalline areas. The cluster model was further developed by Manners and Matheson (1981), who claimed the branch points are arrange in "tiers" or clusters and not distributed randomly throughout the macromolecule.

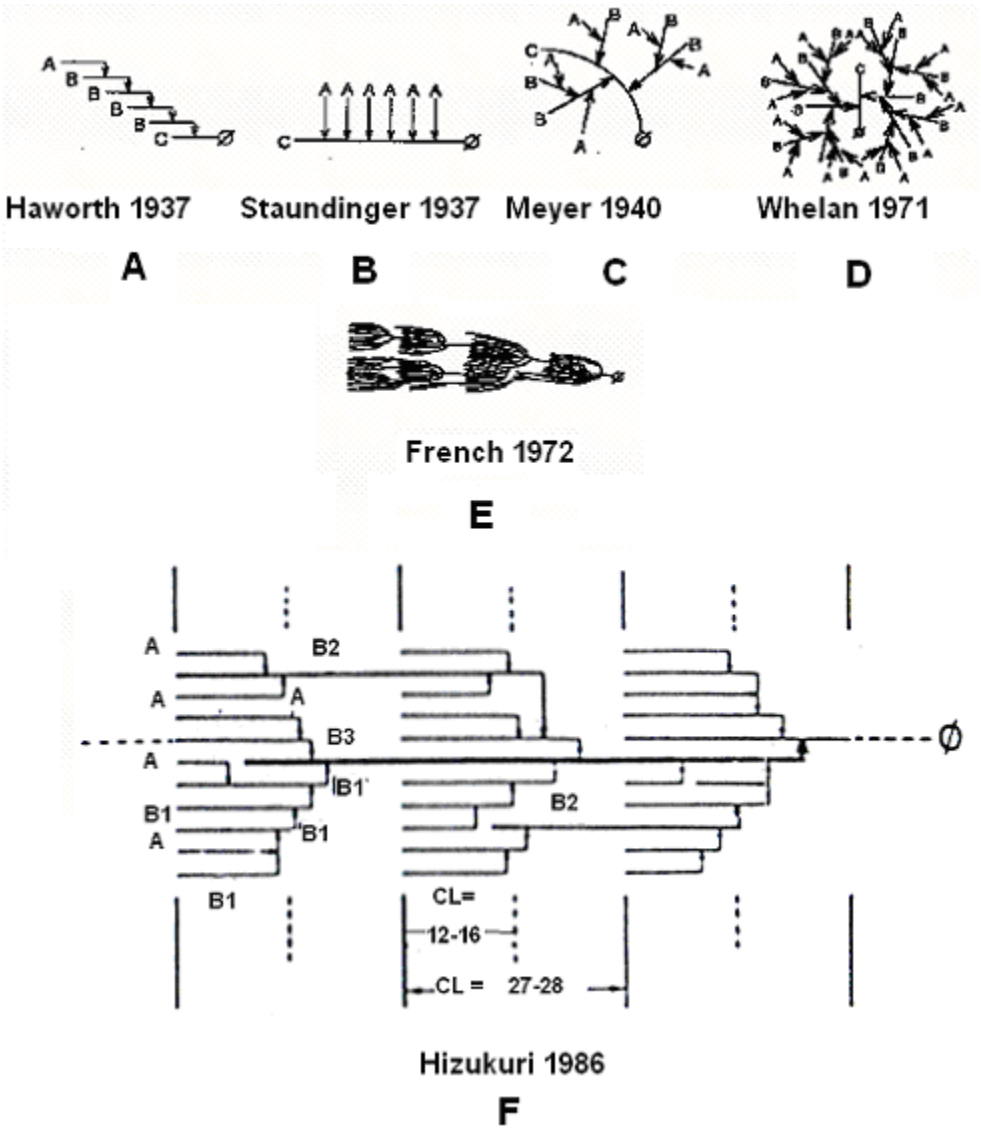

Fig 2.4 Evolution of amylopectin structure proposed by various authors: (A) laminated structure by Haworth, 1937; (B) comb-like model by Staudinger, 1937; (C) bush structure by Meyer, 1940; (D) revision of bush structure, 1971; (E) cluster model by French, 1972; (F) cluster model by Hizukuri, 1986.

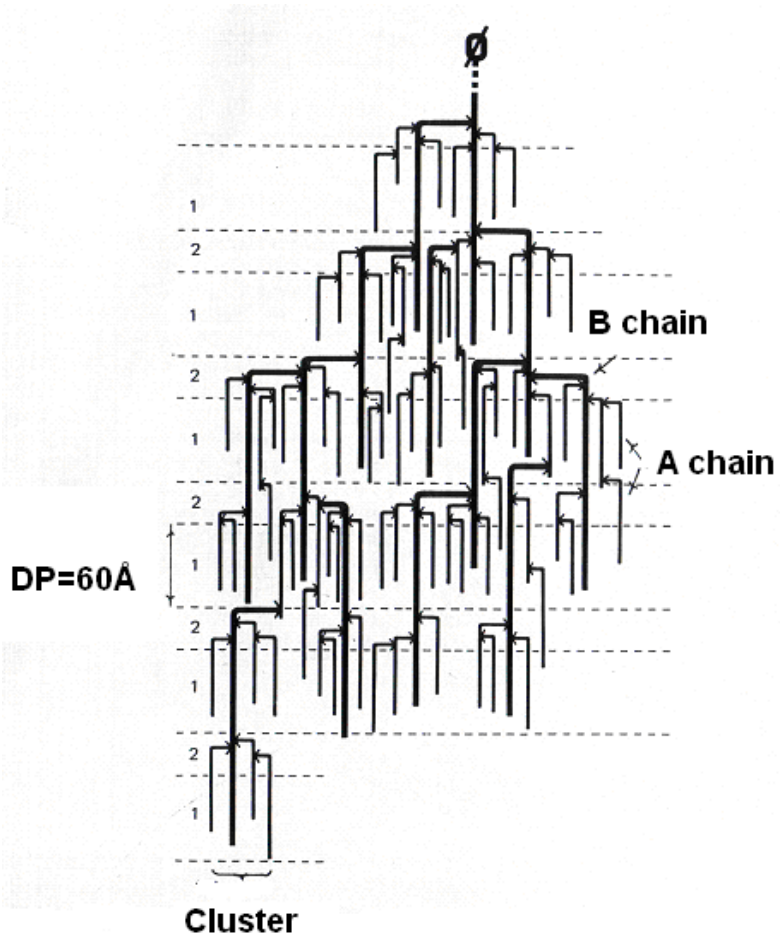

Figure 2.5 Proposed structure for potato amylopectin by Robin *et al*., (1974). 1 = compact area; 2 = less compact area; arrows indicate branching points; 0 = reducing unit.

Hizukuri (1986) used enzymes to debranch amylopectin, followed by high performance liquid chromatography (HPLC) to determine the branch size distribution. Using this method it was possible to separate the chains into two main peaks, one with an average DP of 11-16 and the second with an average dp of 40-45 (Hizukuri, 1986). This study contributed to the formation of the generally accepted cluster model describing the three dimensional structure of amylopectin (Fig. 2.4F). This model proposes that the amylopectin molecules is

made up of three broad classes of glucose chains, A, B and C chains. A-chains are unbranched and are linked to the molecule through their reducing end-group; B chains (B_1-B_4) which are joined to the molecule in the same way but carry one or more A-chains; and one C-chain, which has the reducing end-group of the molecule. Typical chain lengths (CL) for A-, B_1, B_2, B_3, and B_4 chains for starches are 12-16, 20-24, 42-48, 69-75, and 101-119 respectively (Hizukuri, 1986, 1988; Wang and White, 1994; BelloPerez *et al*., 1996). The A and B_1 chains (Fig 2.4F) are the most external (exterior) and form double helices (and crystallites) within the native granules (Tester and Karkalas, 2002). Their CL is typically ~12-24 depending on genetic origin (Hizukuri, 1985, 1993; Mua and Jackson, 1997; Jane, *et al*., 1999; Franco *et al*., 2002; Li *et al*., 2001) and starches with 'A-type' crystallinity, (most cereals) having shorter chain lengths on average than 'B-type' starches (like potato).

2.3 Granular structure of starch

Early microscopic work with plane-polarized light showed that the granules are positively birefringent which implies that molecules are radially oriented (Banks and Greenwood, 1975; Banks and Muir, 1980). Using a more sophisticated microscopy technique, granule internal structure (Fig. 2.6A) was revealed when lightly crosslinked potato starch was attacked by α-amylase (Hollinger and Marchessault, 1975). By using microscopic observation, Yamaguchi *et al.* (1979) reported that granules from waxy maize starch show "rippled" fibrous structures which correspond to growth rings. Their observations

and results had lead to the proposal of various ultrastructure of granule by scientists which will be discussed below.

In 1984, French proposed the growth ring of starch structure which involves possible arrangement of amylopectin clusters (Fig. 2.6B). The growth ring is thought to consist of 16 clusters per growth ring and growth rings 120-400 nm long. The crystalline lamellae consisting of double helices were 5 nm long, and interspersed with amorphous branch regions 2 nm long. This model fitted well either with crystalline polymorphs of A- or B-type.

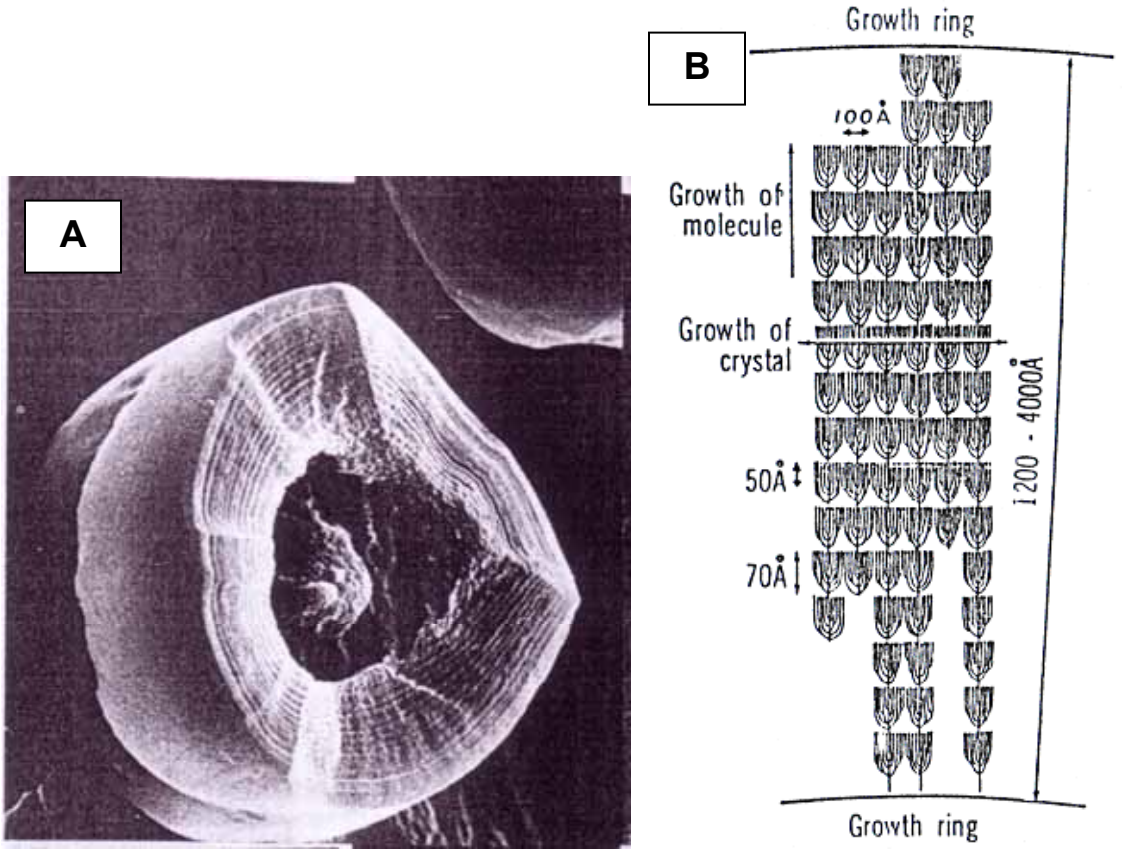

Figure 2.6 (A) Internal structure of crosslinked potato starch after enzyme attack (Hollinger and Marchessault, 1975); (B) Schematic representation of arrangement of amylopectin molecules within a growth ring. The individual molecule may be interwined (French, 1984).

Blanshard (1987) further reviewed starch structure, and presented a model to describe starch structure to accommodate chemical and physical data (expecially X-ray scattering) to provide spatial location for amylopectin (double helices) and amylose (free, as V-type helices with lipids and as double helices as shown in Figure 2.7A. He pointed out that amylose and lipid are arrayed radially which certainly allows for the existence of V-amylose in the native starch granule. In 1987, Eliasson and co-workers combined the cluster structure of amylopectin into the analogy to the quartz structure (Fig. 2.7B).

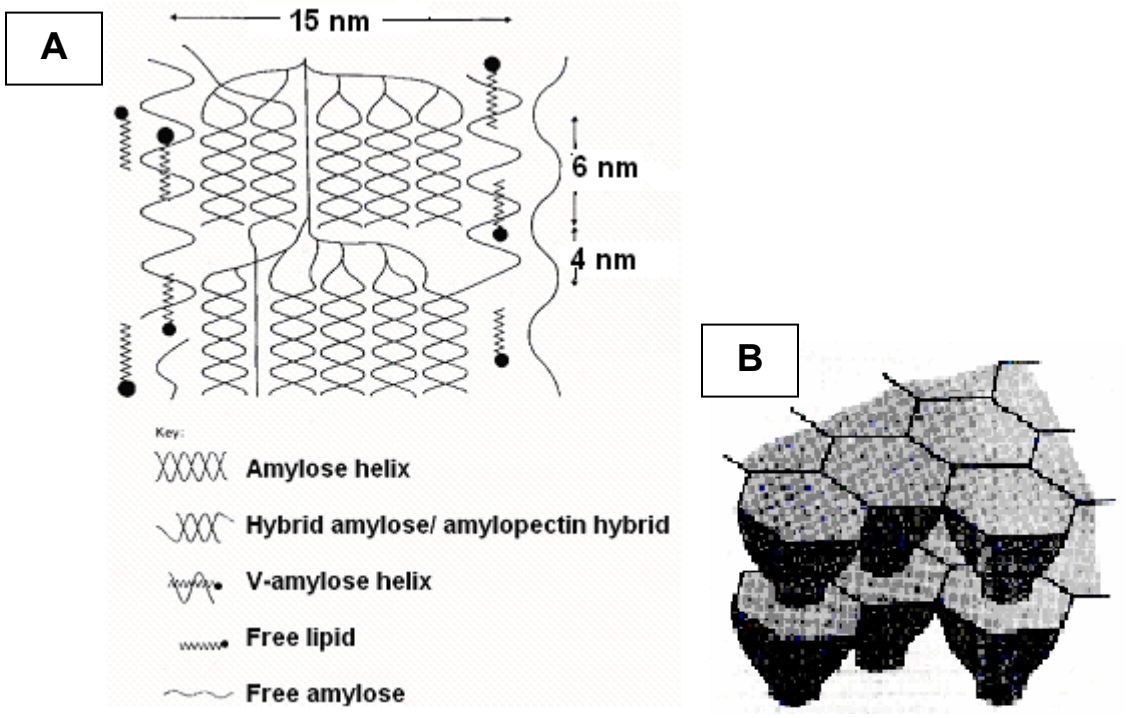

Figure 2.7 (A) Model of starch crystallite showing the possible positioning and interactions of various components (Blanshard, 1987); (B) Cluster arrangement into concentric crystalline layers of the starch granule (Eliasson *et al*., 1987).

In 1993, however, a new idea was proposed by Oostergetel and van Bruggen using electron optical tomography and cryoelectron diffraction. Their data imply a 'super helical' arrangement of both amorphous and crystalline lamellae. Moreover distinct superhelices are interlocked through their respective amorphous and crystalline lamellae to yield a tetragonal symmetry (Fig. 2.8). In this three dimensional arrangement, the double helical glucans are pointing in the axis of the superhelix towards the surface of the granule.

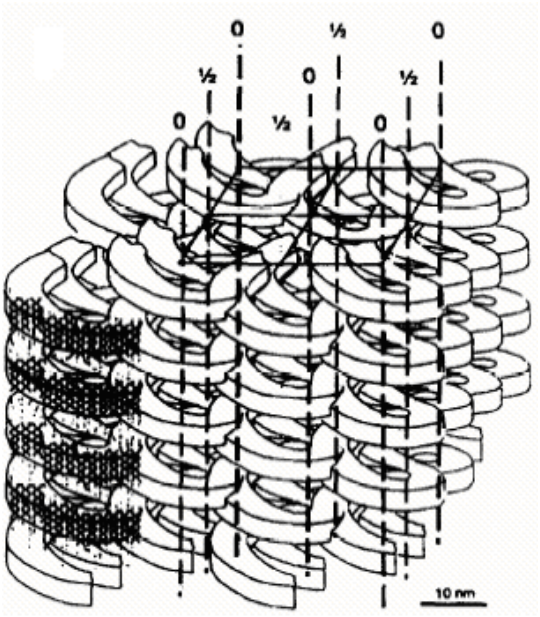

Figure 2.8 Schematic model for the arrangement of amylopectin in potato starch showing the 'super-helical' structure and the amorphous areas inside the crystalline organization (Oostergetel and van Bruggen,1993).

Small angle and wide angle X-ray scattering (Cameron and Donald, 1992; Jenkins *et al*., 1993; Jenkins *et al*., 1994) contribute to the 'lamellar stack' model proposed by Donald *et al.* (1997). Starch granules contain alternating amorphous and crystalline lamellae, with a periodicity of ~9nm as shown in Figure 2.9.