THE IMPACT OF ANNEALING ON THE MOLECULAR STRUCTURE AND PROPERTIES OF DIOSCOREA STARCHES

LUCKSHMAN P. JAYAKODY

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ISBN: 978-0-494-55386-2

THE IMPACT OF ANNEALING ON THE MOLECULAR STRUCTURE AND PROPERTIES OF *DIOSCOREA* STARCHES

by

Luckshman P. Jayakody©

A Thesis Submitted to the School of Graduate Studies in Partial Fulfillment of the Requirement for the Degree of Doctor of Philosophy

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August 2007

St. John's **Newfoundland** **Canada**

Abstract

Dioscorea starches are presently not used in the food industry due to their poor functionality. Annealing has been shown to improve starch functionality. Therefore, the objective of this study was to determine the structural changes within the amorphous and crystalline domains of starches isolated from varieties of *D. esculenta* and *D. alata* tubers on annealing, and the impact of these changes on functional properties. Starches from varieties of *Dioscorea esculenta* (kukulala, java-ala, nattala) and *Dioscorea alata* (hingurala, raja-ala) tubers grown in Sri Lanka were isolated and their yield, morphology, composition (phosphorus, bound lipid, total amylose and lipid complexed amylose), molecular structure and physicochemical properties were studied in their native and annealed states. Among the *D. esculenta* starches, nattala exhibited the highest levels of phosphorus (0.10%), bound lipid (0.44%) and lipid complexed amylose chains (22.01%). However, between the *D. alata* starches, the highest levels of phosphorus (0.05%), bound lipid content (0.25%) and lipid complexed amylose chains (8.34%) was in hingurala. All isolated starches were pure, undamaged and with granule sizes ranging from 3 to 40 μm (*D. esculenta*) and 30 to 45 μm (*D. alata*). The granules had smooth surfaces with shapes ranging from polygonal (*D. esculenta*) to truncated oval or truncated spade in *D. alata* starches. The amylopectin unit chain length distribution and the average chain length of native *D. esculenta* ranged from 24.57-25.85% (Degree of polymerization [DP] 6-12), 56.55-59.64% (DP 13-24), 12.61-13.58% (DP25-36), 3.17-4.41 (DP 37-50) and 17.93-18.33%. However, the corresponding values for

D. alata starches were 17.89-20.68, 57.40-59.76, 17.46-17.47, 4.46-4.87 and 19.29-19.61%. Among the *D. esculenta* starches, the highest proportion of DP 37-50 and average chain length was in nattala starch. *D. esculenta* starches displayed a B-type X-ray pattern. However, a B-type (raja-ala) and C_a-type (hingurala) X-ray patterns were displayed by the *D. alata* starches. Crystallinities ranged from 49-53%, in the *D. esculenta* (nattala>java-ala> kukulala) starches, but were similar (43.0%) in the *D. alata* starches. A V-lipid amylose complex peak was also visible in the X-ray pattern of all starches.

The gelatinization transition temperatures (T_o [onset], T_p [mid point], T_c [conclusion]) and enthalpy (ΔH) of gelatinization of native *D. esculenta* starches ranged from 72.55 to 85.40°C and 17.32-18.07 J/g, respectively. The corresponding values for the *D. alata* starches being 75.45-92.70°C and 18.60-18.98 J/g, respectively. The extent of amylose leaching (AML) at 90°C, ranged from 5.58 to 6.19% and 13.20 to 13.60% in the *D. esculenta* and *D. alata* starches, respectively. Variations in AML among varieties of each species was negligible. Swelling factor at 90°C, ranged from 53.61 to 64.97% in the native *D. esculenta* (nattala>java-ala>kukulala) and from 36.60 to 38.64% in the *D. alata* (hingurala>raja-ala) starches. *D. esculenta* starches exhibited a higher peak viscosity (nattala>kukulala> java-ala), lower pasting temperatures, a greater degree of viscosity breakdown (nattala> java-ala> kukulala) and lower set-back (kukulala>nattala>java-ala) values than *D. alata* starches. A similar trend was also observed for peak viscosity (raja-ala>hingurala), viscosity breakdown (hingurala> raja-ala) and set-back (raja-ala>hingurala) among the *D. alata*

starches. The rate and extent of acid hydrolysis of *D. esculenta* starches (nattala>java-ala>kukulala) were higher than those of the *D. alata* (raja-ala>hingurala) starches. *D. esculenta* (java-ala>kukulala>nattala) starches were more susceptible than *D. alata* (hingurala>java-ala) towards α-amylolysis. The extent of retrogradation of *D. esculenta* (java-ala>kukulala>nattala) was higher than that of the *D. alata* (hingurala>raja-ala) starches.

The second phase involved a study of the impact of annealing on the structure and properties of *Dioscorea* starches. The granule surface, birefringence patterns, concentric growth rings and the amylopectin unit chain length distribution of all starches remained unchanged on annealing. The crystallinity of some varieties of *D. esculenta* (kukulala, java-ala) and *D. alata* (hingurala) starches remained unchanged on annealing. However, crystallinity decreased in nattala (*D. esculenta*) but increased in raja-ala (*D. alata*) starches. In addition crystalline polymorphism remained unchanged on annealing. Annealing increased the gelatinization temperatures to nearly the same extent for all starches. However, the gelatinization temperature range (T_c-T_o) decreased on annealing (hingurala> kukulala>raja-ala>nattala>java-ala). The enthalpy of gelatinization increased marginally (kukulala > hingurala > java-ala > nattala > raja-ala) on annealing. Amylose leaching (AML) and swelling factor (SF) decreased (*D. alata*> *D. esculenta*) on annealing. At 90°C, the decrease in AML in the *D. esculenta* and *D. alata* starches followed the order: nattala~kukulala>java-ala and raja-ala>hingurala, respectively. However, at the same temperature, the decrease in SF for the *D. esculenta* and *D. alata* starches

followed the order: kukulala>nattala>java-ala and raja-ala~hingurala, respectively. In the *D. esculenta* starches, annealing decreased the peak viscosity (kukulala>java-ala>nattala). However, annealing increased peak time (kukulala > nattala > java-ala) pasting temperature (kukulala > nattala~java-ala) and thermal stability (kukulala>nattala>java-ala). The set-back decreased on annealing in kukulala, but increased in both nattala and java-ala (nattala>java-ala) starches. In the *D. alata* starches, annealing decreased peak viscosity (raja-ala>hingurala), but increased peak time (raja-ala>hingurala), pasting temperature (raja-ala>hingurala) and thermal stability (hingurala>raja-ala). However, set-back decreased in raja-ala but increased in hingurala on annealing. In all starches, the acid hydrolysis pattern remained unchanged on annealing. However, the extent of acid hydrolysis decreased (*D. esculenta* [java-ala>nattala>kukulala] ~ *D. alata* [raja-ala~ hingurala]) on annealing. α-amylolysis decreased on annealing. The extent of this decrease was more pronounced in *D. alata* (raja-ala>hingurala) than in the *D. esculenta* (java-ala>kukulala) starches. However, annealing increased the susceptibility of nattala starch towards α-amylolysis. The enthalpy of retrogradation decreased in both *D. esculenta* (kukulala~nattala>java-ala) and *D. alata* (raja-ala>hingurala) starches. The major findings of this research are: (1) the different responses shown by the *Dioscorea* cultivars towards annealing were mainly influenced by differences in their composition and molecular structure, (2) differences in physicochemical properties between native and annealed starches are influenced to a large extent by structural changes within the amorphous (interaction between AM-AM and/or

AM-AMP chains) and crystalline (AM-AMP and/or AMP-AMP interactions, crystallite reorientation, crystallite perfection, reorientation of phosphate groups on amylopectin) domains of the starch granule during annealing, (3) annealing has a major impact on the thermal stability and extent of retrogradation of *Dioscorea* starches. Consequently, annealed *Dioscorea* starches can be used for applications in foods that are subjected to thermal processing and frozen storage.

Acknowledgments

I would like to express my warm thanks to Dr. R. Hoover my thesis supervisor for his excellent supervision, and guidance in the preparation of this thesis. Grateful appreciation is also extended to my supervisory committee members Drs. C. Bottaro and K. Nag for their support and enthusiasm during the development of this work.

Special thanks to Mr. M. Murphy, Mr. P. Mansfield (student labs), Mr. Garry Collins (Dept. of Biology), Mr. R. Ficken (LM & printing facility), Mr. M. Sheffer (SEM facility), Mr. M. Bromberek, Mr. C. Skinner (Amino acid facility), Dr. R. Mason and Ms. L. Thompson (DSC facility) & Ms. H. Gillespie (XRD facility) at MUN & Dr. Q. Liu, and Ms. E. Donner (RVA, HPLC facility) at Agriculture & Agri-Food Canada for their particular expertise. Special thanks to Drs. A. Martin and T. Patel for laboratory equipments.

Thanks to both Ms. S. Katugampola, and Mr. N. Jayatissa (Agrarian Research & Training Institute, Sri Lanka) for plant material and agronomic information. Thanks are also extended to Mr. U. Silva, Ms. M. Thawabalachandran and Dr. M. Jayasuriya (Department of Agriculture, Sri Lanka) for their help in tuber identification and related information.

I am furthermore grateful to Dr. E. Warkentin, Ms. M. Rollmann, Dr. J. M. Snook (Department of German & Russian, MUN), Ms. B. Conran, D. Taylor (QE II Library, MUN) and to L. Thompson and N. Ohara for their help in translating papers published in German, French, and Japanese.

I also wish to express my gratitude to the University of Peradeniya, Sri Lanka for granting me study leave and to journal publishers for permitting reproduction of figures that appear in this thesis. I would like to thank the Department of Biochemistry, School of Graduate Studies and NSERC for academic and financial support, and to the Government of Sri Lanka for providing free education through out my study period.

I am indebted to my loving parents, wife Indrani and daughter Lukindra for their enormous support, patience and encouragement given to me throughout my academic career.

Lastly, and far from least, thank you to my colleagues Madujith, Zhou, Renuka, Lan and Vicky. Thanks also to Mr. D. McInnis and the staff at the technical services, Mr. H. Murphy (Biochemistry stores), Ms. P. Parsons, Ms. S. Wall & M. Hicks (mail room), and Mr. M. Nolan, Ms C. Woolgar, Ms D. Doucette, Mr. B. Gillam, Mr. G. Bourgeois, Mr. M. Nolan, Mr. B. Gillam, Mr. G. Bourgeois, Mr. A. Balsara, Mr. D. Duda, Ms. D. Doucette, Ms C. Woolgar Ms. K. Lippold & Ms. M. March (QE II library) at MUN, and family friends who helped me in various ways.

TABLE OF CONTENTS

List of Figures

List of Tables

List of Abbreviations

AFM	Atomic force microscopy
AM	Amylose
AML	Amylose leaching
AMP	Amylopectin
$\overline{CL}$	Average chain length
BU	Brabender viscoamylogram units
CL	Chain length
CSLM	Confocal scanning laser microscopy
DP	Degree of polymerization
DSC	Differential Scanning Calorimetry
ESEM	Environmental electron microscopy
FAO	Food and Agriculture Organization of the United Nations
GPC	Gel permeation chromatography
HPLC	High performance liquid chromatography
HPSEC	High performance size exclusion chromatography
NIR	Near infrared reflectance
NMR	Nuclear magnetic resonance
RVA	Rapid visco analyser
RVU	Rapid vico analyser units
SAXS	Small angle X-ray scattering
SEM	Scanning electron microscopy
SF	Swelling factor
SP	Swelling power
T_c-T_o	Gelatinization temperature range
TEM	Transmission electron microscopy
T_g	Glass transition temperature
T_o	Onset of gelatinization
T_p	Peak temperature of gelatinization
ΔH	Enthalpy of gelatinization
ΔH_R	Enthalpy of retrogradation

UK	United Kingdom
USA	United States of America
WAXS	Wide angle X-ray scattering
XRD	X-ray diffraction

Dedicated to my loving parents, wife, daughter & to my teachers

CHAPTER 1

Introduction

Starch is a semi-crystalline biopolymer and is stored in various plant locations, for example in cereal grains, roots, tubers, stem-piths, leaves, seed, fruit and pollen. Starch granules in storage tissues can vary in shape, size and composition. The shape and size of the granules depend on the source. The chemical composition, structure and properties are also essentially typical of the biological origin of the starch (Smith 2001). The importance of starch as a food stuff may be judged by the fact that it accounts for over 30% of the average diet on a dry weight basis and more than 25% on an available energy basis (Galliard 1989). Starch granules in higher plants, regardless of the plant source, contain two principal types of polysaccharides, namely amylose (linear) and amylopectin (branched). Both are polymers of α-D glucose connected by α-D-(1→4) linkage chains of varying lengths. Amylopectin, the major component of most starches, consists of a large number of shorter chains that are bound together at their reducing end by α-D-(1→6) linkage, making it extensively branched (Manners 1989). Amylose consists only of either a single or a few long chains, thus making the molecule linear or slightly branched (Hizukuri *et al.*, 2006). The amylose concentration of starches has been found to vary from 17 to 68% (Hizukuri *et al.*, 2006). However, waxy starches contain a much lower amylose content (<1%) or even lack the amylose component completely (Waduge *et al.*, 2006). Several studies have shown that variations in starch properties among cereal, tuber and legume starches and among varieties of a particular starch source is influenced

by differences in: (1) composition, (2) morphology, (3) amylose content, (4) molecular structure and (5) arrangement of amylose and amylopectin within the granule interior.

For many years, tuber and root starches such as yam, taro, arrowroot, cassava and sweet potato have served as the major carbohydrate sources for people in tropical countries. Naturally suited to tropical agro-climatic conditions, they grow profusely with little or no artificial inputs. However, in tropical countries, the very success of starchy crops as staple foods is limiting their potential contribution to agriculture development and economic growth. An extensive search of JSTOR, SciFinder Scholar, Web of Science, Agricola, Food Science & Technology Abstracts (FSTA) and other databases showed that while intensive research has been carried out on the botany, agronomic, and nutritional aspects of yams (*Dioscorea*), they have not benefited from the kind of value added research required for competitiveness in domestic or international markets. As a result wheat, maize, rice and potato continue to dominate lucrative world markets for starches in food and non-food industries. Thus, intensive research is required to elucidate the structure-property relationships of native, as well as modified, *Dioscorea* starches. When aiming at functional properties in starch, most food processors examine the characteristics of competitive starches in specific applications. This establishes a target to shoot for when these characteristics are unattainable with native starches; the only alternative is then some form of physical or chemical modification that would confer desirable properties, e.g. high thermal stability, resistance to shear and pH and low extent of syneresis that add

value to the product. These are properties that the end user requires and is willing to pay for. So far, no research has been carried out to investigate the effect of physical or chemical modification on *Dioscorea* starches. It should be emphasized, that it is not maize, wheat, rice and potato starches themselves that are the competition, but rather the physicochemical characteristics of their value added products.

The world's leading tuber and root starch sources are potato, cassava, sweet potato, yams (*Dioscorea*), and taro, respectively. In Sri Lanka *Dioscorea* is commonly known by its vernacular name, '*Vel-ala*'. It plays an important role in the diet (consumed in the form of a vegetable dish, boiled, or roasted) of individuals living in suburban and rural areas of the island. *Dioscorea alata* (variety: hingurala and raja-ala) and *Dioscorea esculenta* (variety: kukulala, java-ala, nattala) are the two popular yam species widely cultivated in Sri Lanka. A survey of the literature on *Dioscorea* starches have revealed that most of the research on these starches has involved studies on starch property characterization (65%), whereas research on molecular structure analysis (17%) and starch utilization (18%) is limited. The survey also revealed that compared to commercially used starches such as rice, maize, wheat, potato and cassava, there is a dearth of information on the molecular structure, physicochemical properties, functionality and modification of *Dioscorea* starches. Studies have shown that there is a wide diversity in starch characteristics in varieties of potato (Genkina *et al.*, 2004d, Ganga & Corke 1999), sweet potato (Genkina *et al.*, 2004c, Collado 1997) and cassava (Moorthy 1994). However, most studies on

Dioscorea starches have involved only one cultivar. Consequently, it is difficult to ascertain whether the structure-property relationships that have been developed for *Dioscorea* starches reported in the literature are truly representative of the species. In addition, the resulting information on *Dioscorea* starches are from species grown in Africa, South America, China, or India, where environmental conditions are different from those in Sri Lanka. Several researchers (Kiseleva *et al.*, 2004, Genkina *et al.*, 2004d, Protservo *et al.*, 2002, Tester & Karkalas 2001, Haase & Plate 1996, Tester *et al.*, 1995, Shi *et al.*, 1994, Hizukuri 1969) have shown that the environmental conditions influence synthesis, composition, structure and functionality of starches. This suggests that in order to develop value added products from starches isolated from *Dioscorea* tubers grown in Sri Lanka, intensive research is required on their molecular structure and physicochemical properties.

One way of adding value to native *Dioscorea* starches is by a physical modification technique known as annealing. Annealing is a type of hydrothermal treatment where starch is heated with excess (>60% [w/w]) or intermediate (40% [w/w]) water content at a temperature above the glass transition but below the gelatinization temperature (Waduge *et al.*, 2006, Tester & Debon 2000, Jacobs & Delcour 1998). Annealing has been shown to cause changes to starch structure (increase in granular stability, stronger glucan chain interactions, perfection of starch crystallites, formation of new double helices, compartmentalization of amylopectin-amylopectin, amylose-amylopectin and amylose-amylose helices, increase in contrast between crystalline and amorphous lamellae) and properties,

such as elevation of starch gelatinization temperatures, narrowing of the gelatinization temperature range, decrease in swelling factor and amylose leaching and increase in hot and cold paste viscosities. However, discrepancies still exist with regard to the extent of susceptibility of annealed starches towards acid and α-amylase hydrolysis (Nakazawa & Wang 2004, Nakazawa & Wang 2003, Atichokudomchai *et al.*, 2002, Jacobs *et al.*, 1998a, 1998b, Hoover & Vasanthan 1994a) and the role of naturally occurring phosphate esters on annealing properties (Muhrbeck & Wischmann 1998). In many of the above studies, the composition and molecular structure of the starches have not been reported. This makes it difficult to accurately interpret the observed changes on annealing. Surprisingly, retrogradation characteristics of annealed starches have not been explored for cereals, tubers and roots, legumes and non-conventional starch sources. Furthermore, there is a dearth of information on the effect of annealing on: granule morphology, chain length distribution, formation of V-amylose lipid complexes, and crystallinity of pre and post annealed starches. Annealing of cereal starches is well documented, however, among tuber starches, annealing has been studied in detail only in potato starch. There is also some limited information on the effect of annealing on cassava (Gomez *et al.*, 2004) and sweet potato (Genikina *et al.*, 2004b) starches. The effect of annealing on cultivars of other tuber sources has not been investigated. Consequently, the structural changes observed during annealing may not have been truly representative of the species. Tuber starches have been shown (Hoover 2001) to differ from cereal and legume starches with respect to structure

(longer amylose and amylopectin chain length, amylopectin chain length distribution, unit cell structure), crystallinity, composition (higher phosphorous content, trace quantities of lipids) and morphology (larger granule size). Furthermore, variations in the above parameters have also been shown (Hoover 2001) to exist among tuber starches. Consequently, the possibility exists that the extent of interaction between glucan chains and the extent of realignment of double helices during annealing of tuber starches may be different from that observed in cereal starches, and may also vary among tuber starches. Studies on tuber starches would thus provide a deeper understanding of how variations in starch structure, composition and morphology influence changes to physicochemical properties on annealing. It is hypothesized, that if variations exist in composition and molecular structure among varieties of *Dioscorea* starches, then these variations will influence the extent to which glucan chains within the amorphous and crystalline domains realign and interact during annealing. This hypothesis will be tested by probing the molecular structure and physicochemical properties of *Dioscorea* starches before and after annealing using a wide variety of techniques.

Objectives of the present study:

(1) The key advantage of *Dioscorea* starches is that they are low cost sources of carbohydrates. To capitalize on this advantage, there is a need for development of products whose quality and price are comparable to that of cereals. Thus, the first objective of this research was to determine the composition, morphology, molecular structure and physicochemical properties

of starches extracted from different varieties of *Dioscorea esculenta* (kukulala, java-ala, nattala) and *Dioscorea alata* (hingurala, raja-ala), grown in the same location in Sri Lanka, using a wide variety of analytical techniques such as light microscopy, Kofler hot stage microscopy, scanning electron microscopy, rapid viscoanalyzer, high pressure anion exchange chromatography with pulse amperometric detector, powder X-ray diffractrometry, differential scanning calorimetry, susceptibility towards α-amylase and acid, amylose leaching, swelling factor and retrogradation.

2. To determine the types of structural change that could occur when the above starches are subjected to one step annealing.

3. To determine the extent to which structural changes on annealing influence granular swelling, extent of amylose leaching, gelatinization transition temperatures and enthalpy of gelatinization, rheological characteristics, rate and extent of retrogradation, granule crystallinity, X-ray intensities, polymorphic form and susceptibility to acid and α-amylase hydrolysis.

CHAPTER 2

Review of Literature

2.1 *Dioscorea*

Overview

Starchy tubers and root crops are important subsidiary or subsistence foods in tropical countries. They are rich sources of carbohydrates. Proteins, minerals, vitamins, antioxidants, steroids, and saponins, etc. are also present (Jayasuriya 1995, Gunasena 1994, Wanasundera & Ravindran 1992, Tindall 1983). The carbohydrates are mostly starch and are found in storage organs, which may be enlarged tubers, roots, corms, or rhizomes. Although a wide range of tuber crops are commercially grown worldwide, five species account for almost 99 % of the total world production. These are potato (*Solanum tuberosum*) 47%, cassava (*Manihot esculenta*) 29%, sweet potato (*Ipomoea batatus*) 17%, yams (*Dioscorea* spp.) 6%, and taro (*Colocasia*, *Cyrtosperma* and *Xanthosoma* spp.) ~1% (FAO 2006 & 2003).

2.1.1 Classification, center of origin and distribution of *Dioscorea*

Kingdom: Plantae – Plants
Subkingdom: Tracheobionta -- Vascular plants
Superdivision: Spermatophyta -- Seed plants
Division: Magnoliophyta -- Flowering plants
Class: Liliopsida – Monocotyledons
Subclass: Liliidae
Order: Liliales
Family: Dioscoreaceae -- Yam family
Genus: *Dioscorea* L. yam
Species: ~630 spp.

Dioscorea is a large genus consisting of about 630 species (Jayasuriya 1995). Tubers of *Dioscorea*, commonly called true yams, are important food crops in many tropical countries and in some sub-tropical regions. *Dioscorea* tubers are known to contain medicinally important compounds (Riley *et al.*, 2006). Environmentally, they are well adapted to most tropical climates and in many cases they can be eaten as a subsidiary diet.

Dioscorea is a monocotyledous plant. Coursey (1967), Léon (1977) Tindall (1983), and Rajapaksha (1998) indicated four distinct centers of origin for edible yams such as (1) Indo-China Peninsula possibly Burma and Assam [greater or water yam (*D. alata*), lesser or Asiatic yam (*D. esculenta*), aerial or potato yam (*D. bulbifera*), intoxicating yam (*D. hispida*), *D. nummularia* and *D. pentaphylla*], (2) South China [Chinese yam (*D. opposita*) and *D. japonica*], (3) Caribbean area [cuch-cush yam (*D. trifida*), with a subsidiary center in South America] and (4) West African forest belt to the savannah [yellow yam (*D. cayenensis*), white or guinea yam (*D. rotundata*), bitter or wild yam (*D. dumetorum*) and aerial or potato yam (*D. bulbifera*)]. It is believed that *D. alata* and *D. esculenta* possibly originated in Burma and Assam (Rajapaksha 1998, Léon 1977) regions. All the species referred to are found wild in the areas concerned. However, *D. alata* is unknown in the wild state (Coursey 1967). *D. alata*, the greater or water yam is thought to have originated in South-East Asia, possibly in the Assam-Burma region, and reached Africa and tropical America in the sixteenth century (Jayasuriya 1995, Tindall, 1983). Of the species of *Dioscorea*, perhaps *D. esculenta* is the least known (Martin 1977).

2.1.2 World production statistics and current status of *Dioscorea* starches

World yam production is approximately 40 million metric tonnes per year (FAO 2006 & 2003). The bulk of edible yam production comes from the 'yam zone' (Nigeria to Ivory Coast) in West Africa and accounts for approximately 91% of world edible yam production (FAO 2006 & 2003). The worlds leading yam producers are in the area of sub-Saharan Africa: Nigeria (~68%), Ghana (~10%), Ivory Coast (~8%) and Benin (~4%), respectively. Yam is not used for starch production on an industrial scale. Cereal, potato, cassava are the main sources for commercial starch production. However, yam starches have not been thoroughly investigated with respect to their structure, functional properties and industrial application, consequently, these starches have not benefited from the kind of value added research required for competitiveness in domestic or international markets. As a result, potato in temperate climates and cassava and sweet potato in tropical areas continue to dominate the starch for food market for and industrial applications because of their well-documented specific physicochemical and functional properties (Jayakody *et al.*, 2007c & 2005, Sansavani & Verzoni 1998). However, research on *Dioscorea* starches has been steadily increasing (Shujun *et al.*, 2007, Karam *et al.*, 2006, Otegbayo *et al.*, 2006, Peroni *et al.*, 2006, Riley *et al.*, 2006, Shujun *et al.*, 2006a,b,c, Wang *et al.*, 2006, Brunnschwelier *et. al.*, 2006 & 2005, Daiuto *et. al.*, 2005, Srichuwong *et al.*, 2005a,b,c, Freitas *et. al.*, 2004, Iwuoha 2004, Riley *et. al.*, 2004, Amani *et.al.*, 2004, Akissoé *et al.*, 2003, Rolland-Sabaté *et. al.*, 2003, Alves *et. al.*, 2002, Afoakwa & Sefa-Dedeh 2002, Gunaratne & Hoover 2002).

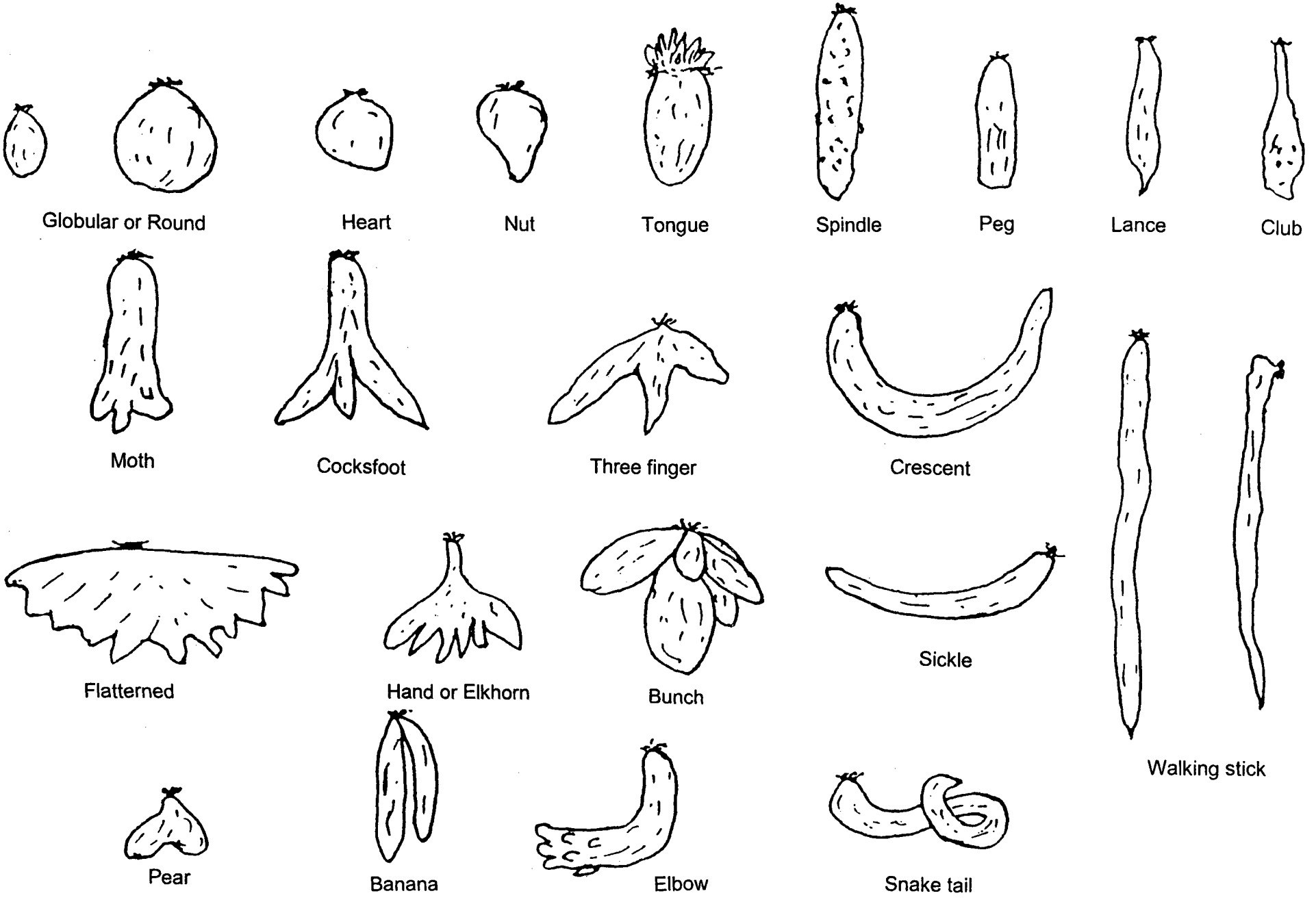
2.1.2.1 Identification and agronomy of popular edible local varieties of *Dioscorea* tubers

A large number of *Dioscorea* species are grown widely in South-East Asia, with nearly 40 varieties grown in Sri Lanka alone (Seneviratne & Appadurai 1966). *Dioscorea* are long, trailing, perennial vines with shiny, heart-shaped leaves, and arise from large underground stems that are technically called tubers.

Dioscorea varieties differ in the color of the flesh or peel and the size of the tuber (Seneviratne & Appadurai 1966), granule size, amylose concentration and physicochemical properties (Rolland-Sabaté *et al.*, 2003). *Dioscorea* tubers generally require a loose, deep and well drained fertile soil. A tropical climate with temperatures of 30° to 34°C and an annual rainfall of 1500 mm are ideal environmental conditions. Regions with a rainfall less than 800 mm per year and higher altitudes (sufficiently great for heavy frost to occur) are considered as unsuitable areas for *Dioscorea* cultivation (Coursey 1967). The daylight should be more than 12h, which is preferable for stem and leaf growth, however, satisfactory tuber formation only occurs during short days (Tindall 1983). *Dioscorea* are well adapted in the traditional agricultural system, because of their high yields, resistance to pest and diseases, and taste qualities (Léon 1977). There is great variation in the number, size, growth habit, skin color, and shape of the tubers produced (Coursey 1967). Shapes of *Dioscorea* tubers are presented in **Figure 2-1**.

Figure 2-1 Shapes of *Dioscorea* tubers

Adapted from Coursey (1967) with permission from Longmans & Green
Information from: Rajapaksha (1998), Gunasena (1994), and Tindal (1983)


Globular or Round
Heart
Nut
Tongue
Spindle
Peg
Lance
Club
Moth
Cocksfoot
Three finger
Crescent
Flatterned
Hand or Elkhorn
Bunch
Sickle
Walking stick
Pear
Banana
Elbow
Snake tail

2.1.2.1.1 Local *Dioscorea* varieties

D. alata L. and *D. esculenta* (Lour.) Burkill, are the most important yam species cultivated in Sri Lanka. The stem of the vine of *D. esculenta* is cylindrical, spiny and twining in an anticlockwise direction. *D. esculenta* forms a tuber cluster (which is comprised of a number of single tubers) per vine. The color of its outer peel (epidermis) is white. Tubers are generally ovoid and cylindrical, and the flesh is white or yellow with a sweet flavor. *Dioscorea* tubers are sensitive to physical tissue damage as a result of harvesting injury, and they are also suceptible to rapid deterioration. *Kukulala*, *java-ala*, and *nattala* belong to *D. esculenta*; *kukulala* is the most popular [Figure 3-1-A, B, C] varieity. The other two types are grown in traditional home gardens, or remain as 'wild types'. The average tuber size of *kukulala* varies from 100 to 300 g, and the potential yield varies from 28-30 tons/ha. *Java-ala* tubers, which are exceptionally large (2-3 kg) are often surrounded with a protective cover of thick spiky roots, and are extremely sensitive to mechanical damage. *Natala* ('nut shaped yam') posesses thick hairs and yellow flesh.

Dioscorea alata is a climber with an aerial stem that is square in cross-section and twining in a clockwise direction. The fleshy underground stem-base contains fibrous roots, whose tips are swollen into tubers. *D. alata* bears a large solitary tuber per vine (Gunasena 1994) and the outer skin (sub-epidermis) color of *Dioscorea alata* tuber is yellow or dark purple. The flesh may be white, cream, or yellow, or may contain anthocyanin, producing a pink or red-purple flesh color (Gunasena 1994, Tindall 1983). The outer peel is much thicker in certain

varieties, and thus damaged tubers deteriorate slowly. *Raja-ala* and *hingurala* belong to *D. alata*. *Hingurala* is a flat-shaped tuber, varying from 300-600g, with the characteristic shape of a palm or fingers [Figure 3-1-D, E]. The outer surface of the tuber is covered with somewhat rough, short hairs. Tuber flesh is hard, white in color, and covered with water soluble mucilage. *Raja-ala* is a fairly large tuber (2-5 kg), and very often the peel or flesh contains anthocyanin pigments. Hence, the flesh color varies from cream to purple. Tuber flesh is generally coarse and the outer skin is always covered with long thick roots. The potential yield of r*aja-ala* and *hingurala* varies from 20 to 24 and 16 to 20 tons/ha, respectively (Kirthisinghe 1994). *Dioscorea* are seasonal crops. The "seed plant" (app. 200 g pieces of the yam) of *Dioscorea* is normally planted with the first rains from the end of March to April (in early *Yala* season). The crop is virtually ready for harvest between December-February (in late *Maha* season) with the signs of yellowing leaves and withered vines.

2.2 Starch utilization and developments

The written history of starch usage for food and medicinal purposes has been documented for over four thousand years (e.g. Susrutha Samhitha [C. 4th Century B.C], Charaka Samhitha [C. 1st century A.D], Sarartha Sanagrahaya [C. 5th century A.D]). The ancient Egyptians [3500-4000B.C] (Whistler 1984), and later the Romans, used starch as an adhesive for paper and papyrus, whilst the ancient Greeks also used it in medical preparations (Pliny 130 B.C). The popularity of starch grew in Europe around the 14th century, owing to its use for stiffening linen, and starch was subsequently adopted for cosmetic purposes

(Radley 1968). Starch has been intensively studied over the last 200 years, probably to a greater extent than any other biopolymer. During the last 20 years, powerful biochemical, chemical and physical research techniques have revolutionised the knowledge of the deposition and characteristics of this renewable biopolymer (Tester & Karkalas 2001).

One of the major uses of starch is to impart viscosity to foods such as soup mixes, fruit pie fillings, gravy, salad dressings and pastes. Granule size has an appreciable influence on its properties (Baldwin 2001, Geddles *et al*, 1965). Starches utilized commercially have large granules (e.g. corn, potato), and these starches show industrially desirable characteristics such as high viscosity, paste clarity, and other functional properties. However, there is a growing interest in small granule starches, which show superior penetration power into the fabric and are less affected by humidity (Lindeboom *et al.*, 2004). Commercial biodegradable plastics are made from mixtures of low density plastic and small granule starches (e.g. amaranth, quinoa) with other additives (Ahamed *et al.*, 1996, Lim *et al.*, 1992). Starch acts as a filler and increases the porous nature of the plastic, consequently plastic degrades faster. There is a growing demand for carbohydrate-based fat replacers for frozen desserts, cookies and cheesecakes (Lindeboom *et al.*, 2004). Small granules are ideal for the manufacture of fine printing paper, formulations of toiletries, "talcum" powders, aerosol sprays, cold water laundry-stiffening agent, and fat replacers (Lindeboom *et al.*, 2004, Malinski *et al.*, 2003, Moorthy 2002, Jayakody 2001). Consequently, the small granule size of *D. esculenta* starches (<10μm) makes them ideal as fillers in

biodegradable plastics and "talcum" powders. However, commercial production and utilization of small granule starches is still hampered by practical limitations of starch isolation, purification, granule loss and the associated costs (Lindeboom *et al.*, 2004). Only two studies have shown that substitution of yam starches in composite bread formulations (Emiola & Delarosa 1981, Rašper *et al.*, 1974) is undesirable, since yam starches exhibit high water-binding capacity resulting in excessive hydration. However, the addition of corn and cassava starch have been shown to decrease the excessive hydration of yam (e.g. *D. alata*) starch (Karam *et al.*, 2006). Thus, mixtures of *D. alata*, cassava and corn starch have been utilized successfully for preparing bread with a good loaf volume (Karam *et al.*, 2006). It has been shown that *Dioscorea* starches can be used as a thickener in canning applications (Emiola & Delarosa 1981) due their high thermal stability. *Dioscorea* and other tropical tuber starches are presently utilized for animal feed formulations (Gallant *et al.*, 1982). Agwunobi (1999) reported that that *D. alata* can replace up to 80% of maize in a laying chicken diet. Furthermore, *D. alata* contains super long amylopectin chains and is relatively high in amylose, hence *D. alata* starches may have a potential use in preparing rapid set elastic gels and edible films (Wang *et al.*, 2006).

2.2.1 Applications and limitations of native starches

Starch can be classified into two types: native and modified. Native starches are produced through the separation of naturally-occurring starch in amyloplasts from different botanical sources. The raw starches produced still retain the original structure and unmodified characteristics and are therefore, called 'native

starches'. The major sources for the industrial production of purified starch are wheat, rice, normal and waxy corn. The production of cassava, arrowroot and sago are also similar (Tester & Karkalas 2001). However, the starch industry is still mainly based on cereals and potato. Furthermore, specific properties of potato starch are profitable in certain applications such as paper manufacturing, adhesives, plastic substitutes, mining, and drilling (Vasanthan *et al.*, 1999, Haase & Plate 1996). Color is an important attribute of starch quality, especially for industrial applications such as paper-making, textiles and sago grains (Moorthy 2002). Native starches from various botanical sources have diverse functional properties. However, native starches cannot be successfully utilized without prior modification for the following reasons : (1) inconsistency of viscosity from one starch to another, region to region and year to year, (2) color inconsistency, (3) poor paste clarity, (4) cool water insolubility, (5) difficulties in obtaining high solid concentrations, (6) high viscosity at a low concentration, (7) viscosity breakdown at low pH, or high processing temperatures and under shear, (8) gelatinized native starches recrystallize or retrograde rapidly upon storage, and (9) rapid polymer re-association leading to syneresis. These impaired properties could be altered by modifying native starches by chemical and/or physical methods. The world leading exporters of modified (chemically or physically) potato and corn starches are Europe and the USA, respectively (Vasanthan *et al.*, 1999).

2.3 Starch granule

2.3.1 Architecture of the starch granule

Starch is a partially crystalline heteropolymer and is a major reserve polysaccharide in higher plants. The hilum is believed to be the botanical center or original growing point of the starch granule (Tester *et al.*, 2001, Hancock & Tarbet 2000, Baldwin *et al.*, 1994, Jane *et al.*, 1994, Lineback & Rasper 1988, French 1984, Lineback 1984) and the center of the 'Maltese Cross' (Baldwin *et al.*, 1994, Lineback & Rasper 1988, Lineback 1984). It has been hypothesized that the central area of the granule surrounding the hilum is the least organized region or the weakest point of the starch granule (Huber & BeMiller 2000, Baldwin *et al.*, 1994, French 1984), This was based on the observation that gelatinization (Baldwin *et al.*, 1994, Hoseney *et al.*, 1986), enzymic hydrolysis (Huber & BeMiller 2000, Baldwin *et al.*, 1994, Fuwa *et al.*, 1978, Leach & Schoch 1961), acid hydrolysis (Chabot *et al.*, 1978) and cavitation (Whistler & Thornburg 1957) all originate around this area. French (1984) reported that the hilum region may contain non-polysaccharide material from the amyloplast. A starch granule is a microcrystalline structure, comprised of alternatively arranged crystalline and non crystalline domains, and may have some transitional regions. Crystalline regions are believed to be mainly constructed of the outermost chains (A chains) of amylopectin [A-chains] and some of the inner chains [exterior B-chains] (Hizukuri 1996, Jenkins *et al.*, 1994). Amylose is thought to be mostly in an amorphous state (Hizukuri 1996). There are three major structural levels in starch granules; (1) microscopic [~0.3-150 μm] (Jayakody 2001, Jane *et al.*,

1994), (2) sub-microscopic: clusters [~10 nm] (Jane 2006, Cameron & Donald 1993a), and (3) lamellae [~4-6 nm] (Kiseleva *et al.*, 2005, Planchot *et al.*, 1997a, Jenkins & Donald 1996) [**Figure 2-2**].

2.3.2 Granular morphology

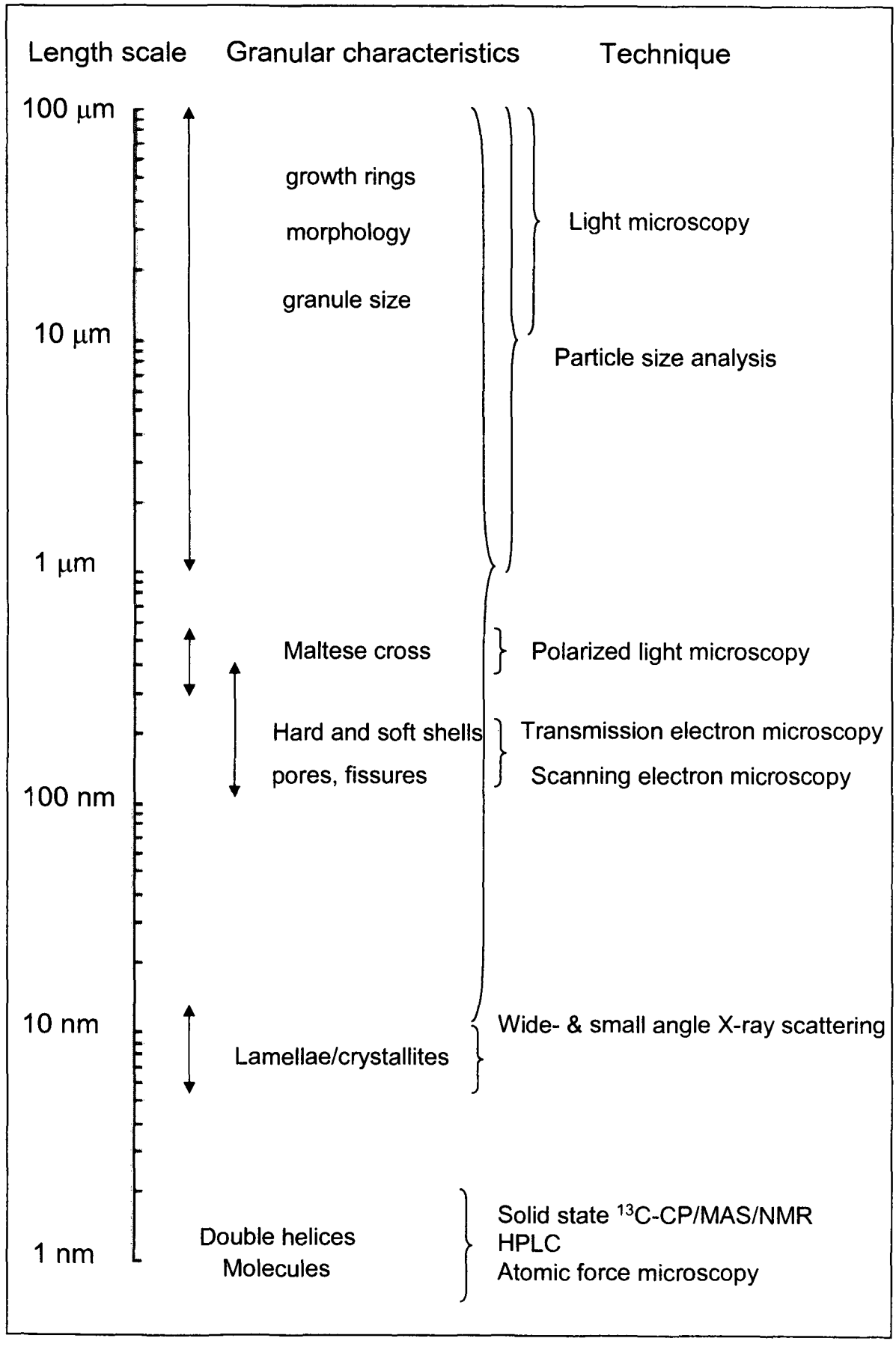
Visualisation of the starch granule is of great importance for a better understanding of the morphology, (Hoover 2001, Jane *et al.*, 1994), size (Jane *et al.*, 1994), surface features (Jayakody & Hoover 2002, Baldwin *et al.*, 1998, BeMiller 1997, Huber & Bemiller 1997, Fanon *et al.*, 1992), internal features (Lineback 1984), behavior of starch and starch pastes (Velde *et al.*, 2002), and topography (Aguilera 2000). Starch granule morphology and granule size are genetically controlled (Takeda *et al.*, 1999, Jane *et al.*, 1994). Radiotracer studies on potato tubers (Badenhuizen & Dutton 1956) and beans (Yoshida *et al.*, 1958) have shown that the granule grows by apposition (concentric deposition of successive layers). However, this process is dependent upon the supply of carbohydrates to the amyloplasts.

2.3.2.1 Microscopic techniques

Light microscopy and/or polarizing light microscopy are frequently used to study the shape, granule size, position of the hilum and size distribution of starch granules from various botanical sources (Jane *et al.*, 1994, Moss 1976, Wivinis & Maywald 1967). The resolution or resolving power (which determines the separating power of two adjacent points) is about 0.1-0.2 μm for an optical system (Velde *et al.*, 2002, Gallant *et al.*, 1997).

Figure 2-2 Comparative representation of starch granule size; techniques for their study and granuler characteristics

Adapted from Tester & Debon (2000) and information from: BeMiller (1997), Jane *et al.*, (1997), & (1994), Hizukuri (1996), Karathanos & Saravacos (1993), Cameron & Donald (1992)


Length scale
Granular characteristics
Technique
100 μm
10 μm
1 μm
100 nm
10 nm
1 nm
growth rings
morphology
granule size
Maltese cross
Hard and soft shells
pores, fissures
Lamellae/crystallites
Double helices
Molecules
Light microscopy
Particle size analysis
Polarized light microscopy
Transmission electron microscopy
Scanning electron microscopy
Wide- & small angle X-ray scattering
Solid state ^{13}C-CP/MAS/NMR
HPLC
Atomic force microscopy

Therefore, the submicroscopic structure of starch granules cannot be successfully ascertained with a light microscope [**Figure 2-2**]. Takaoka (1950) was the first to use the electron microscope to observe surface characteristics of starch granules. Scanning electron microscopy (SEM), environmental electron microscopy (ESEM), transmission electron microscopy (TEM), confocal scanning laser microscopy (CSLM) and atomic force microscopy (AFM) are commonly used instruments for investigation of both surface and submicroscopic features of the starch granules [**Figure 2-2**] (Shujun *et al.*, 2007 & 2006a,c, Ohtani *et al.*, 2000, Jacobs *et al.*, 1998c, Jane *et al.*, 1994, Fannon *et al.*, 1992). Granular morphology and size variation of different botanical sources are presented in **Table 2-1**.

2.3.2.1.1 Advantages, artefact formation and limitations of different microscopic techniques

Starch granules are non-electrical conducting biological particles, therefore, require coating with an electrical conductive material such as gold, palladium, carbon or combination of them. This decreases sample charging and improves the image quality (Baldwin *et al.*, 1998). However, the method of sample preparation, moisture content, accelerating voltage, beam current, vacuum conditions, hardness and softness of the granule, topography of granule clumps, sputter coating (i.e. sole metal or combination of a conductive coating such as carbon and metal), thickness of coating, nature of mount (e.g. electro- conductive or non-conductive double sticky), focusing and scanning speed, resolution, working distance, specimen tilt, and magnitude of magnification are critical

Table 2-1 Granular morphology and their size distribution of various tuber starches

Starch source	Granular shape	Granule size (μm)	Hilum	Specific area (m^2/g)	Reference
D. alata	Elliptical	6.8-47	-	-	Peroni *et al.*, 2006
D. alata	-	8-38	-	0.15	Riley *et al.*, 2006
D. alata	-	25.6	-	-	Riley *et al.*, 2004
D. alata	Round, ovoid	18.6-29.3	-	-	Amani *et al.*, 2004
D. alata	Rod like, round	19-49	-	-	Srichuwong *et al.*, 2005c
D. alata	Oval, shell shaped, ellipsoid	10-14	-	-	Moorthy 2002
D. alata	Oblong to oval	12-100	-	-	Gunaratne & Hoover 2002
D. alata	Ellipsoid, round	10-35	-	-	Farhat *et al.*, 1999
D. alata	Round-oval	6-100			Moorthy 1994
D. alata	-	20-140	-	-	Gallant 1982
D. alata	-	10-70	-	-	Rašper & Coursey 1967
D. esculenta	-	5.4	-	0.63	Riley *et al.*, 2006
D. esculenta	Polygonal	4.9	-	-	Srichuwong *et al.*, 2005c
D. esculenta	-	1-3	-	-	Riley *et al.*, 2004
D. esculenta	Polygonal	5.8-6.2	-	-	Amani *et al.*, 2004
D. esculenta	Round, oval, polyhedral, compound	1-15	-	-	Moorthy 2002
D. esculenta	Round-oval	1-5			Yu *et al.*, 1999
D. esculenta	-	1-5	-	-	Gallant 1982
D. esculenta	-	1-5	-	-	Rašper & Coursey 1967
D. rotundata	-	25.7	-	0.15	Riley *et al.*, 2006
D. rotundata	-	16-32	-	-	Riley *et al.*, 2004
D. rotundata	Flattened, ovoid	20.4-30.9	-	-	Amani *et al.*, 2004
D. rotundata	Oval, polyhedral oval, round, triangular	10-70	-	-	Moorthy 2002
D. rotundata	Polyhedral	10-30	-	-	Farhat *et al.*, 1999
*D. rotundata**	-	7.5-57.5	-		Moorthy & Nair 1989
D. rotundata	-	10-70	-	-	Rašper & Coursey 1967
D. dumetorum	Polygonal	2.2	-	-	Amani *et al.*, 2004
D. dumetorum	Round, polygonal	1-16	-	-	Moorthy 2002
D. dumetorum	Round	3-5	-	-	Farhat *et al.*, 1999
D. dumetorum	-	1-3	-	-	Gallant 1982
D. dumetorum	Round oval	28.5-30.6	-	-	Emiola & Delarossa 1981
D. dumetorum	-	1-5	-	-	Rašper & Coursey 1967

Table 2.1 Granular morphology and their size distribution of various tuber starches (cont.,)

Starch source	Granular shape	Granule size (μm)	Hilum	Specific area (m^2/g)	Reference
D. cayenensis	-	34.5	-	0.12	Riley *et al.*, 2006
D. cayenensis	-	16-42	-	-	Riley *et al.*, 2004
D. cayenensis	Polyhedral, ellipsoid	10-70	-	-	Moorthy 2002
D. cayenensis	Polyhedral, ellipsoid	10-30	-	-	Farhat *et al.*, 1999
D. cayenensis	-	10-70	-	-	Gallant 1982
D. cayenensis	Round-oval	28.5-30.6	-	-	Emiola & Delarossa 1981
D. polygonoides	-	15.3	-	0.26	Riley *et al.*, 2006
D. polygonoides	-	4-17	-	-	Riley *et al.*, 2004
D. opposita	Irregular	8-80			Shujun *et al.*, 2007
D. opposita	Round, polygonal, oval	5-60	-	-	Shujun *et al.*, 2006
*D. opposite**	Oval, polygonal	26-28	-	-	Shujun *et al.*, 2004
D. ballophylla	Elliptical	6-7-50			Soni *et al.*, 1985
D. abyssinica	Round	29.2	-	-	Mariam & Schmidt 1998
Solenostemon rotundifolius	Dome shaped and hemispherical	5-25	-	-	Jayakody *et al.*, 2005
Coleus paraviflorus	-	5-20	-	-	Moorthy 2002 & 1986
Coleus paraviflorus	Spherical to polygonal	2.5-17.5	-	-	Abraham & Mathew 1985
Solanum tuberosum	-	29.9-42.0			Karim *et al.*, 2007
Solanum tuberosum	Oval-spherical	42.3	-	-	Srichuwong *et al.*, 2005c
Solanum tuberosum	Oval-ellipsoidal	17-99-23.06	-	-	Yusuph *et al.*, 2003
Solanum tuberosum	Ellipsoid	12-60	-	-	Farhat *et al.*, 1999
Solanum tuberosum	Oval-spherical	5-100	Eccentric	0.1	Swinkels 1985a,Wivinis *et al.*, 1967
Cana edulis	Broad oval, elliptical	30-100	Centric	-	Jane *et al.*, 1994, Wivinis *et al.*, 1967
Manihot esculenta	Truncated oval	4-35	Centric	0.2	Swinkels 1985a, Wivinis *et al.*, 1967
Maranta arundinacea	Oval, spherical	35.1	Eccentric	-	Srichuwong *et al.*,2005c, Wivinis *et al.*,1967
Metrozylon sagu	Oval, spherical	34.3	Eccentric	-	Srichuwong *et al.*,2005c, Wivinis *et al.*,1967
Ipomea batatas	Polygonal, round	22.7	Centric	-	Srichuwong *et al.*,2005c, Wivinis *et al.*,1967
Musa paradisiaca	Irregular, oval or pear-shaped	-	Eccentric		Wivinis *et al.*, 1967
Xanthosoma sagitifolium	Polygonal to variable	3-10	-	-	Gunaratna & Hoover 2002
Colocasia esculenta (taro)	Polygonal , round to variable	10-50	-		Gunaratna & Hoover 2002
Caladium esculenta (eddo)	Oval, spherical	≤1	-	-	Wivinis *et al.*, 1967

* indicates different varieties

factors that influence the quality of the SEM image (Jayakody *et al.*, 2007a, Velde *et al.*, 2002, Baldwin *et al.*, 1998). A survey of the literature revealed that the conditions used in the visulalization of starch granules by SEM have not been fully reported. For instance, except for accelerating voltage, other experimental conditions are infrequently reported. When a nonconductive specimen such as starch is directly illuminated with an electron beam, its electrons collect locally, thus preventing normal emission of secondary electrons. This phenomenon is called charge-up or charging. Low acceleration voltage decreases beam damage and charge-up, resulting in a clear surface structure, however, it decreases resolution. In contrast, high voltage increases resolution but it also increases charge-up and beam damage. Many researchers have used an accelerating voltage from 10 to 30 kV and half of them were at the higher end. A majority of these SEM images were of poor qualtiy due to charge-up. It has also been reported that sample coating with conductive materials may decrease the visualization of the natural granule surface (Velde *et al.*, 2002), and this could lead to misinterpretation of granule morphology at very high resolutions due to the presence and size of the metal grains (Gunning *et al.*, 1995). Gallant *et al.*, (1997 & 1992) reported that methods used in the drying (e.g. warm air, hot air or by freeze-drying) of extracted starches yields comparable starch granule surface topologies. When observed under a conventional SEM, the surfaces of tuber starch granules appear smooth (Hoover 2001). However, Gallant *et al.*, (1997) observed that at an extremely low primary accelerating beam current ($1x10^{-13}$ A), the surface of potato starch granules is composed of large microscopic pores (up

to 500 nm diameter). Atomic force microscopy (AFM) has shown the presence of large protuberances (30-500 nm in diameter) on the surface of potato starch granules (Juszczak *et al.*, 2003a, Baldwin *et al.*, 1998, Baldwin 1995). These observations clearly show the limitations of different instruments and operational conditions.

In summary, SEM and other microscopic methods are very powerful and apparently non-destructive techniques for visualization of the starch granule surface. The accuracy of observations greatly depend on pre- and post-sample preparation and operational conditions.

2.3.2.2 Granule size and morphology variation

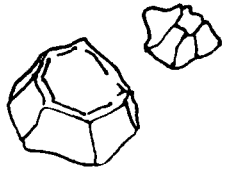
Enzymes involved in starch biosynthesis contribute to the pronounced differences in granule size, shape and characteristics between the various starch types (Baldwin 2001). Granule size and morphology are intimately associated terms. Generally, granule size refers to the average diameter of the starch granules, but may also be expressed as the average length of the major and minor axes. Granule size distribution is classified as monomodal (similar size) or bimodal (e.g. wheat starch large [A granule] and small size [B granule]). Recently Lindeboom *et al.*, (2004) proposed categorization of starch granules based on their size variation (e.g. very small [<5 μm], small [5-10 μm], medium [10- 25 μm], and large [>25 μm]). Aggregated or clustered starch granules are typical for small size granules (Lorenz 1990). The aggregate formation causes difficulty in accurate granule size estimation. Starch granules in plant leaves are generally

smaller (less than 1μm) than those in storage organs and have a distinct macrostructure (Hizukuri 1996, Martin & Smith 1995). *Dioscorea* starches have a quite wide variability in size and shape e.g. *D. esculenta* from 1 to 15 μm (Moorthy 2002) and *D. alata* from ~7 to 140 μm (Moorthy 2002, Gallant 1982). Morphological variations among starch granules from various botanical sources are shown in **Table 2-1** and **Figure 2-3**. *Colocasia* granules are the smallest starch granule reported in storage organs [0.3 μm] (Moorthy 2002).

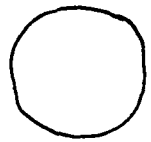
The starch granule is very heterogeneous and its features can vary within the same granule population, or species. Starch granular morphology is a composite picture of several features of the intact granule. However, the classical dictionary meaning of morphology refers only to the 3D view (Council of Science Editors 2006). Granule morphology is a relatively subjective expression, because surface and internal features depend on the quality and extent of reflection and/or refraction of light (e.g. polarized and non-polarized light). Therefore a description of granular morphology should include features such as: (1) external shape, (2) surface characteristics, (e.g. facets, topography, indentation, protrusion, fissures, color, textural appearance [smooth, grainy]), (3) structural anomaly between granules, and (4) refractive features under different light conditions (e.g. hilum, growth rings and birefringence). Pores, channels and central cavities are special anatomical features of starch structure, hence are discussed separately (section 2.3.3.2.1). Most tuber starch granules are oval or

Figure 2-3 Morphological variations among starch granules from various botanical sources

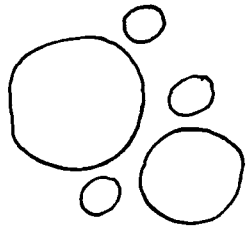
Information from: Jayakody *et al.*, (2005), Jayakody (2001a), Jane *et al.*,(1994), Wivinis & Maywald (1967), Buttrose (1963 & 1960)



Polygonal- Taro



Lence/Round- Triticali



Round-mix-Wheat

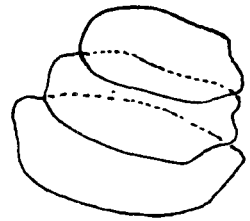
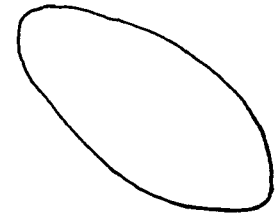
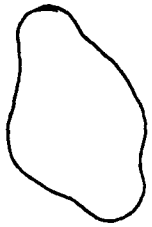


Plate-Shoti

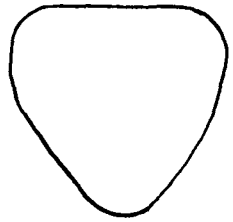
Oval-Canna



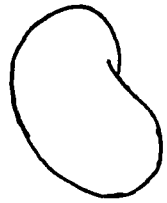
Ellipsoidal-Lotus



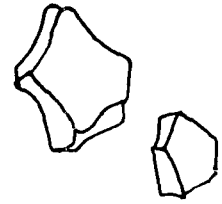
Irregular-oval- Arrowroot



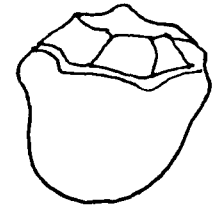
Truncated spade-yam



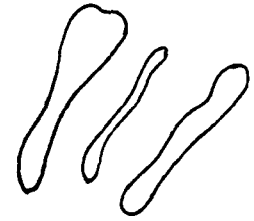
Kidney- Lentil



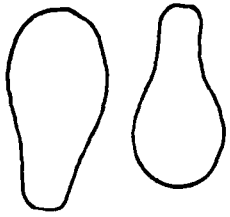
Sharp angular-Rice



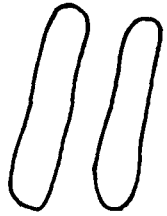
Dome-Innala



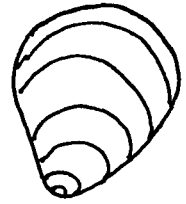
Rod- Highamylose corn



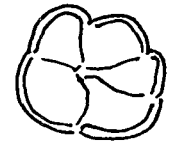
Pear-Kithul



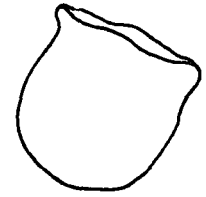
Cylindrical- dumb cane



Oyster shell-Canna

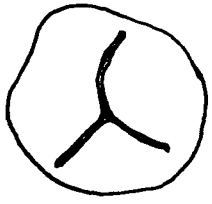


Rounded-rosette (compound) Highamylose pea

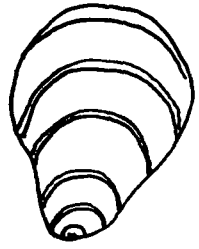


Hat/helmet-Tapioca

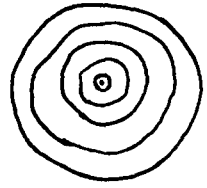
Lobe-Highamylose corn



Deep fissures-Alstroemeria

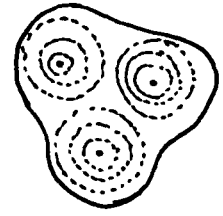


Eccentric hilum-Canna



Centric hila Lentil

Fissured hila-sago



Multiple hila Alstroemeria

Parallel ring-dumb cane

elliptical in shape. However, round, polygonal, truncated, spherical, dome and irregular shaped granules have also been reported. The surface of some starches (e.g. innala *Solenostemon rotundifolius*) have been reported to contain slightly concave facets (Jayakody *et al.*, 2005). In general most of the tuber and root starches are simple granules, however compound granules (e.g. taro) have also been reported (Hoover 2001).

Granule size has an appreciable influence on its properties, because granule composition changes as the granule grows and ages (Baldwin 2001, Geddles *et al*, 1965). Several factors such as starch composition (Meredith 1981, Kulp 1973), amylose-lipid complex (Chiotelli & Meste 2002, Myllärinen *et al.*, 1998a, Eliasson & Larsson 1983), chain length distribution (Naka *et al.*, 1985), gelatinization (Yusuph *et al* 2003, Kulp 1973, Stevens & Elton 1971), crystallinity (Wong & Leliévre 1982), pasting properties (Singh *et al.*, 2006, Jayakody *et al.*, 2005, Goering & DeHass 1972), granule swelling (Liu *et al.*, 2003, Wong & Leliévre 1982), solubility (Lindeboom *et al.*, 2004), susceptibility to enzyme and acid (Jayakody *et al.*, 2005, Kulp 1973), and quality of backed product (Kulp 1973, D'Appolonia & Gilles 1971) have been shown to reflect granule size.

Specific surface area

Specific surface area is another important parameter which influences the rate and extent of certain reactions (e.g. starch solubilization by acid or enzyme). The specific surface area of different starches have been reported (m^2/g): *D. alata* 0.15, *D. esculenta* 0.63, *D. rotundata* 0.15, *D. cayenensis* 0.12, *D. polygonoides* 0.26, (Riley *et al.*, 2006), potato 0.1-0.25 (Singh *et al.*, 2006, Yusuph *et al.*, 2003,

Hellman & Melvin 1950), cassava 0.2-0.25 (Swinkels 1985a, Hellman & Melvin 1950), grass pea 0.16 (Jayakody *et al.*, 2007a), corn 0.48 (Hellman & Melvin 1950), and wheat 0.25-0.9 (Baldwin 2001).

2.3.2.2.1 Effect of environment and growth period on granuler size

Tester and Karkalas (2001) reported that elevated temperatures result in fewer and smaller granules per unit volume of tissue because of the inactivation of specific biosynthetic and thermo-sensitive enzymes, such as soluble starch synthase. It has been reported that elevated temperature tends to decrease the number and/or size of starch granules in potato (Tester *et al.*, 1999, Cottrell *et al.*, 1995, Hizukuri 1969, Nikuni *et al.*, 1969), barley (Myllärinen *et al.*, 1998b, Tester *et al.*, 1991, Tester 1997, MacLeod & Duffus 1988), wheat (Tester *et al.*, 1995, Shi *et al.*, 1994) and corn (Jones *et al.*, 1985). In general the shape of a granule is independent of the environmental temperature (Hizukuri 1969), however, when the plant matures, the average size of the granule increases (Geddes *et al.*, 1965). For instance, Moorthy (2002) reported that granule size of *D. esculenta* and *D. alata* increases up to five months and then remains steady.

In summary, granular morphology and size are probably the most variable factors within the same starch source. Granule size has an appreciable influence on physicochemical properties, however, it is yet unknown what mechanism leads to variation in shapes and sizes of starch granules in the same or various botanical sources, and why such a variability is important to the plant.

2.3.3 Characteristics of granuler surface and interior

2.3.3.1 Starch granular surface

There is a dearth of information on the composition or chemical nature of the starch granule surface. However, it is known that the starch granule surface is mainly carbohydrate (~90-95%) in nature (Baldwin 1995), with lesser quantities of the minor starch components such as protein and lipids (Baldwin *et al.*, 1998). It has been shown that lipid, protein and amylose content increases towards the granule surface, indicating that the chemical nature of the granule surface is different from that of the granule interior. It is now recognized that the granule surface plays a significant role as the primary barrier to granule hydration, enzyme attack and chemical modification (Baldwin 2001).

2.3.3.2 Starch granule interior

2.3.3.2.1 Pores, channels and central cavities

(A) *Pores*

The small openings which are randomly distributed over the surface of the starch granules are called 'micro openings', 'microscopic pores', 'holes' or 'pin holes' (Baldwin 1994, Fannon *et al.*, 1992, Hall & Sayre 1970b). Hall and Sayre (1970b) were the first to report these features by SEM. Pores are present often in clusters (Fannon *et al.*, 1992) and in different quantities (Fannon *et al.*, 1992, Whistler *et al.*, 1958). However, the shape of the pore may vary from botanical source to source. Surface pores have been observed by SEM or environmental scanning electron microscopy (ESEM) in various starches such as innala (Jayakody *et al.*, 2005), rice (Juszczak *et al.*, 2003b, Jayakody & Hoover 2002), corn [normal,

waxy and high amylose] (Jayakody & Hoover 2002, Karathanos & Saravacos 1993, Fannon *et al.*, 1992, Fuwa *et al.*, 1978, Hall & Sayre 1970b), sorghum (BeMiller 1997, Fannon *et al.*, 1992, Hall & Sayre 1970b), barley (Waduge *et al.*, 2006, Li *et al.*, 2001), rye (Juszczak *et al.*, 2003b, Fannon *et al.*, 1992) and millet (Fannon *et al.*, 1992). Pores have been shown to be absent on the surface of potato (Fannon *et al.*, 1992, Hall & Sayre 1970b), cassava (Fannon *et al.*, 1992) and wheat (Hall & Sayre 1970b) starches. However, several other research groups have reported the presence of pores on the surface of potato (Juszczak *et al.*, 2003a,b, Baldwin *et al.*, 1994, Sterling 1973), cassava (Juszczak *et al.*, 2003a,b,) and on the small B granules of wheat (Baldwin *et al.*, 1994) starches. It has been reported that pores are rounded and centered in the potato starch granule at the intersection of the Maltese cross (Baldwin *et al.*, 1994). Sterling (1973) and Karathanos and Saravacos (1993) have shown that the pores on the surface of potato and normal corn starches vary from 0.5 nm to 75 nm and 3 nm to 100 nm, respectively. However, pores have not been reported in oat (Jayakody & Hoover 2002, Fannon *et al.*, 1992 Hall & Sayre 1970b), pinto bean, smooth pea, lentil (Zhou *et al.*, 2004), and grass pea (Jayakody *et al.*, 2007a) starches. Pores on granule surfaces have been shown to be true anatomical features of the native granule structure and not artefacts of starch isolation, drying, specimen preparation or microscopic techniques (Fannon *et al.*, 1992, Whistler *et al.*, 1959). However, it has been suggested (Li 2006) that pores in maize may also occur due to amylase attack on the granule surface during maturation and drying.

(B) *Channels*

The radial tube like connections that penetrate from the external surface inward towards a cavity at the hilum are called 'channels', 'tubelike channels' or 'serpentine channels' or 'twisted tubelike' channels (Huber & BeMiller 1997, Lynn & Stark 1992). Later investigations revealed that not all channels reached the hilum (Huber & BeMiller 2000). The channels are found to vary in depth of penetration from granule to granule (Huber & BeMiller 2000, Huber & BeMiller 1997). Fannon *et al.*, (1993) have postulated that all starch granules have pores and channels that are unobserved either because they are covered over the sputter coating materials or because they are too small to be viewed by SEM, yet large enough for passage for water, reagents, and macromolecules (e.g. enzymes). The presence of pores and channels in starch granules has been shown to influence its chemical reactivity towards modifying reagents (Whistler *et al.*, 1959). Huber and BeMiller (2000) have suggested that channels could provide direct access of reagents to a loosely organized region at the hilum. Channels opening to the granule exterior and penetrating into the granule interior has been found in starch granules *in situ* (i.e. native endosperm tissue). This indicates that the channels are normal structures and not artefacts (Fannon *et al.*, 1993). Clear evidence of channels has been reported for corn (Huber & BeMiller 1997, Karathanos & Saravacos 1993), and sorghum (Huber & BeMiller 1997). It has been shown that channels are more abundant in normal corn than in waxy corn (Huber & BeMiller 2000) starch. Channels have been shown to cross over the entire granule matrix, from the outer surface to the central cavity.

However, these pores and channels could be altered or enlarged by drying (Baldwin *et al.*, 1994, Karathanos & Saravacos 1993, Fannon *et al.*, 1992, Whistler *et al.*, 1958), moisture content (Whistler *et al.*, 1958) or by the method of starch extraction (Baldwin *et al.*, 1994). Gallant *et al.*, (1997) hypothesized that channels are not void spaces, but instead may contain amorphous material. The biological origin of channels remains unknown.

(C) *Cavities*

Central cavities were first reported by Reichert (1913). Cavities may not always be visible within hydrated granules due to complications of refractive index (Baldwin *et al.*, 1994). Cavities have been reported in granules of potato (Baldwin *et al.*, 1994, Hall & Sayre 1970a), canna (Hall & Sayre 1970a), normal corn (Zhao *et al.*, 1996, Hall & Sayre 1973), waxy corn (Chabot *et al.*, 1978), sorghum (Hall & Sayre 1973), barley (Hall & Sayre 1973), lentils (Revilla & Tárrago 1986) and rice (Baldwin *et al.*, 1994) starches. Cavity size in potato starch has been reported to vary from granule to granule (Baldwin *et al.*, 1994). No apparent relationship between granule size and cavity size has been reported, and it seems that granules of all sizes possess cavities (Baldwin *et al.*, 1994). Overall, the available evidence suggests that cavities located at the hilum of certain native starch granules are actual granule features and not artefacts of specimen preparation. However, Baldwin *et al.*, (1994), Whistler and Thornburg (1957) have suggested that formation and development of cavities in native starch granules occur on drying at elevated temperatures.

2.3.4 Starch damage

Brown and Heron (1879) identified that damaged starch granules result in greater rate of diastatic activity. Their efforts greatly contributed to the establishment of basic concepts for the study of starch properties such as absorption of water, gelatinization characteristics, granule swelling, pasting properties, enzyme susceptibility and dye absorption. A necessary prerequisite to starch property characterization is the isolation of starch granules without any damage (Karlsson & Eliasson 2003, Vasanthan *et al.*, 1999, Evers *et al.*, 1984). Several types of granule damage have been reported such as radial cracking, chipping or splitting, abrasions, and squashing or flattening (Williams 1969). Vasanthan and Hoover (1992a) reported the possibility of cross-contamination of surface (free and bound) with internal (free and bound) lipids, *vice versa*, during cold and hot solvent extractions of damaged starches. Furthermore, solvent extraction of physically damaged starches under certain conditions has been reported to be extract amylose with other starch components (Stark & Yin 1986). Morrison *et al.*, (1994) have shown that the double helical content and crystallinity decreases with the extent of starch damage.

2.3.4.1 Techniques of starch damage determination

Damaged starch differs from intact granules in a number of ways such as water absorption (Stevens & Elton 1971, Williams 1969, Sandstedt & Mattern 1960), solubility (Stark & Yin 1986, Craig & Stark 1984), susceptibility to staining with iodine and certain dyes [e.g. Schultze's solution, Congo red] (Williams 1969, Dadswell & Gardner 1947), and susceptibility towards amylase hydrolysis (Greer

& Stewart 1959, Sandstedt & Fleming 1954, Dadswell & Gardner 1947). Damaged granules can be distinguished either microscopically [e.g. preferential staining or partial or total loss of birefringence] (Stevens & Elton 1971, Williams 1969) or by susceptibility towards amylolytic enzymes (Jayakody *et al.*, 2005, Stark and Yin 1986, Stevens & Elton 1971, Sandstedt & Mattern 1960). It has been shown that methods based on amylolytic activity are more suitable due to their reliability, reproducibility, simplicity, convenience and minimum requirement for special equipment (Blish & Sandstedt 1933). Microbial amylases (e.g. fungal α-amylase *Aspergillus oryzae*) are more effective for short term hydrolysis than pancreatic amylases (Valetudie *et al.*, 1993) and are cost effective. Thus microbial amylases are widely used for damaged starch estimations.

2.3.5 Growth rings

Overview

Examination of hydrated or chemically treated (enzyme eroded or acid treated) (Jenkins & Donald 1996) starch granules under an optical (Tester & Karkalas 2001, French 1984) or electron microscope (Tester & Karkalas 2001, Gallant *et al.*, 1997, French 1984) has shown the presence of broad amorphous zones (so called light regions) that are interspersed with alternating crystalline and amorphous laminate structure (so called dark regions) that create concentric rings or growth rings (Tester & Karkalas 2001, Jenkins & Donald 1996, Jenkins *et al.*, 1993, French 1984, Lineback 1984, Yamaguchi *et al.*, 1979).

2.3.5.1 Theories of growth ring formation

The origin of growth rings remains obscure. Cameron and Donald (1992) and French (1972) have suggested that the rings represent periodic growth which reflects daily fluctuation (diurnal rhythm) in carbohydrate availability for starch deposition. However, this is not a new theory, since it was originally proposed by Meyer (1895). Yamaguchi *et al.*, (1979) suggested that part or all of the amylose and amylopectin molecules terminate at the boundary of a ring. Thus, the length of a starch molecule (i.e. amylose and amylopectin) would be similar to the thickness of a growth ring. It is believed that the molecular chains of starch are fairly perpendicular to these growth rings and to the surface of the granule (Yamaguchi *et al.*, 1979, French 1972, Kreger 1951). Extensive studies by small angle X-ray scattering (SAXS) and electron microscopy revealed that the semi-crystalline rings are composed of stacks of alternating crystalline and amorphous lamellae (Jenkins & Donald 1996). These lamellae are distinctly visible when they are oriented parallel to the electron beam (Yamaguchi *et al.*, 1979). The currently accepted crystalline structure model consists of a radial arrangement of clusters of amylopectin proposed originally by Robin *et al.*, (1974 & 1975).

The average thickness of the semi-crystalline growth rings in certain starch granules (e.g. wheat) increases with increasing amylose content (Yuryev *et al.*, 2004). The thickness and hardness of the growth rings differ with botanical origin; thickness tends to decrease towards the edges of the granules while hardness increases (Ridout *et al.*, 2003, Szymńoska & Krok 2003, Tang *et al.*, 2001a,b, Kaker *et al.*, 2001, Vasanthan & Bhatty 1996). There are no

recognizable growth rings at the center of the granule near the hilum (Ridout *et al.*, 2003, Tang *et al.*, 2001a,b).

2.3.5.2 Birefringence

The phenomenon of double refraction (i.e. two unequally reflected or transmitted waves) of light wave fronts in a molecularly ordered material is called birefringence. The starch granule has a high degree of molecular order. Thus, the ordering present in starch granules may be visualized at the molecular level as birefringence (Marchant & Blanshard 1980). Native starch granules are birefringent in polarized light and they exhibit the so called 'Maltese Cross', which may be centric or eccentric [**Table 2-1**]. Birefringence indicates the orientation of starch molecules (radial direction) and the intrinsic semicrystalline nature of the native starch granule (Cameron & Donald 1992). Birefringence and crystallinity are not essentially related to each other (Hoseney 1994, Lineback & Rašper 1988). Birefringence implies that a high degree of molecular order exists in the granule, without any relation to crystalline form. There is a lack of information on the birefringence and growth rings of *Dioscorea* starches.

In summary, growth rings are common features of starch granules. The interplay of circadian rhythms, physical mechanisms, and perhaps diurnal rhythms possibly contribute to the control of growth ring formation in starch granules (Matheson 1996). However, the exact mechanism of growth ring formation still remains unknown.

2.3.6 Granuler composition

Overview

Starch has been the subject of intensive scientific investigation for over two centuries. There have been a number of hypotheses put forward to explain the origin, nature and structure of the starch granule (Schimper 1880, Nägeli 1874, Maschke 1852, Payen 1838, Fritsche 1834). However, much of the early work is confusing. For instance, all varieties of sugar and sugar-like substances derived from starch were called amylose (Wiley 1881). Nägeli, in 1858, constructed a theory concerning the chemical nature of the starch granule, its manner of origin and subsequent growth. He postulated that the starch granule is made up of two substances called cellulose and granulose (Kraemer 1902, Gregory 1895). However, in later literature it was indicated that granulose gives a blue color with iodine (Kraemer 1902), what is now called amylose. Meyer (1895) postulated that starch is mainly composed of two substances and named them as α-amylose and β-amylose. Maquennel and Roux (1903) separated starch into two fractions by a precipitation method and called these fractions as 'amylose' and 'amylopectin'. Mayer and Bernfeld (1940) showed that amylose (linear molecule) and amylopectin (branched molecule) are two different fractions of starch. The molecular features of amylose and amylopectin were first studied by Hirst *et al.*, (1932). Branching of amylose was first suggested by Peat *et al.*, (1952).

2.3.6.1 Major components

2.3.6.1.1 Amylose

Amylose is synthesized by granular-bound starch synthase (Jane 2006, Smith 2001, Denyer *et al.*, 1999, Ball *et al.*, 1998, Martin & Smith 1995). Amyloses from various botanical sources are not exactly identical showing variation in molecular size and branching (Hizukuri 1996). The molecular weight and its distribution are characteristic of the amylose source (Takeda *et al.*, 1984, Hizukuri *et al.*, 1981). Amylose is found with molecular weights ranging from 1x 10^5 to 2x 10^6 Da (Tester & Karkalas 2001, Hizukuri *et al.*, 1989). In general, the molecular size of amylose is in the range 200-20,000 DP (degree of polymerization), however amyloses from tuber and root starches have fewer small amylose molecules than cereal amyloses (Hizukuri 1996). Generally, the distribution of molecular size of amylose by gel permeation chromatography or HPLC is monomodal (Takeda *et al.*, 1986). Molecular characteristics of amyloses are presented in **Table 2-2**.

Normal starches and high-amylose starches contain ~16-40% and ~40-92% amylose, respectively (Jayakody 2001, Shi *et al.*, 1998). Amylose is considered to be essentially a linear biopolymer, but it also contains a few branches. Tester and Karkalas (2001) reported that about 99:1 ratio of α-D-(1→4) to α-D-(1→6) bonds are present in the amylose molecule. It has been reported that hot-water soluble potato amylose is more branched than a similar amount of amylose containied in cereal starch (Murugesan *et al.*, 1993). Colonna and Mercier (1984) reported 2-3 branch points/molecule for pea starches. The side chains of rice

Table 2-2 Molecular characteristics of amylose and amylopectin from various tuber starches

Botanical source	Amylose			Amylopectin			β amylolysis	Reference
	Apparent (%)	Total (%)	DP_n	$\overline{CL}$	ECL	ICL	(%)	
D. alata	28.5	-	-	-	-	-	-	Karam *et al.*, 2006
D. alata	32.6	-	-	-	-	-	-	Peroni *et al.*, 2006
D. alata	32.2-34.3	-	-	-	-	-	-	Wang *et al.*, 2006
D. alata	23.4-25.4	-	-	-	-	-	-	Brunnschweiler *et al.*, 2005
D. alata	-	20.8	-	-	-	-	-	Srichowong *et al.*, 2005a
D. alata	25.3-27.4	-	-	-	-	-	-	Amani *et al.*, 2004
D. alata	21.0	-	-	-	-	-	-	Riley *et al.*, 2004
D. alata	36	-	-	-	-	-	-	Freitas *et al.*, 2004
D. alata	25-27	-	-	-	-	-	-	Rolland-Sabaté *et al.*, 2003
D. alata	21-30	-	-	-	-	-	-	Moorthy 2002
D. alata	24.6	28.5	1800	29	18.5	9.5	57 ± 4	Gunaratne & Hoover 2002
D. alata	21.5	-	-	-	-	-	-	Valetudie *et al.*, 1993
D. alata	-	-	2000	-	-	-	-	Suzuki *et al.*, 1986
D. alata	24.1	-	-	-	-	-	-	Soni *et al.*, 1985
D. alata	~30	-	-	-	-	-	-	Gallant *et al.*, 1982
D. alata	22.2	-	-	-	-	-	-	Emolia & Delarosa 1981
D. alata	~21	-	-	-	-	-	-	Rašper & Coursey 1967
D. esculenta	14.2	-	-	-	-	-	-	Srichowong *et al.*, 2005a
D. esculenta	14.1-15.7	-	-	-	-	-	-	Amani *et al.*, 2004
D. esculenta	11.1	-	-	-	-	-	-	Riley *et al.*, 2004
D. esculenta	16	-	-	-	-	-	-	Rolland-Sabaté *et al.*, 2003
D. esculenta	14	-	-	-	-	-	-	Gallant *et al.*, 1982
D. esculenta	14.2	-	-	-	-	-	-	Rašper & Coursey 1967
D. rotundata	25.2-28.8	-	-				-	Amani *et al.*, 2004
D. rotundata	22.1	-	-	-	-	-	-	Riley *et al.*, 2004
D. rotundata	25-29	-	-	-	-	-	-	Rolland-Sabaté *et al.*, 2003
D. rotundata	21.1-25.3	-	-	-	-	-	-	Moorthy 2002
D. rotundata	20.9-24.6	-	-	-	-	-	-	Moorthy & Nair 1989
D. rotundata	22.4	-	-	-	-	-	-	Emolia & Delarosa 1981
D. rotundata	20.9-24.6	-	-	-	-	-	-	Rašper & Coursey 1967
				-	-	-	-	
D. dumetorum	16.6	-	-	-	-	-	-	Amani *et al.*, 2004
D. dumetorum	17	-	-	-	-	-	-	Rolland-Sabaté *et al.*, 2003
D. dumetorum	25.4	-	-	-	-	-	-	Emolia & Delarosa 1981
D. dumetorum	10-15	-	-	-	-	-	-	Gallant *et al.*, 1982

Table 2-2 Molecular properties of amylose and amylopectin from various tuber starches (cont.,)

Botanical source	Amylose			Amylopectin			β amylolysis	Reference
	Apparent (%)	Total (%)	DP_n	$\overline{CL}$	ECL	ICL	(%)	
D. cayenensis	25.6	-	-	-	-	-	-	Brunnschweiler *et al.*, 2005
D. cayenensis	26.5	-	-	-	-	-	-	Riley *et al.*, 2004
D. cayenensis	23.2-27	-	-	-	-	-	-	Moorthy 2002
D. cayenensis	27						-	Gallant *et al.*, 1982
D. cayenensis	21.6	-	-	-	-	-	-	Emolia & Delarosa 1981
D. polygonoides	11.9	-	-	-	-	-	-	Riley *et al.*, 2004
D. polygonoides	21.17-25	-	-	-	-	-	-	Shujun *et al.*, 2006a
D. polygonoides	20.7-25.9	-	-	-	-	-	-	Shujun *et al.*, 2006c
D. abyssinica	29.7	-	-	-	-	-	-	Moorthy 2002
D. batata	34.0-34.6	-	-	-	-	-	-	Wang *et al.*, 2006
D. opposita				21.3			-	Hizukuri *et al.*, 1983
S. rotundifolius	16.25-22.95	18.7-25.2					-	Jayakody *et al.*, 2005
Coleus paraviflorus	-	33	-	-	-	-	-	Moorthy 1986
Coleus paraviflorus	18.5	-	-	-	-	-	-	Abraham & Mathew 1985
Solanum tuberosum	24.5	-	-	-	-	-	-	Brunnschweiler *et al.*, 2005
Solanum tuberosum	18-7-23.9	-	-	22.5-22.9	-	-	-	Kim *et al.*, 2007, Hizukuri 1983
Solanum tuberosum	-	-	2110-4920	-	-	-	-	Hoover 2001
Solanum tuberosum	18-33	-	-	-	-	-	-	Yusuph *et al.*, 2003
Solanum tuberosum	25.2	28.1	4850 ± 75	28.1	18.6	8.5	-	Gunaratna & Hoover 2002
Cana edulis	-	27.9					-	Srichowong *et al.*, 2005a
Cana edulis				27			-	Hizukuri *et al.*, 1983
Manihot esculenta	-	17.9	-	-	-	-	-	Srichowong *et al.*, 2005a
Manihot esculenta	19.8	22.4	2500 ± 62	24.5	16.2	7.3	58 ± 2	Gunaratna & Hoover 2002
M. arundinacea	-	20.0	-	-	-	-	-	Srichowong *et al.*, 2005a
Metrozylon sagu	-	21.9	-	-	-	-	-	Srichowong *et al.*, 2005a
Ipomea batatas	19.8	-	-	20.3-20.9	-	-	-	Hizukuri 1983
X. sagitifolium	19.8	-	-	-	-	-	-	Valetudie *et al.*, 1993
X. sagitifolium	26.1	29.3	2775	24.2	15.8	7.4	57 ± 2	Gunaratna & Hoover 2002
C. esculenta (taro)	22.3	26.4	2200	26.1	16.6	8.4	56 ± 4	Gunaratna & Hoover 2002

Apparent & total amylose content was determined by I_2 binding before and after removal of bound lipids, respectively

DP_n: number average degree of polymerization $\overline{CL}$: average chain length ECL: external chain length ICL: internal chain length

amylose has been reported to range from ~10 to 4000 DP, however the majority of side chains are less than 100 DP (Hizukuri 1996). The molar ratio of super long (DP>200), long (30-200 DP) and short (10-30 DP) rice amylose side chains are 4:3:20, respectively. This suggests that predominant side chains are similar to amylopectin (Takeda *et al.*, 1993).

2.3.6.1.2 Location of amylose in starch granule

The exact location of amylose and amylopectin in native granule is still in dispute. Jane and Shen (1993) have shown using stepwise chemical [$CaCl_2$ 4M, at 23°C] gelatinization, that potato and corn starch granules have increasing amylose concentration towards the granule periphery relative to that at the core. Studies on amylose leaching, V-complex formation, and iodine vapour complexing suggests that amylose is separated from amylopectin in normal corn starch but interspersed among amylopectin in potato starch (Zobel 1988). Blanshard (1986) proposed that amylose is present in the amorphous area partially co-crystallized with amylopectin (e.g. potato starch). Jane *et al.*, (1992a) have postulated that amylose (e.g. from potato, and corn starch) could be randomly mixed together with individual molecules of amylopectin rather than being in bundles of amylose.

2.3.6.1.3 Factors influencing amylose concentaration in various botanical sources

Amylose content has been shown to be influenced by the environmental temperature (Debon *et al.*, 1998, Cottrell *et al.*, 1995, Asaoka *et al.*, 1987, Hizukuri 1969), date of planting (Hizukuri 1969, Zuber 1965), seasonal variation [e.g. wet or dry season] (Madamba & San Pedro 1976), age of crop (Moorthy

2002), age of tuber (Sugimoto *et al.*, 1987, Geddes *et al.*, 1965), harvesting period (Liu *et al.*, 2003), granule age and size (Jane 2006, Shujun *et al.*, 2006c, Wasiluk *et al.*, 1994, Zayas *et al.*, 1994, Jones *et al.*, 1992, Matheson 1971, Geddes *et al.*, 1965) and length of storage (Sriroth *et al.*, 1999).

2.3.6.1.3.1 Amylose determination techniques

Different methods such as (1) colorimetry (Jayakody *et al.*, 2005, Juliano *et al.*, 1981, Sowbhagya & Bhattacharya 1971, Williams *et al.*, 1958, McCready & Hassid 1943), (2) potentiometry (Bates *et al.*, 1943), (3) semi-micro differential potentiometry (Banks & Greenwood 1975), (4) amperometry (Larson *et al.*, 1953), (5) differential scanning calorimetry [DSC] (Moorthy *et al.*, 2006, Sievert & Lausanne 1993, Kugimiya & Donovan 1981), (6) high performance size exclusion chromatography [HPSEC] (Grant *et al.*, 2002), or gel permeation chromatography [GPC] (Sargeant 1982), (7) near infrared reflectance [NIR] (Delwiche *et al.*, 1995), (8) preferential precipitation (Yun & Matheson 1990) and (9) polarimetry (Shuman & Plunkett 1964) have been used for amylose determination.

All of the above methods are useful but suffer from some inherent drawbacks. Colorimetry is very popular due to its versatility and simplicity. However, the major objection to the colorimetric method is the strong interference of amylopectin in the iodine-amylose color at low pH and difficulties in solubilizing the sample. The amylose-iodine color is blue at neutral to basic pH but is green at acid pH (Perez & Juliano 1978). However, it has been shown by a cooperative

study (Juliano *et al.*, 1981), that phosphate buffers greatly increase the stability of the starch iodine-blue color complex. Other negative features of the colorimetric method are the tendency of amylose to retrograde in aqueous solution, and the susceptibility to interference by bound lipids (Morrison & Laignelet 1983, Sargeant 1982). The amperometric/potentiometric method shows high accuracy, but the process is tedious and the broad inflection point can lead to inaccuracies. DSC has proven to be satisfactory for high amylose starches. Total amylose determination by DSC does not require a prior defatting step. However, it gives an over estimation for low amylose starches (Sievert & Lausanne 1993). The NIR procedure is rapid but requires tedious standardization and validation processes, and also gives a poor response to high-amylose starches (Polaske *et al.*, 2005). The GPC and HPSEC methods are reliable, but the GPC procedure is laborious and needs costly enzymes (Fredriksson *et al.*, 1998). The HPSEC method is simple and uses deionized water as the eluant but amylose is not stable in water (Grant *et al.*, 2002). The preferential precipitation method essentially precipitates the amylopectin fraction, but it needs costly enzymes and is time consuming. The polarimetric method is sensitive, however the downside of this method is the requirement for heavy metal containing reagents (e.g. $HgCl_2$).

2.3.6.1.4 Amylose conformation

The amylose molecule is presently believed to be arranged in a left-handed helix due to the natural twist present in the chair conformation of glucose (Kowblansky 1985). The helix consists of six glucose units per turn, with exterior, and central cavity diameters of 12.97 Å, and 5 Å, respectively (Fonslick & Khan 1989, Rundle

& French 1943). Takeo and Kuge (1969) and Krog (1971) reported that the inside diameter of the helix can vary between 4.5~6.0 Å, where the helix consists of 6 or 7 glucose units per helical turn. The amylose helix is stabilized by hydrogen bonds between the hydroxyl groups of adjacent glucosyl residues and inter-turn hydrogen bonds located on the outer surface of the helix (Banks & Greewood 1975). Studies of light scattering, viscosity analysis and molecular weight have shown that the conformation of the amylose helix appears to be either a random helical coil (6 glucose units per turn) [**Figure 2-4-A-(a)**] (Banks & Greenwood 1971), interrupted helix [i.e. segregated helical with 10-15 turns and linear parts in the same molecule] [**Figure 2-4-A-(b)**] (Szejtli & August 1966), or a deformed helix/worm-like coil (Rao & Foster 1963) in aqueous solution [**Figure 2-4-A-(c)**]. Amylose in solid state shows two X-ray diffraction patterns which are similar to the A- and B-type of amylopectin crystallites in native starches (Wu & Sarko 1978a).

2.3.6.1.5 Co-crystallization of amylose

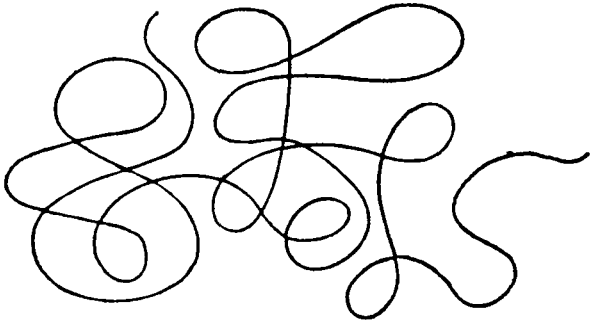
The effects of varying amylose content on the internal granular structure of normal, waxy and high amylose corn starches have been studied by small angle X-ray scattering (Jenkins & Donald 1995). These authors showed that an increase in amylose content increases the size of the crystalline portion of the amylopectin cluster and that amylose acts to disrupt the packing of the amylopectin double helices within the crystalline lamellae. Jenkins and Donald (1995) have suggested that the disrupting effect of amylose on amylopectin

Figure 2-4 Models proposed for **(A)** amylose conformation in aqueous solutions: (a) random coil, (b) interrupted helix and (c) deformed helix/worm-like coil **(B)** amylose-lipid complex **(C)** linear-chain structure of amylose

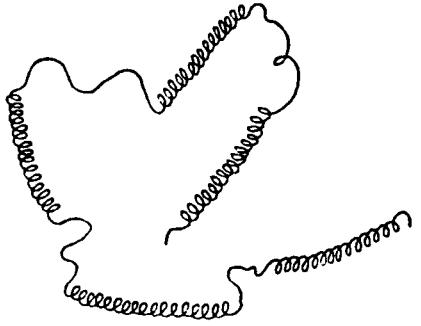
Adapted from Banks & Greenwood (1975), and Carlson *et al.*, (1979) with permission from: Edinburgh University Press, & Wiley InterScience

(A) Amylose conformation

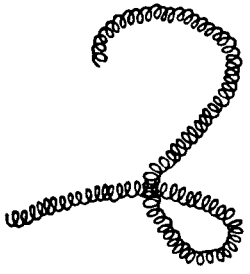
(a)



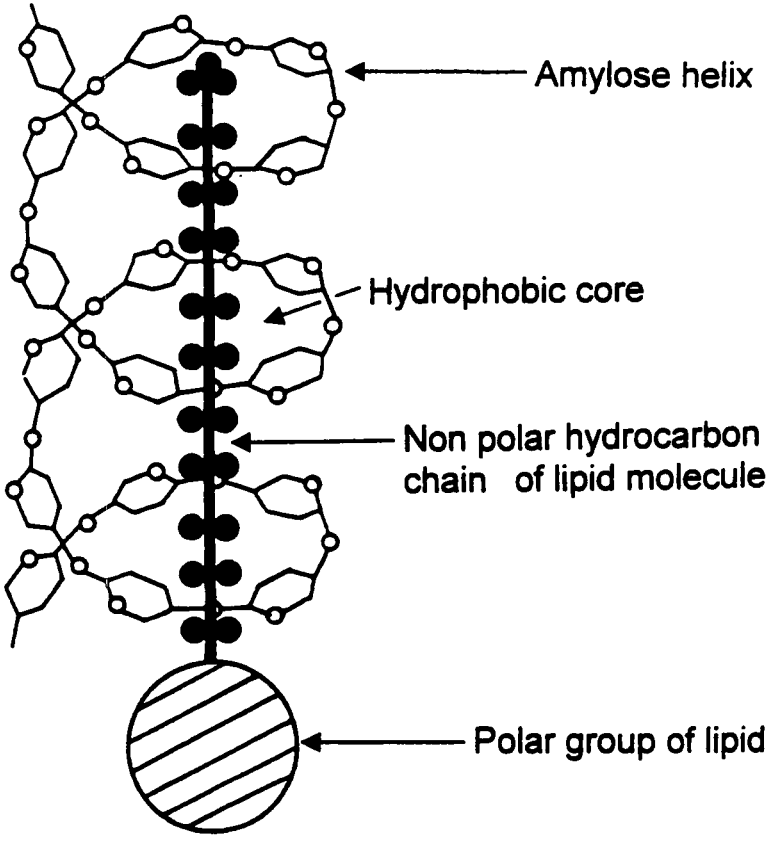
(b)

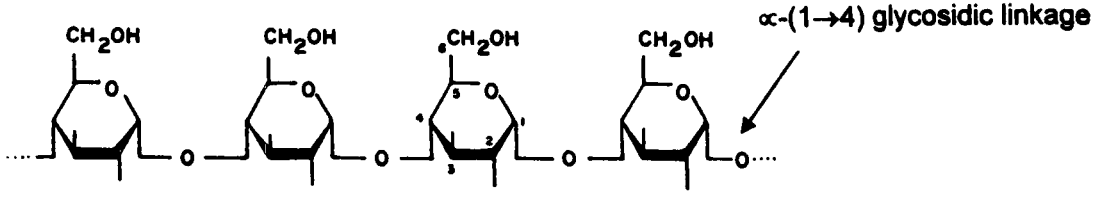


(c)



(B) Amylose-lipid complex





(C) Linear-chain structure of amylose

structure could be due to (1) co-crystallization of a portion of an amylose chain into a hybrid amylose/amylopectin helix within the crystalline lamellae, or (2) penetration of amylose into the amorphous regions where the a α-D-(1→6) branch points are located. However, Jane *et al.*, (1986) have postulated that amylose in the normal starch interacts with amylopectin, thereby preserving starch granule integrity. However, it is not clear whether both mechanisms occur concurrently or separately. Waduge *et al.*, (2006) suggested that when the amylose content reaches a certain threshold, amylose chains may interact with amylopectin.

2.3.6.1.6 Amylose inclusion complexes

The formation of inclusion complexes between glucan chains and a hydrophobic guest molecule has long been known (Mikus *et al.*, 1946). The long linear nature of amylose chains gives them some unique properties, such as the ability to form complexes with iodine, fatty acids, organic alcohol, or acids. Such complexes are called *clathrates* or helical inclusion compounds (Hoseney 1994).

2.3.6.1.6.1 Amylose-lipid complex

It has been known for many years that saturated distilled monoglycerides form insoluble inclusion complexes with amylose. Complex formation between corn starch and fatty acids was first reported by Schoch and Williams (1944). The core of the amylose helix consists solely of C-H bonds and is thus hydrophobic [**Figure 2-4-B**]. Consequently, the hydrophobic part of the fatty acid chain has a great affinity for the core of the amylose helix. However, Waduge *et al.*, (2006)

reported lipids in starches may also be present trapped in the spaces between amylose and amylopectin. Potato starches include little or no lipids, and other tuber and root starches contain less lipids than those from cereals (Hizukuri 1996). Complex formation with amylose is dependent on the length of the fatty acid molecule. It has been reported that lipids and surfactants are required to have a minimum of 8 carbons in the fatty acid chain (Yamamoto *et al.*, 1984) to form a complex. However, chain lengths between 12-18 (Kowblansky 1985, Krong 1970), 14-18, (Russell 1983) or carbons ≥ 12 (Tufvesson *et al.*, 2003) are required for optimum complexing effect. It has been reported that optimum complexing occurs when the fatty acid chain length is between C-12 & C14 (Hoover & Hadziyev 1981).

The crystalline melting temperature of amylose-lipid complex has been reported to occur in the range ~85-125°C (Waduge *et al.*, 2006, Nakazawa & Wang 2004, Jacobs *et al.*, 1998a, Karkalas *et al.*, 1995, Slade & Levine 1988, Biliaderis *et al.*, 1986b, Biliaderis *et al.*, 1985, Kugimiya & Donovan 1981, Russell 1987b). Amylose-lipid complexes exhibit a V-type X-ray diffraction pattern centered at ~20 2θ (Evans 1986, Kowblansky 1985, Jane & Robyt 1984, Buléon *et al.*, 1984, Yamamoto *et al.*, 1984). The V-type crystalline structure of fatty acid-amylose complexes is formed by single helices with six anhydroglucose monomer resides per helical turn (Gallant *et al.*, 1992). Amylose-lipid complexes have been shown to greatly restrict the hydration capacity, and granule swelling of starch (Hoover 2001, Zheng & Sosulski 1997, Swinkels 1985a), thus amylose-lipid complex greatly influences the functional properties of starch.

2.3.6.1.6.2 Iodine-starch complex

The formation of a blue color when starch is reacted with iodine was first observed in 1814 (Colin & Claubry 1814). Until 1937, it was a classical test for identification of starch. However, Hanes (1937) hypothesized that the blue color may be due to complex formation between amylose & iodine with iodine) occupying the helical cavity of the amylose helix. It was later established that Hanes (hypothesis was correct (Rundle *et al.*, 1944, Rundle & Baldwin 1943, Rundle & French 1943, Rundle & Edwards 1943). The actual nature of the iodine chromophore within the amylose helix has been the subject of considerable speculation and controversy. One of the classical schools of thought is that the triiodide ion (I_3^-) must be present to form a blue color with the iodine-starch complex (John *et al.* 1983, Hatch, 1982, Zitomer & Lambert 1962, Lambert 1951). However, Teitelbaum *et al.*, (1980 & 1978) have postulated that the principal chromophore was the pentaiodide (I_5^-) ion. The amylose-iodine complex is pH sensitive and is thermally unstable. The complex is stable below 15°C (Fonslick & Khan 1989). Teitelbaum *et al.*, (1980) have shown that I_5^- ion breaksdown into the triiodide (I_3^-) ion and molecular iodine at elevated temperatures. Monoacyl lipids (free fatty acids and lysophospholipids) competitively inhibit the accessibility of iodine into the amylose helix, and thus reduces its ability to form a complex (Morrison & Laignelet 1983, Mius *et al.*, 1946). However, lipid interferences can be overcome by defatting native starch.

2.3.6.1.6.2.1 Effect of chain length, composition of amylose & amylopectin on color intensity

The color of the iodine-amylose complex may vary as a result of the chain length (DP) of amylose, which influences its binding capacity with iodide anions. John *et al.*, (1983) showed that the color of the complexes changes from blue (DP>47), blue-violet (DP 39-46), red-violet (DP 30-38), red (DP 25-29), brown (DP 21-24), and finally no color (achroic limit [where the iodine coloration disappears]) at a DP below 20. The most stable complex of linear amylose with iodine is formed above DP>40 (Yamamoto *et al.*, 1984).

Studies on amylopectin have shown that a minimum of 15-20 glucose units of the outer branches are required for complex formation with iodine at low temperatures (Banks *et al.*, 1970). In general, amylopectin fractions of high-amylose cereal and legume starches have longer A- and B-chains than other starches. For instance, super long amylopectin B-chains (average chain length of DP 85-180) have a very high affinity for iodine (Takeda *et al.*, 1987). These chains could bind iodine resulting in an overestimation of amylose content (Morrison & Karkalas 1990).

In general, the absorbance maxima (λ_{max}) value for normal starch is greater than 600 nm [**Table 2-3**]. However, several researchers have shown that branched amylose molecules greatly decrease the λ_{max} of the starch-iodine complex (Zeeman *et al.*, 2002, Fuwa *et al.*, 1999). The λ_{max} also depends on the proportion of amylose and amylopectin in the mixture [**Table 2-3**] (McGrance *et al.*, 1998). The solubility of starch will influence variability between samples.

Table 2-3 Absorbance maxima of amylose (AM) and amylopectin (AMP) from various starch sources

Starch source	λ_{max} (nm)	Reference
Amylose (purity not indicated) from Yam, Nagaimo	658	Hizukuri 1996
100% amylose from potato	636	McGrance *et al.*, 1998
100% amylose from potato	618	Hovenkamp-Hermelink *et al.*, 1998
Amylose from potato*	660	Hizukuri 1996
Amylose from lily*	648	Hizukuri 1996
Amylose from edible canna*	654	Hizukuri 1996
Amylose from cassava*	662	Hizukuri 1996
Amylose from sago*	653-656	Hizukuri 1996
Amylose from kuzu*	656	Hizukuri 1996
Amylose from water chestnut*	640	Hizukuri 1996
Amylose from chestnut	655	Hizukuri 1996
100% amylose from rice (Japonica)	656-658	Takeda *et al.*, 1987
100% amylose from (Indica)	653-657	Takeda *et al.*, 1989 & 1987
Amylose from wheat*	636-648	Shibanuma *et al.*, 1994
Amylose from barley*	652-653	Hizukuri 1996
Amylose from normal maize*	643-645	Hizukuri 1996
Amylose from high amylomaize*	645-650	Hizukuri 1996
100% amylose from smooth-pea	625	Colonna & Mercier 1984
100% amylose from wrinkled-pea	630	Colonna & Mercier 1984
100% amylopectin from *D. alata*	556-558	Wang *et al.*, 2006
100% amylopectin from *D. batata*	555	Wang *et al.*, 2006
100% amylopectin from potato	552	McGrance *et al.*, 1998
100% amylopectin from potato	550	Hovenkamp-Hermelink *et al.*, 1998
100% amylopectin from potato	548	Jarvis & Walker 1993
Amylopectin from potato*	560	Hizukuri 1996
Amylopectin from edible canna*	558	Hizukuri 1996
Amylopectin from sago*	528-532	Hizukuri 1996
Amylopectin from kuzu*	556	Hizukuri 1996
Amylopectin from water chestnut*	554	Hizukuri 1996
100% amylopectin from rice (Japonica)	531-542	Takeda *et al.*, 1987

Table 2-3 Absorbance maxima of amylose (AM) and amylopectin (AMP) from various starch sources (cont.,)

Starch source	λ_{max} (nm)	Reference
100% amylopectin from (Indica)	542-575	Takeda *et al.*, 1989 & 1987
Amylopectin from wheat*	547-560	Shibanuma *et al.*, 1994
Amylopectin from barley*	540-546	Hizukuri 1996
100% amylopectin from corn	521	McGrance *et al.*, 1998
Amylopectin from normal maize*	554	Hizukuri 1996
Amylopectin from high amylomaize*	573-575	Hizukuri 1996
100% amylopectin from smooth-pea	560	Colonna & Mercier 1984
100% amylopectin from wrinkled-pea	560	Colonna & Mercier 1984
75% potato AM + 25% potato AMP	628	McGrance *et al.*, 1998
50% potato AM + 50% potato AMP	613	McGrance *et al.*, 1998
25% potato AM + 75% potato AMP	592	McGrance *et al.*, 1998
10% potato AM + 90% potato AMP	566	McGrance *et al.*, 1998
70% corn AM + 30% corn AMP	604	McGrance *et al.*, 1998
Corn starch (amylose content not specified)	606	McGrance *et al.*, 1998
Corn starch (normal)	590-593	Fuwa *et al.*, 1999
Corn starch (waxy)	535-536	Fuwa *et al.*, 1999
Wheat starch (amylose content not specified)	617	McGrance *et al.*, 1998
Wrinkled pea starch (amylose content not specified)	605	Colonna & Mercier 1984
Smooth pea starch (amylose content not specified)	595-600	Colonna & Mercier 1984
Intermediate material	570-580	Hizukuri 1996

* Purity was not available Absorbance maxima: (λ_{max})

Absorbance maxima of iodine solution (0.0025M I_2/0.0065M KI): 352 nm (McGrance *et al.*, 1998)

This must be kept in mind during amylose quantification.

The stability of amylose and complexes follows the order: pure anhydrous amylose crystals (Biliaderis *et al.*, 1986a, & 1985) >> amylose-lipid complex (Slade & Levine 1987) > hydrated amylose (Biliaderis *et al.*, 1986a) > amylose-iodine complex (Yamamoto *et al.*, 1984).

2.3.6.1.2 Amylopectin

Amylopectin is one of the largest branched biomolecules found in nature (Falk *et al.*, 1996). It is synthesized by soluble starch synthases (Jane 2006), which is responsible for the biosynthesis of super-long branch chains of amylopectin (Denyer *et al.*, 1999). The initial proof of α-D-(1→6) branch linkage in amylopectin was provided by oxidation of the methylated amylopectin molecule with periodic acid (Halsall *et al.*, 1947). The final proof of the presence of the α-D-(1→6) linkage was demonstrated by the isolation of panose from a partial acid hydrolysis of waxy corn amylopectin (Thompson & Wolform 1951). The molecular weight (weight average) of amylopectin can vary from 10^8 to 10^9 Da for cereal, tuber and root starches (Jane 2006, Hoover 2001). The molecular weights of normal and high-amylose starch amylopectins are smaller than that of the waxy starch amylopectin (Jane 2006). Amylopectin molecules radiate from the hilum (Tester & Karkalas 2001, Hancock & Tarbet 2000). Amylopectin is an important component in food as it can lead to time dependent changes in the texture and digestibility of food (Kalichevsky *et al.*, 1990). Molecular characteritics of amylopectin are summarized in **Table 2-2**.

2.3.6.1.2.1 Structure of amylopectin

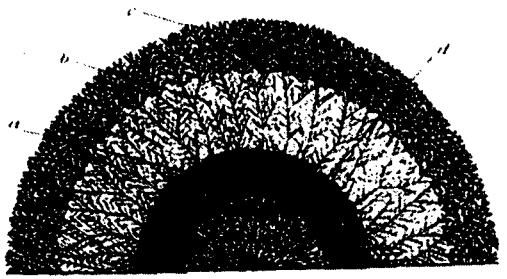
The concentric trichitic model was first suggested by Arthur Mayer in 1895 [**Figure 2-5-a**]. Since then, a number of models of amylopectin have been proposed including; (1) the laminated structure [**Figure 2-5-b**] (Haworth *et al.*, 1937), though not intended as a complete representation of the true molecule, (2) the herring-bone structure Staudinger and Husemann (1937), in which the single main chain carries all the branch linkages [**Figure 2-5-c**], and (3) the randomly-branched or tree like structure [**Figure 2-5-d**] (Meyer & Bernfeld 1940).

2.3.6.1.2.1.1 Development of cluster model

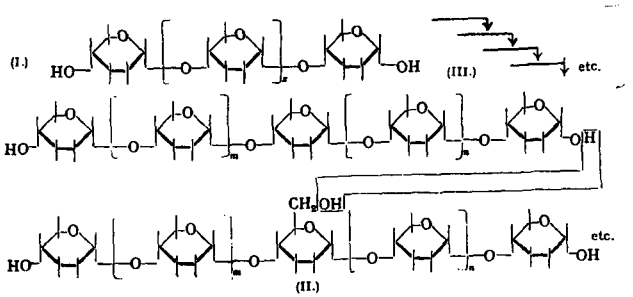
The Mayer and Bernfeld (1940) model has been significantly amended since the late 1960's due to its inability to explain certain structural and physicochemical property related issues such as: (1) how amylopectin molecules make a crystalline structure (Kainuma & French 1972, Mark 1940, Katz & van Itallie 1930), (2) why a bimodal chain length distribution exists in the amylopectin fraction (Gunja-Smith *et al.*, 1970) and (3) why amylopectin molecules show higher viscosities ([η] ~90-150 cm^{-3}/g) than glycogen ([η] ~6-13 cm^{-3}/g) of similar molecular weight (Yamaguchi *et al.*, 1979). However, thirty years later a revised version of Meyer and Bernfeld's model was proposed by Gunja-Smith *et al.*, (1970) [**Figure 2-5-e**]. Nikuni's (1969) [**Figure 2-5-f**], French's (1972) [**Figure 2-5-g**] and later Hizukuri's (1986) [**Figure 2-5-i**] remarkable contribution led to the development of the cluster model [**Figure 2-6**].

Figure 2-5 Schematic representation of the models proposed for amylopectin structure

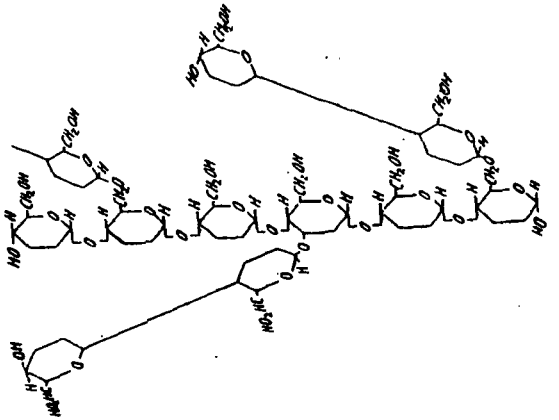
Adapted with permission from Marcel Dekker, Inc.,



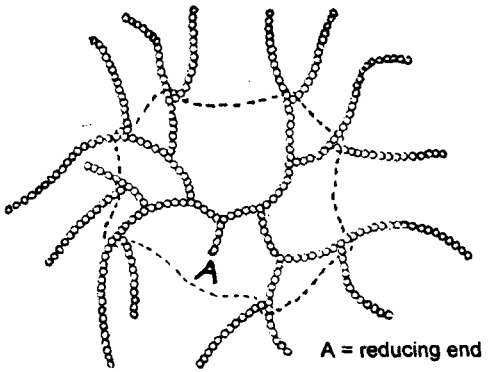
(a) Arthur Mayer-1895



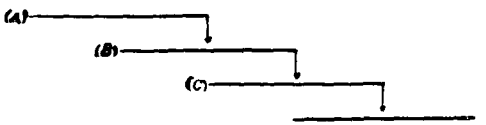
(b) Haworth *et al.*,-1937



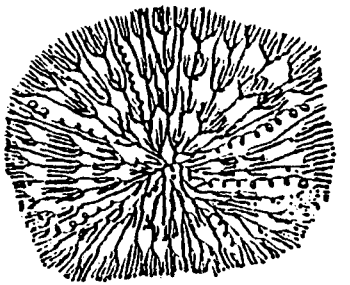
(c) Staudinger & Husemann-1937



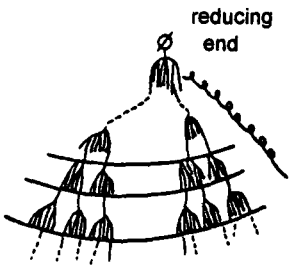
(d) Meyer & Bernfeld-1940



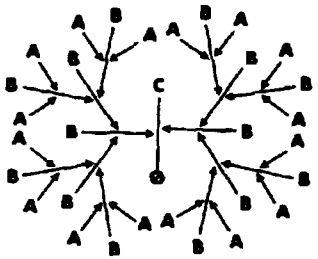
Halsall *et al.*,-1947



Nikuni-1969

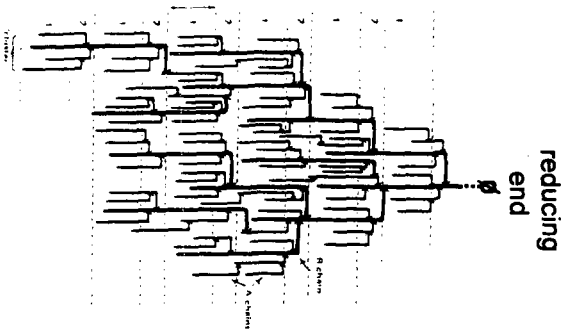


(e) Nikuni-1969

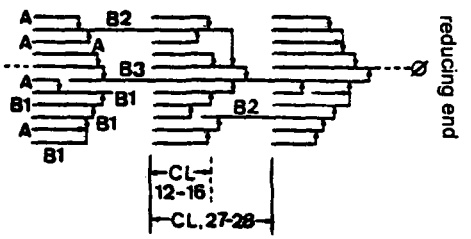


(f) Gunja-Smith *et al.*,-1970

(g) French-1972



(h) Robin *et al.*, -1974



(i) Hizukuri-1986

This model was able to answer the above questions successfully: (1) the double helices of amylopectin form the crystallites (French 1972), (2) the amylopectin molecule is composed of varying chain lengths which correspond to bimodal or polymodal distributions (Hizukuri 1986, Sargeant 1982), and (3) the viscosity of amylopectin depends on molecular shape, chain-length distribution (longer the chain length higher the viscosity), amount of ionized groups (e.g. phosphate groups) and on its molecular weight (Hizukuri 1996).

2.3.6.1.2.1.2 Nomenclature and classification of branch chains

Amylopectin has three types of chains kown as A-, B- and C-chains. Peat *et al.*, (1956) developed a systematic nomenclature for these chains. A-chains are the outer most unbranched and are linked to the rest of the molecule through their reducing end-group. B-chains could be linked in similar way but carries other A- and/or B-chains at one or more of its primary hydroxyl groups (Peat *et al.*, 1956). However, the ratio of the A to B varies chains greatly with different botanical sources [A: B: 1~2:1] (Hizukuri 1996). Every amylopectin molecule has a single C-chain with a free reducing end group [**Figure 2-6**].

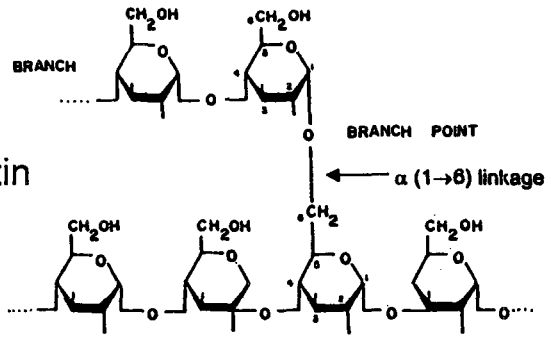
2.3.6.1.2.1.2.1 Chain length distribution and cluster model

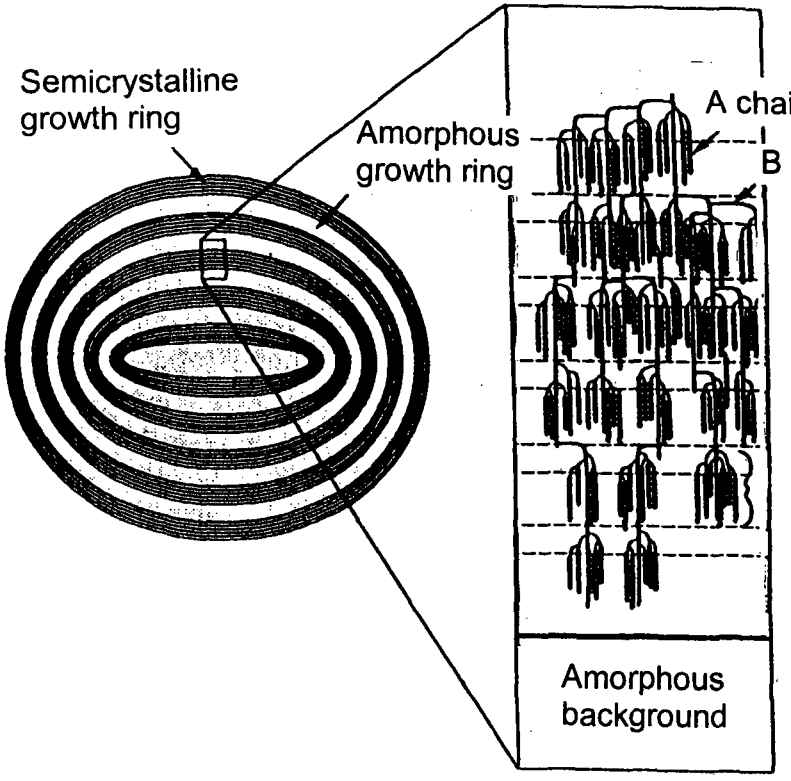
Enzymatic methods which use specific α-D-(1→6) glucosidases, collectively called debranching enzymes, have been developed to study the fine structure of amylopectin. The common debranching enzymes are isoamylase and pullulanase (Gunja-Smith *et al.*, 1970). Isoamylase is more useful since it completely debranches the amylopectin molecule.

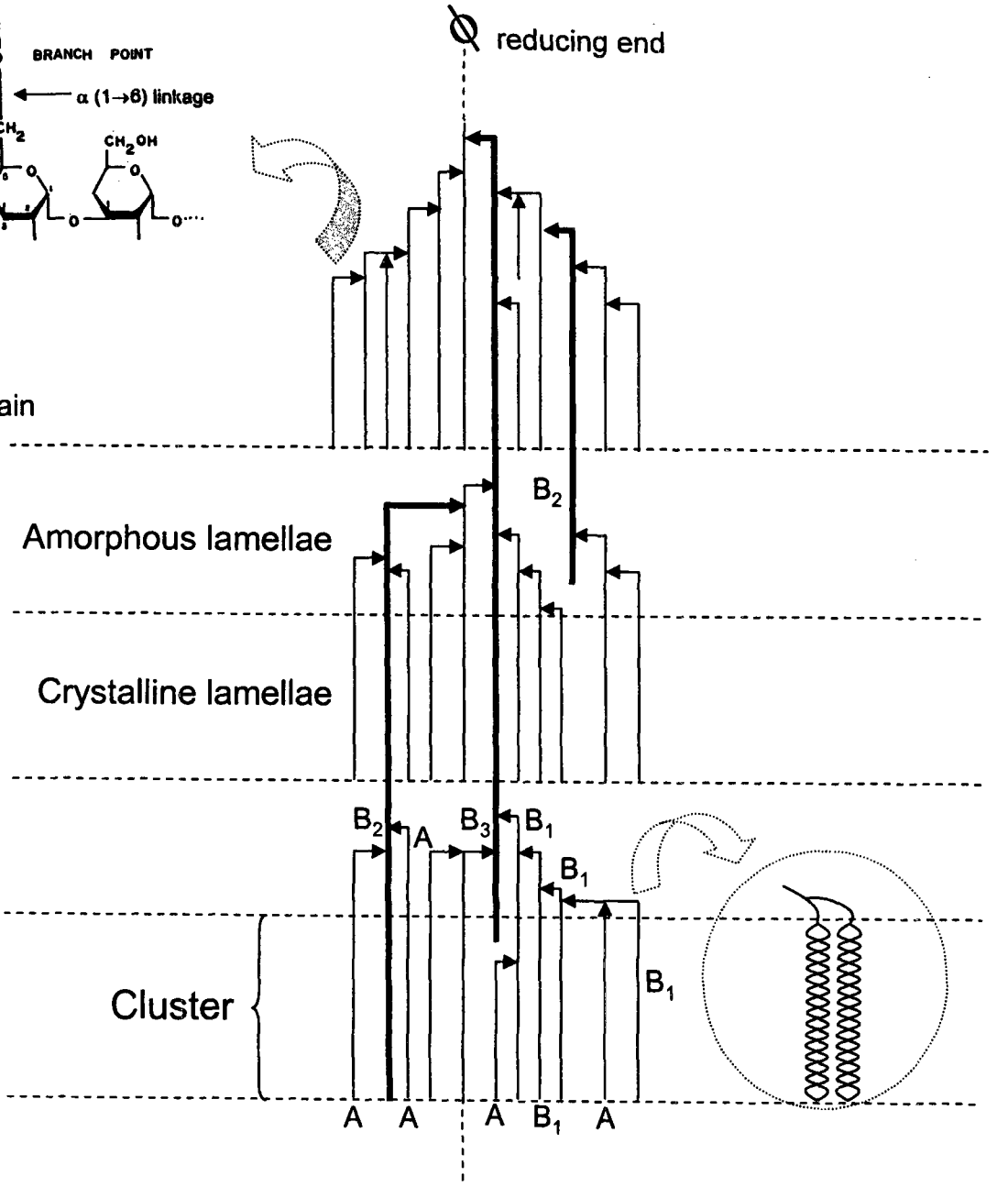
Figure 2-6 Schematic diagram of starch granule structure and branch chain length distribution of amylopectin

Adapted from Jenkins *et al.*, (1994) and Hizukuri (1986) with permission from Wiley InterScience and Elsevier

Branch structure of amylopectin







Gel permeation chromatography has shown a bimodal distribution (FII & FIII) of chain length which corresponds to the A-chains (FIII: DP<18) and B-chains (FII: 18>DP<60). However, more segregated polymodal distribution of B-chains (such as B_1, B_2, B_3) [**Figure 2-6**] has been reported by HPLC techniques (Hizukuri 1986). The compact parts of oriented chains present between adjacent branch points is called a cluster and it is the basic unit of the cluster model (Hizukuri 1986). These primary units are linked by long chains which traverse into two or more clusters. A single cluster is composed mainly of A and B_1 (80-90%) chains, and approximately 10% of B_2 chains connect two clusters, 1-3% of B_3 and very few (0.1-0.6%) B_4 chains connect three and four clusters, respectively (Jane *et al.*, 1999, Jane *et al.*, 1997, Hizukuri 1996) [**Figure 2-6**]. It is envisaged that within a single cluster, chains have an average DP of 14-18, while longer chains with an average DP of 45-55 interconnect multiple clusters (Vermeylen *et al.*, 2004). Shorter chains with DP 6-24 consist of A and B_1 chains (Hanashiro *et al.*, 1996, Hizukuri 1986). The average chain lengths of B_1, B_2, and B_3 fractions are in the range DP: 20-24, 42-48, and 69-75, respectively, and the relative lengths are approximately 1:2:3. The A and B_1 are side or lateral chains while B_2 to B_4 basically act as connecting chains in the amylopectin molecule [**Figure 2-6**]. It has been estimated that 80-90% of the total number of chains in an amylopectin molecule are involved in forming the lateral chain clusters, whilst the remaining 10-20% of chains form the inter-cluster connections (Manners 1989).

2.3.6.1.2.1.2.1.1 Average chain length

The average chain length of most amylopectins are in the range DP 18 to 24 (Hizukuri 1996). In general the average chain length of cereal starches (mainly A-type) are shorter than tuber (mainly B-type) and legume starches (mainly C-type) (Hizukuri 1985). Average chain length of *Dioscorea* starches has been shown to vary from DP 18 to 29 (Wang *et al.*, 2006, Gunaratne & Hoover 2002, McPherson & Jane 1999, Emiola & Delarossa 1981). Little or no influence of environmental temperature on average chain length of potato starch has been observed (Hizukuri 1969, Nikuni *et al.*, 1969). However, Geddes *et al.*, (1965) reported that average amylopectin chain lengths of starch decreases with increasing tuber maturity stage.

2.3.6.1.2.1.2.1.2 Long, super long B chains and lamellae formation

A single super long amylopectin chain extends over several lamellae, and double helical branches in adjacent crystalline lamellae are covalently bound to each other through amylopectin α-D-(1→6) branch points (Hizukuri 1986). Branch points reside mainly in the amorphous lamellae [Figure 2-11-A] (Jenkins *et al.*, 1994, Oostergetel *et al.*, 1989, Robin *et al*, 1974), whereas amylopectin double helices (formed by short chains) are present in the crystalline lamellae (Jane 2006, Jane *et al.*, 1997, Jenkins & Donald, 1996 & 1995, Jenkins *et al.*, 1994, Robin *et al.*, 1975). Jane *et al.*, (1997) have suggested that α-D-(1→6) linkages are more confined to the amorphous lamellae in B-type than in A-type starches [Figure 2-11-A]. **Figure 2-6** depicts an expanded view of the internal granular structure. Crystalline double helices are called short-range order, whereas, long

range order refers to alternating of crystalline and amorphous lamellae (Jenkins *et al.*, 1994). However, according to Russell (1987a,b) short-range ordering involves both bulk amorphous regions and intercrystalline areas, permitting double helix formation between amylose and amylopectin (i.e. co-crystallization). It is clear that long chains are mandatory for the formation of long range order in starch structure. Short A-chains form clusters while long B-chains help to connect the cluster. The super long chain (DP>100) content is significantly higher in *Dioscorea* starches than in cassava, kuzu (Hizukuri 1986), rice (Lu *et al.*, 1997b), wheat (Shibanuma *et al.*, 1996), and potato (Hizukuri 1986) amylopectins. However, no definitive DP limits have been set for short, long and super long unit chains.

2.3.6.1.2.1.2.2 Periodicity or repeat distance

It has been hypothesized, that the starch granule is composed of 10-40 clusters (~15 nm wide) and the length of the amylopectin molecule is believed to correspond to the thickness of the growth rings (Jenkins & Donald 1995, Martin & Smith 1995). Studies on small angle X-ray scattering (SAXS) have shown that long-range (alternatively arranged crystalline and amorphous lamellae) periodicities in various moist starch granules vary from 9 to 11 nm for potato, sweet potato cassava, rice, barley, wheat, normal corn and waxy corn starches (Vermeylen *et al.*, 2004, Jenkins *et al.*, 1997, Blanshard *et al.*, 1984, Hizukuri *et al.*, 1964, Sterling 1962). Jenkins *et al.*, (1997) postulated that the uniform periodicity or repeat distance (combined size of crystalline and amorphous lamellae) is independent of the botanical source and it is found to be 9 nm.

Kiseleva *et al.*, (2005) reported that the amylopectin cluster size is approximately 10 nm and cluster thickness is not influenced by amylose content. However, lamellae thickness depends on the maturity stage of the plant. For instance, lamellae thickness of fully matured pea starch is 9.1 nm, whereas at the milky stage of development it is 6.3 nm (Yurvev *et al.*, 2002, Kozhevnikov *et al.*, 2001). This indicates that double helical formation and crystallite perfection is influenced by the thickness of the crystalline lamellae. However, the authors have not mentioned variations in the thickness of the intercrystalline lamellae during the maturation period.

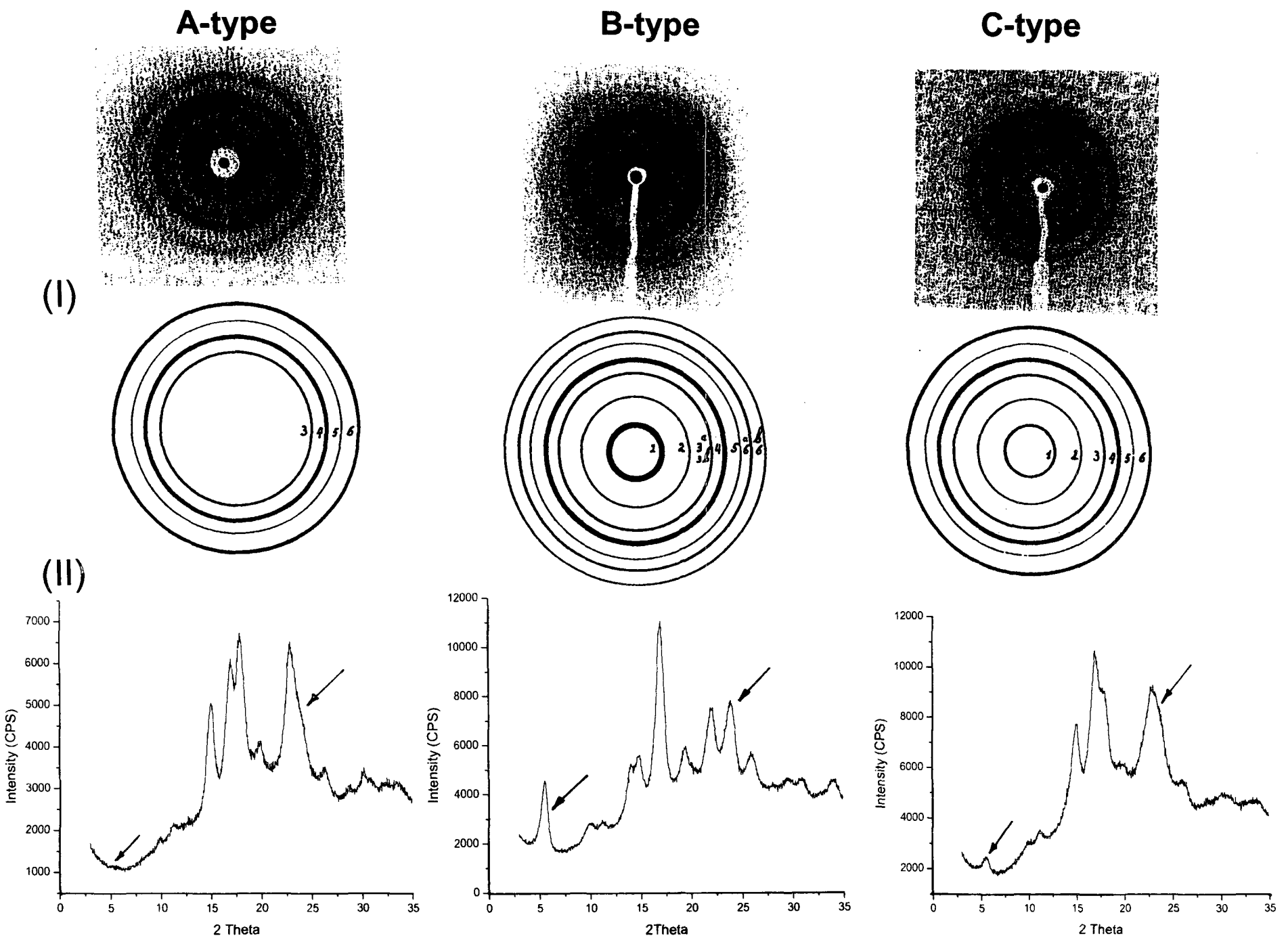
2.3.6.1.2.2 Powder X-ray diffraction and crystallinity

Overview

The semi-crystalline nature of the starch granule was first demonstrated by X-ray diffraction (Scherrer 1920). Náray-Szabó (1928) analyzed different starches, and such as yam, potato, canna, cassava, turmeric, sweet potato, and cereals. He was the first to suggest 'potato group' (*kartoffelgruppe*) and 'rice group' (*reisgruppe*) X-ray diffraction patterns in native starches [**Figure 2-7-I**]. The classical work of Katz and van Itallie (1930) established that starch granules contain sufficiently ordered crystalline areas to diffract X-rays, and were the first to introduce three types of X-ray diffraction patterns [*Röntgenspektrum*] or "spectrums" designated as A- (wheat, rice, corn, rye & oat), B- (potato, canna & common hourse-chestnut) and C-patterns (arrowroot, cassava, banana & sago) and V-spectrum ('V' refers to 'Verkleisterung' which means agglutination or 'complexing'). **Figure 2-7** shows the original Katz and van Itallie (1930)

Figure 2-7 Debye-Scherrer **(I)** and digital spectrums **(II)** of A-, B-, & C-type starches

Adapted from Katz & van Itallie (1930) and Jayakody *et al.*, (2007c, & 2006)



Arrows indicate major differences in peak intensities in A, B, and C type starches

Debye-Scherrer patterns of A-, B-, & C-type [**Figure 2-7-I**] and their modern digital spectra [**Figure 2-7-II**].

2.3.6.1.2.2.1 Wide angle X-ray scattering (WAXS) & small angle X-ray scattering (SAXS)

Wide angle X-ray diffraction patterns of various botanical sources are presented in **Table 2-4**. Wide angle X-ray scattering (WAXS) quantifies crystalline order throughout starch granules, but small angle X-ray scattering (SAXS) quantifies differences (periodicity) at the level of amorphous-crystalline lamellae radiating from the hilum to the periphery of starch granules (Tester & Debon 2000). Thus, SAXS is a versatile tool for the determination of scattering density of absorbed water within the crystalline lamellae, amorphous lamellae and amorphous background of the starch granule (Jenkins & Donald 1996). Cameron and Donald (1992, 1993a, & 1993b) developed a model fitting technique to generate electron density profiles from SAXS. Low electron density profiles have been reported in more water rich areas of the starch granule (e.g. corn starch). The crystalline lamellae contain the highest density of carbohydrate and the lowest density of water, whereas, the reverse order has been reported for the amorphous lamellae (Perry & Donald 2000). SAXS has shown that more water is present within the amorphous lamellae than in the amorphous background region of the granule (Perry & Donald 2000, Jenkins & Donald 1996). The above authors suggested that the amorphous lamella is less compact than the amorphous growth ring.

Table 2-4 Polymorphic patterns, crystallinity, method of moisture equilibration & diffractormeter settings for various starches

Starch source	Polymorphic pattern	Crystallinity (%)	Moisture equilibration	Diffractometer settings	Reference
D. alata	B	*	90 min over water	40kV, 35mA, 4-30° 2θ	Brunnschweiler *et al.*, 2005
D. alata	B	29.5	**	30kV, 10mA, 4-30° 2θ	Srichowong *et al.*, 2005a
D. alata	B,C	31-41	Moisture 16-18%	40kV, 30mA, 3-30° 2θ	Amani *et al.*, 2004
D. alata	B	*	Over saturated $CuSO_4$, 2 Wks	-, -, 4-38° 2θ	Riley *et al.*, 2004
D. alata	B	32	**	40kV, 100mA, 3-35° 2θ	Gunaratne & Hoover 2002
D. alata	C	*	Over saturated $CuSO_4$, 2 Wks	40kV, 50mA, 4-38° 2θ	Farhat *et al.*, 1999
D. alata	B	*	**	40kV, 50mA, -	Valetudie *et al.*, 1993
D. alata	B	*	-	-	Gallant *et al.*, 1982
D. esculenta	C	27.8	**	30kV, 10mA, 4-30° 2θ	Srichowong *et al.*, 2005a
D. esculenta	B,C	26-35	Moisture 16-18%	40kV, 30mA, 3-30° 2θ	Amani *et al.*, 2004
D. esculenta	C	*	Over saturated $CuSO_4$, 2 Wks	-, -, 4-38° 2θ	Riley *et al.*, 2004
D. esculenta	C	*	Over saturated $CuSO_4$, 2 Wks	40kV, 50mA, 4-38° 2θ	Farhat *et al.*, 1999
D. esculenta	B	*	-	-	Gallant *et al.*, 1982
D. rotundata	B,C	27-45	Moisture 16-18%	40kV, 30mA, 3-30° 2θ	Amani *et al.*, 2004
D. rotundata	B	*	Over saturated $CuSO_4$, 2 Wks	-, -, 4-38° 2θ	Riley *et al.*, 2004
D. cayenensis	B	*	90 min over water	40kV, 35mA, 4-30 2θ	Brunnschweiler *et al.*, 2005
D. cayenensis	B	*	Over saturated $CuSO_4$, 2 Wks	-, -, 4-38° 2θ	Riley *et al.*, 2004
D. cayenensis	B	*	-	-	Gallant *et al.*, 1982
D. cayenensis	B	*	Over saturated $CuSO_4$, 2 Wks	40kV, 50mA, 4-38° 2θ	Farhat *et al.*, 1999
D. dumetorum	A	37	Moisture 16-18%	40kV, 30mA, 3-30° 2θ	Amani *et al.*, 2004
D. dumetorum	A	*	Over saturated $CuSO_4$, 2 Wks	40kV, 50mA, 4-38° 2θ	Farhat *et al.*, 1999
D. dumetorum	A	*	-	-	Gallant *et al.*, 1982
D. oposita	C	43.1	**	40kV, 40mA, 4-40° 2θ	Shujun *et al.*, 2007
D. oposita	C	34-49	**	40kV, 40mA, 4-40° 2θ	Shujun *et al.*, 2006a
D. oposita	C	31.5-45.9	**	40kV, 40mA, 4-40° 2θ	Shujun *et al.*, 2006b
D. oposita	C	32.99-50.2	**	40kV, 40mA, 4-40° 2θ	Shujun *et al.*, 2006c
Dioscorea (spp. NA)	C_a	*	100% RH, 24h	50kV, 27mA, 4-40° 2θ	McPherson & Jane 1999

Table 2-4 Polymorphic patterns, crystallinity, method of moisture equilibration & diffractormeter settings for various starches (cont.,)

Starch source	Polymorphic pattern	Crystallinity (%)	Moisture equilibration	Diffractometer settings	Reference
D. hispida Dennst	B	*	**	40kV, 30mA, 5-45° 2θ	Tattiyakul *et al.*, 2006
D. polygonoides	A	*	Over saturated. $CuSO_4$, 2 Wks	-, -, 4-38° 2θ	Riley *et al.*, 2004
S. rotundifolius	C_a	37-40	Over saturated $BaCl_2$, 1 Wk	40kV, 100mA, 3-35° 2θ	Jayakody *et al.*, 2005
Coleus paraviflorus	A	*	-	-	Abraham & Mathew 1985
Solanum tuberosum	-	29.8	**	40kV, 20mA, 5-35° 2θ	Zhang *et al.*, 2006
Solanum tuberosum	B	43	Over Sat. NaCl, -	NA, NA, 6.5-33° 2θ	Vermeylen *et al.*, 2006
Solanum tuberosum	B	29.8	**	30kV, 10mA, 4-30° 2θ	Srichowong *et al.*, 2005a
Solanum tuberosum	B	30	**	40kV, 100mA, 3-35° 2θ	Gunaratne & Hoover 2002
S. tuberosum- normal	B	*	Over 100 RH, 24h	50kV, 27mA, 4-40° 2θ	McPherson & Jane 1999
S. tuberosum- waxy	B	*	Over 100 RH, 24h	50kV, 27mA, 4-40° 2θ	McPherson & Jane 1999
Solanum tuberosum	B	24-32	**	40kV, 40mA, 4-30° 2θ	Yusuph *et al.*, 2003
Solanum tuberosum	B	*	**	40kV, 50mA, -	Valetudie *et al.*, 1993
Cana edulis	B	27.2	**	30kV, 10mA, 4-30° 2θ	Srichowong *et al.*, 2005a
Manihot esculenta	A	35.8	**	30kV, 10mA, 4-30° 2θ	Srichowong *et al.*, 2005a
Manihot esculenta	A	37	**	40kV, 100mA, 3-35° 2θ	Gunaratne & Hoover 2002
Manihot esculenta	C_a	*	**	40kV, 50mA, -	Valetudie *et al.*, 1993
Maranta arundinacea	A	34.6	**	30kV, 10mA, 4-30° 2θ	Srichowong *et al.*, 2005a
Metrozylon sagu	A	32.9	**	30kV, 10mA, 4-30° 2θ	Srichowong *et al.*, 2005a
Ipomea batatas	A	34.4	**	30kV, 10mA, 4-30° 2θ	Srichowong *et al.*, 2005a
Ipomea batatas	C	*	Over 100 RH, 24h	50kV, 27mA, 4-40° 2θ	McPherson & Jane 1999
Ipomea batatas	C_a	*	**	40kV, 50mA, -	Valetudie *et al.*, 1993
Kudzu	A	34.4	**	30kV, 10mA, 4-30° 2θ	Srichowong *et al.*, 2005a
Lathyrus sativus L.	C	33-34	Over saturated K_2SO_4, 1 Wk	40kV, 100mA, 3-35° 2θ	Jayakody *et al.*, 2007a
Caryota urenes	C	42	Over saturated K_2SO_4, 1 Wk	40kV, 100mA, 3-35° 2θ	Jayakody *et al.*, 2007b
Borassus flabellifer L.	C	39	Over saturated K_2SO_4, 1 Wk	40kV, 100mA, 3-35° 2θ	Jayakody *et al.*, 2007b
Artocarpus heterophyllus	C	35	Over saturated K_2SO_4, 1 Wk	40kV, 100mA, 3-35° 2θ	Jayakody *et al.*, 2007b
X. sagitifolium	A	33.2	**	30kV, 10mA, 4-30° 2θ	Srichowong *et al.*, 2005a
X. sagitifolium	A	45	**	40kV, 100mA, 3-35° 2θ	Gunaratne & Hoover 2002
X. sagitifolium	C_a	*	**	40kV, 50mA, -	Valetudie *et al.*, 1993
Colocasia esculenta	A	31	**	40kV, 100mA, 3-35° 2θ	Gunaratne & Hoover 2002
Colocasia esculenta	A	35.3	**	30kV, 10mA, 4-30° 2θ	Srichowong *et al.*, 2005a

* Not determined -: Data not available ** Moisture equilibration was not carried out prior to X-ray diffraction

2.3.6.1.2.2.1.1 Nomenclature of polymorphic patterns and diffraction angles

The modern wide angle powder X-ray diffractometer produces a digital signal. Consequently, it is relatively easy to determine polymorphic patterns and granule crystallinity. Most cereal, tuber and legume starches exhibit A-, B-, and C-type X-ray diffraction patterns, respectively [**Figure 2-7**], although some exceptional cases have been reported (Jayakody *et al.*, 2007b, Jayakody & Hoover 2002, Cheetham & Tao 1998, Hizukuri 1969 & 1960). For instance, X-ray diffraction patterns of high amylose cereal and legume starches show a B-type polymorphic pattern and low crystallinity (Jayakody & Hoover 2002). The A- and B-types are believed to be independent. However, the C-type is suggested to be a superposition of the A- and B-types in various proportions (Buléon *et al.*, 1998). The C-type has been further classified into subgroups as C_a (>95% A-type), C_b, and C_c (50% A-type) based on the extent of their relative resemblance to A-type and B-type or between the two types, respectively (Hizukuri *et al.*, 1960). In general, the scanning range of WAXS varies from 3 to 35° 2θ [**Table 2-4**], which encompasses all major diffraction peaks. Tuber starches have been shown to exhibit a B-type X-ray pattern with reflections centered at ~5.5, 15.0, 17.0, 19.7, 22.2 and 24° 2θ angles. The A-type starches exhibit reflections at ~15.3, 17.0, 18.0, 20.0 and 23.4° 2θ angles, whereas, C-type starches exhibit major peaks centered at ~5.5, 17.0, 18.0, 20.0 and 23.5° 2θ (Jayakody *et al.*, 2005, Cheetham & Tao 1998, Shi & Seib 1992, Zobel 1988, Hizukuri *et al.*, 1983a). Peak intensities and 2θ values of various starches and the influence of amylose content (within a particular species) on the WAXS are shown in **Table 2-5**.

Table 2-5 Effect of amylose content on major X-ray diffraction intensities and diffraction patterns

Starch source	2θ							X-ray pattern	Reference
	~5°	~15°	~17°	~18°	~20°	~22°	~23°		
Waxy maize (0% amylose)	-	14.86	16.7	17.84	19.70	-	22.86	A	Cheetham & Tao 1998
Normal maize (amylose 28%)	-	14.96	16.96	17.78	19.70	-	22.86	A	Cheetham & Tao 1998
Maize (amylose 40%)	5.24	14.66	16.80	-	19.46	-	22.60	C	Cheetham & Tao 1998
High amylose maize (amylose 56-84%)	5.3 to 5.6	14.42 to 14.68	16.74 to 16.96	-	19.50 to 19.60	21.84 to 21.98	23.6 to 23.8	B	Cheetham & Tao 1998
Smooth pea (amylose ~35%)	5.49	14.97	16.89	-	19.53	-	22.86	C	Jayakody *et al.*, 2006
Grass pea (Lath 96) (amylose~38%)	5.52	14.97	16.95	-	19.80	-	22.98	C	Jayakody *et al.*, 2007a
Grass pea (NC-8A) (amylose~38%)	5.58	14.91	17.01	-	19.62	-	22.89	C	Jayakody *et al.*, 2007a
Wrinkled pea (amylose ~80%)	5.46	14.88	16.92	-	19.47	-	21.90	B	Jayakody *et al.*, 2006
Innala (*Bola*) (amylose~19%)	5.52	14.97	17.04	-	19.92	-	22.92	C_a	Jayakody *et al.*, 2005
Innala (*DIK*) (amylose ~25%)	5.52	15.0	17.04	-	20.01	-	22.80	C_a	Jayakody *et al.*, 2005

In maize starch, the WAXS pattern changes from A-type (waxy maize -0% amylose) to B-type (high amylose maize - 56-84% amylose), whereas, in pea starches, the trend is for a C-type (smooth pea - 35% amylose) to B-type (wrinkled pea- 80% amylose) [**Table 2-5**].

2.3.6.1.2.2.2 Influence of amylopectin chain length on double helix formation and starch polymorphism

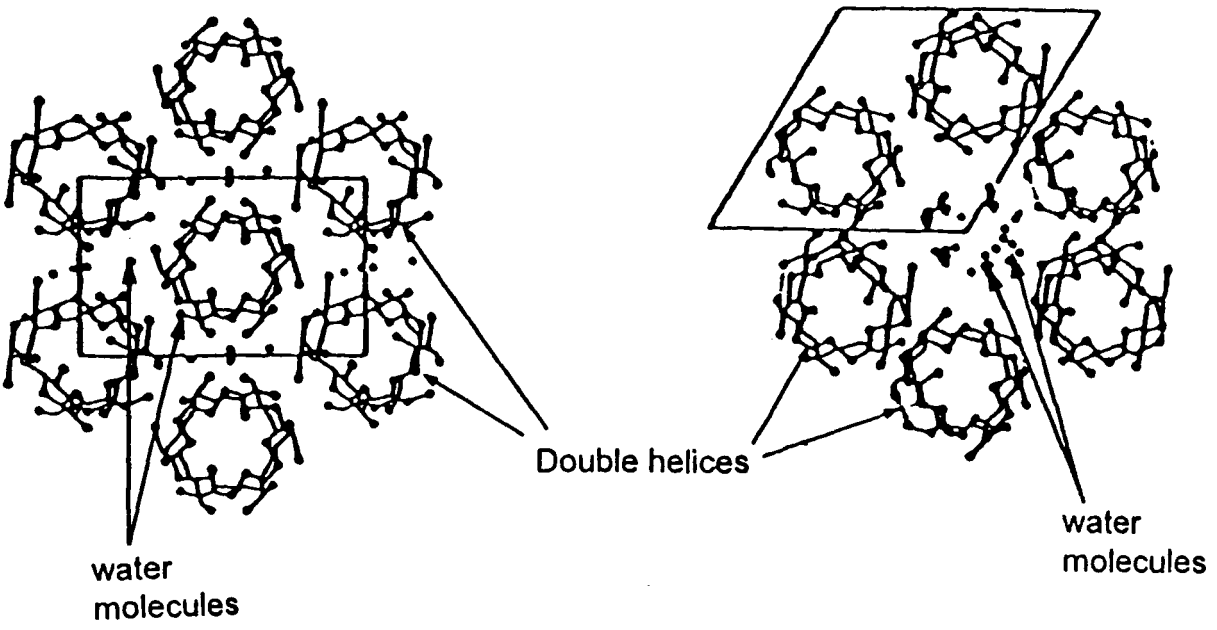
Nakamura *et al.*, (2002) and Martin and Smith (1995) have shown that the double helical formation occurs completely during starch biosynthesis, while Gidley and Bulpin (1987) indicated that a minimum chain length of DP 10 is required for double helical formation. The double helices of amylopectin are left-handed, parallel-stranded and are packed in a parallel manner. Hydrogen bonds are found only in inter-strands between O-2 and O-6, which tightly binds the double helices. Lineback (1984) postulated that branch points of the amylopectin molecule are the initiative sites of double helical formation. The packing of double helices in A- and B-type starches are shown in **Figure 2-8**. Polymorphic variation is mainly controlled by packing differences of double helices in the granule (Imberty *et al.*, 1988, Imberty & Pérez 1988). Starch crystallinity is positively correlated with the proportion of the short chain fraction in amylopectin (Cheetham & Tao 1998). The average chain length of amylopectin has been found to influence crystalline polymorphism. In general, branch chain-length distributions for the different type of starches show that the A-type polymorphic starch has a larger population of short branch chains (CL≤~20.0) (Srichuwong *et al.*, 2005a, Hizukuri *et al.*, 1983a)

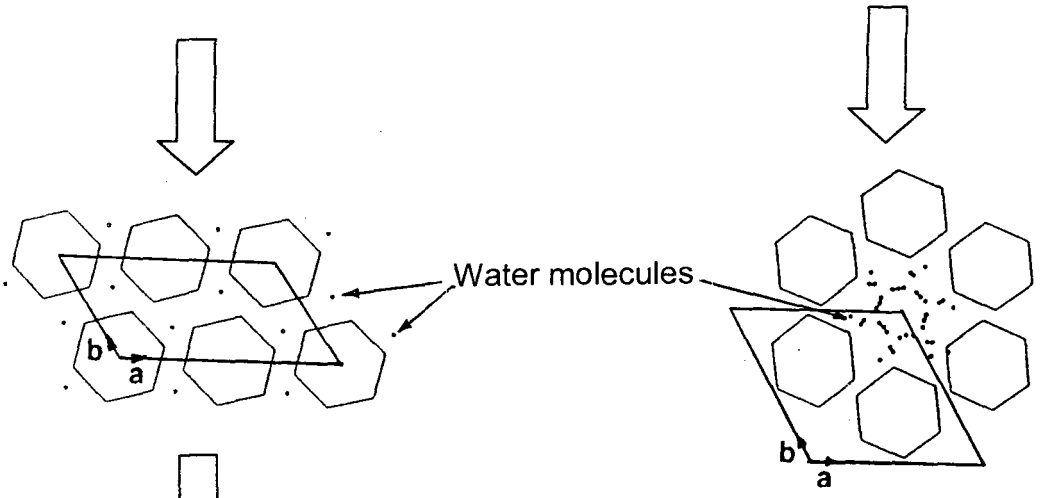
Figure 2-8 Hexagonal packing arrangement of double helices in A-type and B-type
starches (dots indicate water molecules)

Adapted from Wu & Sarko (1978a, b) with permission from Elsevier

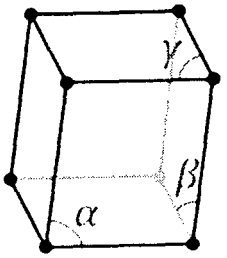
A-type
(Top view)

B-type
(Top view)



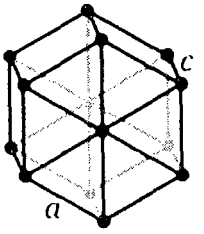


$\alpha \neq 90°$
$\beta, \gamma = 90°$



Monoclinic unit cell

$a \neq c$



Hexagonal unit cell

and is more densely packed in helical structures (Perry & Donald 2000, Wang *et al.*, 1998, Gidley & Bulpin 1987), whereas B-type (tuber & high-amylose) starches have fewer short branch chains, but more long branch or linear chains [CL≥~22] (B_2, B_3 & longer chains)(Jane *et al.*, 1999, Jane *et al.*, 1997, Hizukuri 1986). The intermediate chain length [CL ~20.0 and ~22] is associated with C-type (e.g. legume starches) crystallinity (Hizukuri 1985, Hizukuri *et al.*, 1983a). The super long chains seem to have no effect on polymorphism.

A-type starches have been shown to have less hydration capacity than B-type starches (Hizukuri 1996). The presence of shorter amylopectin chains in A-type starch allows effective packing of helices and the incorporation of only four water molecules per 12 glucose molecules per A-type unit cell [**Figure 2-8**], whereas B-type starches have longer amylopectin branches and exhibit less effective helical packing, resulting in the incorporation of 36 molecules of water molecules for the same amount of glucose residues per B-type unit cells [**Figure 2-8**] (Perry & Donald 2000, Hizukuri 1996, Imberty *et al.*, 1988, Imberty *et al.*, 1987, Wu & Sarko 1978a,b). The double helices of A-type and B-type are packed in monoclinic and hexagonal arrays, respectively [**Figure 2-8**].

2.3.6.1.2.2.3 Crystalline structure and crystallinity

Kainuma and French (1972) were the first to suggest that the crystalline orientation of starch is due to both parallel and anti parallel arrangement of double helices. The double helices of the outer branch chains of amylopectin form the crystalline structure of the starch granule. (Srichuwong *et al.*, 2005a).

During crystallization, portions of the glucan chains align themselves in a parallel array and, many crystallites ordinarily grow radially from a common nucleus resulting in the formation of a 'spherulitic' aggregate (Flory 1967). Spherulitic aggregates are structures that are made up of many crystallites that are large enough to diffract X-rays and are responsible for the crystallinity of starch. Crystallites are densely packed in crystalline lamellae while amorphous areas invariably fill the intervening space between the crystallites or crystalline regions (Flory 1967) Thus, it is now widely accepted that the amylopectin fraction of starch mainly contributes to granule crystallinity (Tang *et al.*, 2006, Zoble 1988a). However, crystallinity is also dependent on the chain length of the amylopectin and its degree of branching and is thus, an indirect measurement of the above two factors (Cottrell 1995). Among the tuber starches, *D. alata* starches appears to have the maximum variability in crystalline structure (Moorthy 2002).

2.3.6.1.2.2.3.1 Factors influencing granule crystallinity

Starch granule crystallinity is influenced by several factors: the method of sample preparation (Cottrell *et al.*, 1995), moisture content (Jayakody *et al.*, 2007a, 2006 & 2005, Buléon *et al.*, 1998, Cheetham & Tao 1998, Buléon *et al.*, 1987, Nara *et al.*, 1978, Hermans & Weidinger 1948), method of starch drying (Ahmed & Leliévre 1978), granule size (Wong & Leliévre 1982), chain length of amylopectin (Biliaderis 1981a, Biliaderis *et al.*, 1980), degree of amylopectin branching (Cottrell., 1995), crystal size (Jayakody *et al.*, 2005, French 1984), orientation of the double helices [within the crystallites] (Jayakody *et al.*, 2005), number of crystallites (Jayakody *et al.*, 2005), extent of packing of double helices within the

crystalline lamella (Jayakody *et al.*, 2005), amylose content (Waduge *et al.*, 2006, Jayakody *et al.*, 2006, Jayakody & Hoover 2002, Cheetham & Tao 1998), extent of disruption of amylopectin crystallites by amylose (Jenkins & Donald 1995, Jenkins 1994) and the X-ray diffractometer settings (Jayakody *et al.*, 2006).

Interpretation of X-ray data is difficult for starch due to its small crystallite size and crystallite imperfections (French 1984). The X-ray diffractograms of starch granules are somewhat diffuse, indicating that the crystalline domains are not perfectly developed. Broad diffraction peaks indicate either imperfect or relatively small crystallites while sharp peaks indicate more perfect or sufficiently large crystallites (Cooke & Gidley 1992). Crystallinity progressively decreases with increase in amylose content (Waduge *et al.,* 2006, Jayakody 2001).

Crystallinity of native starch granules could vary from 15 to 45% (Zobel 1988). Granular crystallinity plays a significant role in the granular architecture and physicochemical properties, such as the susceptibility to acid and enzyme (Jayakody *et al.*, 2005, Tang *et al.*, 2002), insolubility in cold water (Tang *et al.*, 2006), granular integrity (Jayakody *et al.*, 2005), granule swelling (Jayakody *et al.*, 2005), and pasting properties (Jayakody *et al.*, 2005). Wong and Leliévre (1982) reported that in wheat starch, small granules are more crystalline than large granules. It has also been reported that crystallinity is decreased by high phosphate levels (Muhrbeck *et al.*, 1991). Several researchers have shown by studies on potato (Tester *et al.*, 1999, Cottrell *et al.*, 1995), rice (Asaoka *et al.*, 1985 & 1984, Suzuki & Murayama 1967) and sweet potato (Noda *et al.*, 1997)

that crystallinity is influenced by environmental conditions and harvesting time. Polymorphic transition by thermal treatment has been observed to follow the order: B-, C-, and A-types (Jacobs & Delcour 1998, Hizukuri *et al.*, 1980). A- and B-type spherulites were found to melt at 90°C and 77°C, respectively (Whittam *et al.*, 1990). The A-type crystallinity is more thermodynamically stable and cannot be converted to the B- or C-type by hydrothermal treatment (Kiseleva *et al.*, 2004, Tester & Karkalas 2001, Jacobs & Delcour 1998, Eliasson & Gudmudsoon 1996). However, the exact mechanism of polymorphic transformation under hydrothermal treatment is not known.

2.3.6.1.2.2.3.1.1 Effect of moisture content on crystallinity

Hermans and Weidinger (1946 &1948) have shown that hydration increases crystallinity. In native dry starch, the double helices are not properly aligned (side by side), however, the alignment of double helices improves with increased moisture content. Consequently, diffraction of X-rays is higher in hydrated starch than in the dry starch (Hermans & Weidinger 1948, 1946). Diffraction peaks become sharper and peak resolution is more pronounced upon hydration (Jayakody *et al.*, 2007c, 2006, & 2005, Cheetham & Tao 1997, Sievert *et al.*, 1991). However, an increase in moisture content does not always increase crystallinity. Several factors such as: botanical source, the type of unit cell, amylose content, lipid content, relative humidity (RH), and the storage temperature have also been shown to influence crystallinity (Jayakody *et al.*, 2007a,b & 2006 Jayakody & Hoover 2002, Cheetham & Tao 1998, Hizukuri 1996).

2.3.6.1.2.2.4 Absolute crystallinity versus relative crystallinity

Hermans and Weidinger (1946) were the first to discuss crystallinity in biopolymers (e.g. cellulose). Later, different authors expressed crystallinity of starch in different ways such as (1) percent relative crystallinity (Gunaratne & Hoover 2002, Vasanthan *et al.*, 1999, Vasanthan 1994, Wakelin *et al.*, 1959), (2) percent absolute crystallinity (Tester *et al.*, 2004, Moorthy 2002, Blanshard 1986), (3) percent crystallinity (Jayakody *et al.*, 2007c, Hoover 2001), (4) percent crystalline order (Cooke & Gidley 1992), (5) percent degree of crystallinity (Cheetham & Tao 1998), (6) degree of crystallinity (Hermans & Weidinger 1948), (7) crystal index (Nara & Komiya 1983), and (8) percent crystallinity index (Ahmed & Leliévre 1978). Two different methods have been used to express crystallinity. One method using the area corresponding to the respective contributions of amorphous and crystalline scattering, was first introduced by Hermans and Weidinger (1948). This measurement was expressed as degree of crystallinity and later called absolute crystallinity (Wakelin *et al.*, 1959). In the second method crystallinity was expressed as relative crystallinity (Wakelin *et al.*, 1959). Relative crystallinity of starches was determined by calculating the proportion of crystallinity within the starch granules using reference materials with zero percent representing a fully amorphous material (freeze-dried gelatinized starch) and 100 percent crystallinity representing quarts (Vasanthan 1994) or starch in which all the amorphous material had been eroded by acid hydrolysis (Tester *et al.* 2004).

2.3.6.2 Minor components

In addition to the major components, the starch granule also contains minor components such as protein, lipids, and minerals [ash] [**Table 2-6**]. Minor components have been shown to influence physicochemical properties (granular swelling, gelatinization, amylose leaching, acid and enzyme digestibility, paste clarity, pasting properties, and retrogradation), and the quality of starch-derived products (Han & Hamaker 2002, Baldwin 2001, Jayakody 2001, Appelqvist & Debet 1997, Skerritt *et al.*, 1990, Craig *et al.,* 1989, Galliard & Bowler 1987). Baldwin (2001) hypothesized, that, higher levels of minor components may have a structural role in maintaining granule integrity, resistance to hydration and enzymes.

2.3.6.2.1 Lipids

The existence of lipids as an inherent part of the starch has long been known (Taylor & Nelson 1920). Lipids associated with starch can be classified into three categories: (1) surface (lipids present on the surface of the granule), (2) internal, bound or starch lipid (lipids present complexed with glucan chains), and (3) non-starch lipids (present in the aleurone layers and germ of the grain) (Morrison 1988b). Surface lipids are mainly triacylglycerols, followed by free fatty acids, glycolipids and phospholipids (Vasanthan & Hoover 1992b, Galliard & Bowler 1987, Morrison 1981) and can be extracted by a mixture of chloroform and methanol in the ratio of 2:1 [v/v] (Folch *et al.*, 1957). However, any unbound lipids, despite their location, could be dissolved in this solvent, hence, 'free lipid' would be a more appropriate term rather than surface lipid. The exact location

Table 2-6 Composition (%, dry basis) of some minor components of various starches

Starch source	Lipid (%)	Nitrogen (%)	Ash (%)	Reference
D. alata	0.50	0.03	0.13	Karam *et al.*, 2006
D. alata	0.10	0.01	0.22	Peroni *et al.*, 2006
D. alata (different varieties)	0.09-0.11	0.03-0.05	0.08-0.38	Amani *et al.*, 2004
D. alata	-	0.02	-	Freitas *et al.*, 2004
D. alata	0.01	0.05	0.12	Gunaratne & Hoover 2002
D. alata	0.09	0.03	0.12	Valetudie *et al.*, 1993
D. alata	0.03	0.01	-	Emolia & Delarosa 1981
D. alata (different varieties)	-	0.021-0.024	0.2-0.23	Rašper & Coursey 1967
D. esculenta (different varieties)	0.06-0.09	0.01-0.06	0.21-0.23	Amani *et al.*, 2004
D. esculenta	-	0.013	0.44	Rašper & Coursey 1967
D. rotundata	0.03-0.10	0.02-0.04	0.06-0.26	Amani *et al.*, 2004
D. rotundata	0.04	0.005	-	Emolia & Delarosa 1981
D. rotundata (different varieties)	-	0.008-0.24	0.15-0.28	Rašper & Coursey 1967
D. dumetorum	0.04	0.01	-	Emolia & Delarosa 1981
D. dumetorum	-	0.24	0.29	Rašper & Coursey 1967
D. ballophylla	1.08	0.41	0.27	Soni *et al.*, 1985
Solenostemon rotundifolius (variety-*Dik*)	0.07	0.07	0.10	Jayakody *et al.*, 2005
Solenostemon rotundifolius (variety-*Bola*)	0.05	0.05	0.09	Jayakody *et al.*, 2005
Solanum tuberosum	0.12	0.09	0.25	Gunaratne & Hoover 2002
Solanum tuberosum (different varieties)	-	0.02-0.14	-	Vasanthan *et al.*, 1999
Solanum tuberosum	0.16	-	0.19	Valetudie *et al.*, 1993
Solanum tuberosum	0.05	0.06	0.4	Swinkels 1985a
Maranta arundinacea (arrowroot)	0.17	0.02	0.28	Peroni *et al.*, 2006
Ipomea batatas	0.14	0.02	0.21	Peroni *et al.*, 2006
Ipomea batatas	0.36	0.02	0.08	Valetudie *et al.*, 1993
Colocasia esculenta (taro)	0.04	0.03	0.14	Gunaratne & Hoover 2002

Table 2-6 Composition (%, dry basis) of some minor components of various starches (cont.,)

Starch source	Lipid (%)	Nitrogen (%)	Ash (%)	Reference
Manihot esculenta	0.28	0.02	0.22	Karam *et al.*, 2006
Manihot esculenta	0.15	0.03	0.21	Peroni *et al.*, 2006
Manihot esculenta	1.2	0.02	-	Freitas *et al.*, 2004
Manihot esculenta	0.08	0.02	0.11	Gunaratne & Hoover 2002
Manihot esculenta	-	0.03	-	Thitipraphunkul *et al.*, 2003
Manihot esculenta	0.08	0.02	0.09	Valetudie *et al.*, 1993
Manihot esculenta	0.34	0.02	0.22	Soni *et al.*, 1985
Manihot esculenta	0.10	0.10	0.20	Swinkels 1985a
Xanthosoma sagitifolium	0.30	0.08	0.15	Gunaratne & Hoover 2002
Xanthosoma sagitifolium	0.01	0.03	0.11	Valetudie *et al.*, 1993
Cana edulis	0.19	0.2	0.24	Peroni *et al.*, 2006
Cana edulis (different varieties)	0.014-0.019	~0.01	-	Thitipraphunkul *et al.*, 2003
Lathyrus sativus L.(grass pea) NC-8A	0.8	0.11	0.15	Jayakody *et al.*, 2007a
Lathyrus sativus L.(grass pea) Lath 96	0.8	0.03	0.18	Jayakody *et al.*, 2007a
Mung bean	0.14	0.9	-	Thitipraphunkul *et al.*, 2003
Pisum sativum L. - Wrinkled pea	0.8	0.03	0.1	Zhou *et al.*, 2004
Pisum sativum L. - Smooth pea	0.47-0.48	0.02-0.08	0.02	Zhou *et al.*, 2004
Pisum sativum L. - Green pea	0.15	0.13	0.09	Hoover & Manual 1996
Lens esculenlus - Lentil	0.72-0.81	0.04-0.05	0.03	Zhou *et al.*, 2004
Lens esculenlus - Lentil	0.09	0.06	0.11	Hoover & Manual 1996
Artocarpus artilis (bread fruit)	0.51	0.26	0.35	Adebowale *et al.*, 2005
Caryota urines (Kithul)	-	-	0.24	Jayakody *et al.*, 2007b
Borassus flabellifer L. (Palmyhra)	-	-	0.13	Jayakody *et al.*, 2007b
Artocarpus heterophyllus (Jack fruit)	-	-	0.07-0.16	Jayakody *et al.*, 2007b

of the lipids on the granule surface is still unknown (Buléon *et al.*, 1998). In cereal (e.g. wheat, barley, and rye) starches, the internal or bound lipids are predominantly lysophospholipids (Morrison 1988b, Swinkels 1985a, Morrison 1981, Hargin & Morrison 1980). More polar solvents (e.g. *n*-propanol-water or water saturated butanol) and a long refluxing time (~72h) are needed to completely extract bound lipids from native starches (Vasanthan & Hoover 1992b). Normal cereal starches contain approximately up to 1.5% of lipid by weight (Tester & Karkalas 2001). Lipids reduce water binding capacity (Tester & Morrison 1990a) and increase opaqueness of a starch paste (Swinkels 1985a). Free fatty acids contribute up to 30-50% of lipids in normal maize and rice starches (Morrison 1988b). Amylomaize starches contain 40-70% more lipids than normal maize (Jayakody & Hoover 2002, Jayakody 2001). Trace quantities of lipids (mainly phospholipids) are present in tuber starches (0.1-0.2%) (Jayakody *et al.*, 2005, Hoover 2001, Vasanthan & Hoover 1992b) [**Table 2-6**]. However, very little is known about the deposition of lipid or its regulation during starch biosynthesis.

Starch-lipids improve the textural properties of various foods (Moorthy 2002). However, the high amount of lipid has certain unfavourable effects such as undesirable flavors (due to oxidation of lipids), opaque or cloudy appearance of starch films (due to amylose-lipid inclusion compounds), reduction of granular swelling, solubilization and water-binding capacity of starches (Hoover 2001, Swinkels 1985a).

2.3.6.2.2 Proteins

Nitrogen is generally considered to be present as protein, but it may also be part of lipids (e.g. lysophosphatidylcholine in wheat starch) (Morrison 1988a). It has been postulated that all major granule associated proteins are remnants of proteins associated with amyloplast during starch biosynthesis (Rahman *et al*, 1995, Galliard 1983). Protein is intimately associated with starch granule matrix as storage protein (e.g. gluten and gliadin) and starch granule-associated protein. Storage proteins remain adsorbed to the granule surface while starch granule-associated proteins occur as 'internal' granule-associated proteins (Li *et al.*, 2003) and 'surface' granule-associated proteins (Baldwin 2001, Skerritt *et al*, 1990). The 'surface' granule-associated proteins are low molecular weight proteins (M_w < 30 kDa) and 'internal' granule-associated proteins are high molecular weight proteins (60<M_w>149 kDa) (Baldwin 2001).

Morrison (1988a & 1981) reported that nitrogen content of isolated starches represents contamination from storage proteins, lipids that contain choline, ethanolamine and serine, and other proteins located inside the starch granules. Thus, the amount of protein present in purified starch is a good indicator of starch purity. In general, purified starches contain less than 0.6% protein (Tester *et al*, 2004). Average nitrogen content of well purified starches is 0.05-0.06%, and 0.25-0.5% in potato and cereal starches, respectively (Baldwin 2001, Martin & Smith 1995, Skerritt *et al.*, 1990, Swinkels 1985a, Lowy *et al.*, 1981) [**Table 2-6**]. Most of the starch granule associated proteins are easily removed by repeated washing in water (Lowy *et al.*, 1981). Surface proteins can be readily extracted

with diluted NaCl, aqueous alkali solution or 1-2% sodium dodecyl sulfate solution at room temperature (Seguchi & Yamada 1989, Lowy *et al.*, 1981). However, internal protein can be extracted only after starch gelatinization (Lowy *et al.*, 1981, Mu-Forster & Wasserman 1998). This indicates, internal proteins are interspersed within the starch matrix, whereas surface proteins are deposited on the granule surface as aggregates (Mu-Forster & Wasserman 1998).

Several physicochemical properties, such as gelatinization, pasting, and enzyme resistance characteristics, could be influenced by the presence, orientation and nature of starch granule-associated proteins (Baldwin 2001). These proteins may contribute to the flavor, foam formation and color of starch (Martin & Smith 1995, Galliard & Bowler 1987, Swinkels 1985a).

2.3.6.2.3 Phosphorous

Tuber starches contain significantly more esterified monophosphate groups than other starches [**Table 2-7**]. The phosphorous content of tuber starches varies from 0.003 to 0.08% (Moorthy 2002). Potato starch has a relatively high ash content because of large number of phosphate groups. For instance, starch bound phosphorous content of a potato starch is ~0.1% of the dry matter (Galliard & Bowler 1987). Phosphorous is present mainly on amylopectin, however, pure amylose is phosphorous free (Sherman & Baker 1916). Phosphorus in native starch is found in three major forms: (1) phosphate monoester (bound to amylopectin), (2) phospholipids (complexed with lysophospholipids) and (3) inorganic phosphate [**Table 2-7**].

Table 2-7 Phosphorous content (%, dry basis) of various starches

Starch source	Total phosphorous (%)		Phosphate monoester (%)	Phospholipids (%)	Inorganic phosphorous (%)	Reference
	Colorimetric	^{31}P NMR				
D. alata	0.022	-	-	-	-	Peroni *et al.*, 2006
D. alata (native starch)	0.0302	-	-	-	-	Srichuwong *et al.*, 2005c
D. alata (defatted starch)	0.0285	-	-	-	-	Srichuwong *et al.*, 2005c
D. alata (2 varieties)	0.032-0.074	-	-	-	-	Wang *et al.*, 2005
D. alata	0.03	-	-	-	-	Gunaratne & Hoover 2002
Dioscorea (spp. not specified)	0.012	0.012	0.011	Not detected	0.001	McPherson & Jane 1999
D. alata	0.017	-	-	-	-	Moorthy 1991
D. esculenta (native starch)	0.0290	-	-	-	-	Srichuwong *et al.*, 2005c
D. esculenta (defatted starch)	0.0282		-	-	-	Srichuwong *et al.*, 2005c
D. esculenta	0.017	-	-	-	-	Moorthy 1991
D. rotundata	0.011-0.015	-	-	-	-	Moorthy 2002
D. rotundata	0.012					Moorthy 2002
D. dumetorum	0.003	-	-	-	-	Moorthy 2002
D. ballophylla	0.005	-	-	-	-	Soni *et al.*, 1985
D. batata (2 varieties)	0.006-0.01	-	-	-	-	Wang *et al.*, 2005
Solanum tuberosum	0.051-0.094	-	-	-	-	Karim *et al.*, 2007
Solanum tuberosum (normal)	0.075	-	-	-	-	Srichuwong *et al.*, 2005c
Solanum tuberosum (defatted)	0.0691	-	-	-	-	Srichuwong *et al.*, 2005c
S. tuberosum (diff. stored temp.)	0.041-0.075		-	-	-	Tester *et al.*, 2005
Solanum tuberosum	0.038-0.069	-	-	-	-	Liue *et al.*, 2003
Solanum tuberosum (varieties)	0.039-0.075	-	-	-	-	Yusuph *et al.*, 2003
Solanum tuberosum	0.01-	-	-	-	-	Gunaratne & Hoover 2002
Solanum tuberosum - normal	0.075	0.075	0.073	Not detected	0.001	McPherson & Jane 1999
Solanum tuberosum - waxy	0.066	0.069	0.069	Not detected	0.001	McPherson & Jane 1999
S. tuberosum (small granules)	0.059-0.101	-	-	-	-	Vasanthan *et al.*, 1999
S. tuberosum (large granules)	0.049-0.079	-	-	-	-	Vasanthan *et al.*, 1999
Solanum tuberosum	0.061-0.078	-	-	-	-	Debbon *et al.*, 1998
Solanum tuberosum	0.090	0.091	0.086	Not detected	0.0048	Kasemsuwan & Jane 1996
Solanum tuberosum *	0.046-0.059	-	-	-	-	Haase & Plate 1996
Solanum tuberosum **	0.07-0.1	-	-	-	-	Cottrell *et al.*, 1995
Solanum tuberosum	-	0.090	0.089	Not detected	0.001	Lim *et al.*, 1994
Solanum tuberosum **	0.053-0.093	-	0.032-0.058	-	-	Nikuni *et al.*, 1969

Table 2-7 Phosphorous content (%, dry basis) of various starches (cont.,)

Starch source	Total phosphorous (%)		Phosphate monoester (%)	Phospholipids (%)	Inorganic phosphorous (%)	Reference
	Colorimetric	^{31}P NMR				
Solanum tuberosum (varieties)	0.046-0.075	-	0.031-0.053 [C-6]	-	-	Hizukuri *et al.*, 1970
Potato amylopectin	0.0477	-	0.0322 (C-6)	-	-	Hizukuri *et al.*, 1970
Ipomea batatas	0.014	-	-	-	-	Peroni *et al.*, 2006
Ipomea batatas (native starch)	0.0226	-	-	-	-	Srichuwong *et al.*, 2005c
Ipomea batatas (defatted starch)	0.0221	-	-	-	-	Srichuwong *et al.*, 2005c
Ipomea batatas	0.020	0.021	0.020	Not detected	0.001	McPherson & Jane 1999
Ipomea batatas	-	0.012	~0.011	Not detected	Not detected	Lim *et al.*, 1994
Ipomea batatas (diff. temp. levels)	0.009-0.0123	-	0.006-0.009 [C-6]	-	-	Hizukuri *et al.*, 1970
Cana edulis	0.031	-	-	-	-	Peroni *et al.*, 2006
Cana edulis (different varieties)	0.036-0.04	-	-	-	-	Thitipraphunkul *et al.*, 2003
Cana edulis (native)	0.0339	-	-	-	-	Srichuwong *et al.*, 2005c
Cana edulis (defatted starch)	0.0313	-	-	-	-	Srichuwong *et al.*, 2005c
Cana edulis	0.01-0.08	-	-	-	-	Moorthy 2002
Manihot esculenta	0.007	-	-	-	-	Peroni *et al.*, 2006
Manihot esculenta (native)	0.0113	-	-	-	-	Srichuwong *et al.*, 2005c
Manihot esculenta (defatted)	0.0087	-	-	-	-	Srichuwong *et al.*, 2005c
Manihot esculenta	0.007	0.006	0.0062	Not detected	Trace	Kasemsuwan & Jane 1996
Manihot esculenta	-	0.009	0.008	Not detected	0.001	Lim *et al.*, 1994
Manihot esculenta	0.0075	-	-	-	-	Soni *et al.*, 1985
Nelumbo nucifera - lotus	-	0.005	0.005	Not detected	Not detected	Lim *et al.*, 1994
Maranta arundinacea - arrowroot	-	0.022	0.021	Not detected	0.001	Lim *et al.*, 1994
Trapa natans – water chestnut	-	0.011	0.004	Not detected	0.007	Lim *et al.*, 1994
Metrozylon sagu (native starch)	0.0110	-	-	-	-	Srichuwong *et al.*, 2005c
Metrozylon sagu (defatted starch)	0.0107	-	-	-	-	Srichuwong *et al.*, 2005c
Pueraria hirsuta - Kuzu	0.0092	-	0.005 [C-6]	-	-	Hizukuri *et al.*, , 1970
Xanthosoma sagitifolium	0.02					Gunaratne & Hoover 2002
Colocasia esculenta (taro)	0.02					Gunaratne & Hoover 2002
Cucurbita maxima - winter squash	0.022-0.026	-	-	-	-	Stevenson *et al.*, 2005
S. rotundifolius, variety-*Dik*	0.02	-	-	-	-	Jayakody *et al.*, 2005
S. rotundifolius, variety-*Bola*	0.01	-	-	-	-	Jayakody *et al.*, 2005
Phaseolus aureus	0.012	0.012	0.0083	0.0006	Not detected	Kasemsuwan & Jane 1996
Phaseolus aureus	-	0.013	0.011	0.001	0.001	Lim *et al.*, 1994
Caryota urines - kithul	0.007	-	-	-	-	Jayakody *et al.*, 2007b
Borassus flabellifer L. -palmyrah	0.012	-	-	-	-	Jayakody *et al.*, 2007b

* Different fertilizer levels ** Different temperature levels

Phosphorus in tuber (potato), root (sweet potato, cassava, water chestnut and lotus), rhizome (arrowroot), legume (green pea, lima bean, mung bean, lentils), waxy maize, and amaranth starches contain mainly starch phosphate monoesters (Hizukuri 1996, Bay-Smidt *et al.*, 1994, Kasemsuwan & Jane 1996, Lim *et al.*, 1994, Takeda & Hizukuri 1982, Tabata & Hizukuri 1971, Hizukuri *et al.*, 1970, Schoch 1941) with some inorganic phosphate, however, no phospholipids has been reported for the above starches (Lim *et al.*, 1994) [**Table 2-7**]. Normal cereal (maize, wheat, rice, oat and millet) and waxy (*du*-waxy maize, rice) starches contain phosphorous in the form of phospholipids such as lysophospholipids (Hizukuri 1996, Lim *et al.*, 1994, Morrison 1988b, Morrison & Gadan 1987, Swinkels 1985a, Hizukuri *et al.*, 1983b, Morrison 1981).

2.3.6.2.3.1 Location of phosphorus in starch granule

Amylose is nearly free from covalently bound phosphate. However, Hizukuri (1996) has reported that organic phosphorous content of amylose varies from 1 to 14 ppm for various botanical sources. On average, 1 of every 200 to 500 glucose residues of amylopectin is phosphorylated (Jacobson *et al.*, 1998, Nielsen *et al.*, 1994, Swinkels 1985a). On a macromolecular basis, the monophosphate esters have been reported to be distributed over the long B chains of amylopectin except in the vicinity of the branch points (Hizukuri 1996, Takeda & Hizukuri 1982). It has been shown that in amylopectin, phosphate groups are mainly located at C-6 (61%), C-3 (38%), and C-2 (1%) positions (Bay-Smidt *et al.*, 1994, Hizukuri 1996, Lim *et al.*, 1994, Takeda & Hizukuri 1982, Tabata & Hizukuri 1971, Hizukuri *et al.*, 1970, Hizukuri 1969). Lim *et al.*, (1994)

reported that tuber and root starches contain more phosphate derivatives on C-6 than at the C-3 position. However, physical and biochemical methods indicate that phosphorylation at the C-6 position varies between potato cultivars, but the C-3 phosphorylation level remains almost constant (Bay-Smidt *et al.*, 1994, Muhrbeck & Tellier 1991). Several authors have shown that severe heat treatments (e.g. autoclaving, heat-moisture treatment) and acid hydrolysis liberate inorganic phosphate mainly from C-3 position (Jacobson *et al.*, 1998, Nielsen *et al.*, 1994, Tabata & Hisukuri 1971, Hizukuri *et al.*, 1970). Phosphate groups have been shown to be about nine glucosyl residues away from a branch point and no phosphate groups are present in the unit chains with a DP less than ~20 (Takeda & Hizukuri 1982). Furthermore, Takeda and Hizukuri (1982) concluded that about one third of the phosphate groups are present in the inner regions of the B-chains, and two thirds in the A-chains and the outer regions of the B-chains. This fact suggests that phosphate groups are mainly present bound to B chains (~88%) and with trace quantities associated with A-chains (~12%). It has been hypothesized, that phosphate groups are not evenly distributed thought the amylopectin molecule and it might be densely localized on the surface of the starch granule (Yamada *et al.*, 1987).

2.3.6.2.3.2 Factors influencing phosphorylation & phosphorous content in starch granule

The degree of phosphorylation depends on cultivar (Haase & Plate 1996, Nielsen *et al.*, 1994, Muhrbeck & Teiller 1991, Veselovsky 1940), granule size (Vasanthan *et al.*, 1999, Nielsen *et al.*, 1994, Jane & Shen 1993), growth

conditions (Cottrell *et al.*, 1995, Nikuni *et al.*, 1969), type and level of fertilizer (Jacobsen *et al.*, 1988, Haase & Plate 1996, Edelbauer 1988), growth temperature (Cottrell *et al.*, 1995), and storage (Hizukuri 1996). Vasanthan *et al.*, (1999) observed that in potato starch, smaller granules contain more phosphorous than the larger granules. It has also been reported that in potato starch, small granules starch contain more C-6 phosphate (~25% more) than the larger granules (Nielsen *et al.*, 1994, Jane & Shen 1993). Furthermore, Nielsen *et al.*, (1994) indicated that phosphorylation constantly occurs during tuber development, but the efficiency of phosphorylation decreases with starch granule development. Prolonged storage of starch at room temperature results in a release of organic phosphate at the C-6 position and an increase in the relative amount of inorganic phosphate (Hizukuri 1996). The biochemical pathway for starch phosphorylation in potato tubers and the exact function of starch phosphorylation in the plant metabolism remains unknown (Jacobson *et al.*, 1998). However, it is believed that the level of phosphorylation could be manipulated either through plant breeding or by agronomic means (Haase & Plate 1996).

2.3.6.2.3.3 Functional properties and stability of starch phosphates

Phosphate monoesters in tuber and root starches have been shown to increase water holding capacity (Swinkels 1985a), swelling factor/power (Karim *et al.*, 2007, Jayakody *et al.*, 2005, Singh *et al.*, 2005, Swinkels 1985a), paste clarity (Lim *et al.*, 1994, Jane *et al.*, 1996), and gel strength (Moorthy 2002). Many desirable starch properties such as low rates of retrogradation (Jayakody *et al.*,

2005, Kasemsuwan & Jane 1996), increased paste clarity and light transmittance (Singh *et al.*, 2006, Lim & Seib 1993, Craig *et al.*, 1989), increased peak viscosity (Wang *et al.*, 2006, Noda *et al.*, 2004, Moorthy 2002, Jacobson *et al.*, 1998, Bay-Smidt *et al.*, 1994, Kim *et al.*, 1995, Swinkels 1985a), decreased gelatinization temperature (Palasinsky 1980, Veselovsky 1940), resistance to freezing and thawing (Wang *et al.*, 2006, Hoover *et al.*, 1988), and improved textural properties (Vasanthan *et al.*, 1999) have been attributed to starch phosphate content. Phosphate esters bound to C-6 carbons have been shown to reduce the gelatinization enthalpy, whereas phosphate esters at C-3 have very little influence on starch gelatinization (Muhrbeck & Eliasson 1991). Phosphate esters have been shown to influence starch crystallinity (Muhrbeck & Eliasson 1991, Muhrbeck *et al.*, 1991).

2.3.6.2.3.4 Techniques of phosphorous determination

A survey of the literature revealed that colorimetry [~76%] and nuclear magnetic resonance [23%] (^{31}P-NMR) have been used widely for quantification of phosphorous in starches. Colorimetric methods are based on analysis of inorganic phosphrous liberated from organic matter incineration or wet digestion (Jayakody *et al.*, 2005, Singh & Ari 1987, Kovacs 1986, Totani *et al.*, 1982, Hizukuri *et al.*, 1970, Laws 1965, Smith & Caruso 1964, Morrison 1964, Fleischer *et al.*, 1956, Chen *et al.*, 1956, Beveridge & Johnson 1949, Berenblum & Chain 1938, Fiske & Subbarow 1925). The phosphorous content determined by ^{31}P-NMR is in good agreement with that determined by colorimetry **Table 2-7**.

2.4 Physicochemical properties and starch functionality

Overview

The physicochemical properties of starches are differentiated on the basis of their responses to physical or/and chemical stimuli, which may be applied in various ways such as heat (e.g. gelatinization, granular swelling, amylose leaching, viscosity, retrogradation), shear (pasting properties), light (birefringence) or X-ray scattering (crystallinity), and susceptibility to acid and enzymes. The physicochemical parameters of starches are dependent mainly on genetic factors and to some extent are affected by environmental (Hizukuri 1969) and other non-genetic factors (Hizukuri 1969) such as starch modification and processing conditions (Jul *et al.*, 1996, Kempf *et al.*, 1961, Wegner 1957). Environmental temperature has been shown to influence starch structure by mediation of endogenous enzymes (Tester & Karkalas 2001). Physicochemical properties of starch may vary with maturity of the granule, because granules of different maturity may have differing quantities of major and minor components, and hence, different structural characteristics (Baldwin 2001, Cottrell *et al.*, 1995).

2.4.1 Gelatinization

Starch gelatinization is the collapse (disruption) of molecular order within the starch granule manifested in irreversible changes in granular properties such as granular swelling, native crystallite melting, loss of birefringence, and starch solubilization. The point of initial gelatinization (T_o) and the range over which it occurs is governed by starch concentration, granule type, pre-treatments and

heterogeneities within the granule population under observation (Nakazawa & Wang 2004 & 2003, Jacobs *et al.*, 1998a,b,c, Atwell, *et al.*, 1988, Cooke & Gidley 1992).

2.4.1.1 Mechanism of starch gelatinization

Gelatinization is a cooperative event between the amorphous and crystalline region in starches. Hydration first occurs in the amorphous background followed by the hydration of the crystalline lamellae (Tester & Debon 2000, Jenkins & Donald 1997, Cameron & Donald 1993). The onset of gelatinization reflects initiation of granule swelling which is closely related to the state of plasticization of the amorphous domains. As the crystalline domains are closely associated with the amorphous domains, the swelling and movement in the amorphous domains tears the crystallites apart, a phenomenon is called solvation assisted melting (Donovan 1979). Crystallites melt cooperatively (smaller crystallites are destroyed first) at a lower temperature than they would if isolated from the amorphous region. It has been shown that water content in the amorphous growth ring in B-type starches are much higher than in A-type starches [where water density/carbohydrate density is ~1.6 and ~1.0 for potato and waxy corn starch, respectively] (Perry & Donald 2000). Since the amorphous growth ring is well plasticized at room temperature, less thermal energy input is required to initiate gelatinization of the starch. Plasticization increases chain mobility within the crystalline regions, thus it could lead to a loss of crystalline order at lower temperatures. Cottrell *et al.*, (1995) have shown that the onset gelatinization temperature of tuber starches are significantly lower than those of cereal

starches. If water content is low then the non-cooperative melting endotherm occurs at higher temperatures.

2.4.1.2 Techniques of measuring gelatinization characteristics

Granular property changes during starch gelatinization are monitored using different analytical probes such as visco-amylograph (measures changes in viscosity as result of granule swelling and solubilization), thermal analysis (quantifies both melting of amylopectin crystallites and conformational disordering of double helices), loss of granule birefringence (determines randomization of crystallite orientation induced by the initial stages of granule swelling or crystallites melting), swelling power or swelling factor (measures granular volume changes) (Ziegler *et al.*, 1993, Tester & Morrison 1990a). The most commonly used thermal analysis technique in the study of starch gelatinization is differential scanning calorimetry (DSC) (Tester & Karkalas 2001, Atwell, *et al.*, 1988). In addition, several other methods have been used for measuring gelatinization such as Kofler hot stage microscopy [measures loss of birefringence] (Emiola & Delarosa 1981, Watson 1964), confocal scanning laser microscopy (CSLM) [measures swelling and expansion of the granule with time] (Velde *et al.,* 2002), WAXS [measures loss of granule crystallinity] (Jacobs *et al.*, 1998b, Zobel *et al.,* 1988), SAXS [measures electron density differences between crystalline and amorphous lamellae] (Jenkins & Donald 1996, Cameron & Donald 1992, 1993a, & 1993b), proton NMR [measures increase in mobility and hydration of glucan chains] (Da Silva *et al.*, 1996, Leliévre & Mitchell 1975), spectrophotometry [measures changes in light reflectance] (Howitt *et al.*, 2005),

viscosity analyzer [measures viscosity changes] (Ross *et al.*, 1987, Schoch 1959), enzyme susceptibility [measures reducing sugar content] (Chiang & Johnson 1977), paste clarity [measures loss of clarity] (Cook & Axtmayer 1937), swelling [measures changes in granule dimensions] (Collison & Elton 1961), solubility [measures rapid increases in soluble material] (Collison 1968a), and absorption of dyes [measures staining power of starch] (Jones 1940). Zobel *et al.*, (1965) were the first to use differential thermal analysis (DTA) for measurement of starch gelatinization.

2.4.1.2.1Differential scanning calorimetry (DSC) & gelatinization parameters

Stevens and Elton (1971) were the first to use DSC to study starch gelatinization. DSC measures the gelatinization parameters such as temperatures onset (T_o), midpoint or peak (T_p), conclusion (T_c) and enthalpy (ΔH) of gelatinization. The gelatinization temperature range (T_c-T_o) reflects the crystallites stability. Gelatinization is a moisture dependent process. A single endotherm is formed in the presence of excess water. The overall DSC endotherm reflects endothermic transitions of a heterogeneous granule population (Karim *et al.*, 2007). The gelatinization endotherm reflects endothermic [melting of crystallites, loss of double helical order, granule swelling] and exothermic [hydration of starch molecules and formation of amylose-lipid complex] events (Cooke & Gidley 1992, Stute 1992, Tester & Morrison 1990a,b Kugimiya *et al.*, 1980). Gelatinization parameters and DSC settings are summarized in **Table 2-8.** Tester and Morrison (1990a) have postulated that ΔH reflects the overall crystallinity (quality and quantity of starch crystallites) of amylopectin. Noda *et al.*, 1996) have

Table 2-8 Gelatinization parameters and scanning conditions of various tuber starches

Starch source	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	Starch:H_2O	Scanning conditions (DSC/Kofler)	Reference
D. alata	73.0	76.3	82.5	20.1	1:9	30-120°C, 10°C/min	Karam *et al.*, 2006
D. alata	70.8	74.5	77.8	14.3	1:3	25-100°C, 10°C/min	Peroni *et al.*, 2006
D. alata	-	81.6	-	19.9	~1:2.3	4-180°C, 10°C/min	Brunnschweiler *et al.*, 2005
D. alata	78.2	81.0	91.4	19.4	1:2	15-120°C, 2°C/min	Srichuwong *et al.*, 2005a
D. alata	69.0	77.0	72.5	11.5	1:2	Not available, 2.5°C/min	Freitas *et al.*, 2004
D. alata	69.0	77.0	72.5	11.5	1:2	Not available, 3°C/min	Freitas *et al.*, 2004
D. alata	69.0	78.0	72.5	12.0	1:2	Not available, 4°C/min	Freitas *et al.*, 2004
D. alata	69.5	79.0	73.0	11.5	1:2	Not available, 5°C/min	Freitas *et al.*, 2004
D. alata	~75-76	-	-	16.1-18.1	1:5	25-160°C, 10°C/min	Amani *et al.*, 2004
D. alata *	~70-77	~74-82	~80-85	11.6-20.9	Not available	Not available	Moorthy 2002
D. alata	74.4	78.8	84.9	17.3	1:9	20-120°C, 10°C/min	Alves *et al.*, 2002
D. alata	75	80	91.2	17.8	1:3	20-120°C, 10°C/min	Gunaratne & Hoover 2002
D. alata	76.5	78.8	81.9	~19.8	~1:2.3	2-100°C, 10°C/min	Farhat *et al.*, 1999
D. alata	70.2	74.4	80.9	20.9	1:4	30-180°C, 1°C/min	Valetudie *et al.*, 1995
D. alata	65	69	71.5	-	-	Kofler hot stage microscopy	Emolia & Delarosa 1981
D. esculenta	71.9	74.8	82.4	14.3	1:2	15-120°C, 2°C/min	Srichuwong *et al.*, 2005a
D. esculenta	72-74	-	-	19.5-20.3	1:5	25-160°C, 10°C/min	Amani *et al.*, 2004
D. esculenta *	75.9	79.8	85.7	13.3-13.6	Not available	Not available	Moorthy 2002
D. rotundata	70-77	-	-	13.7-16.7	1:5	25-160°C, 10°C/min	Amani *et al.*, 2004
D. rotundata *	72-79	75-83	81-88	10.3-15.0	Not available	Not available	Moorthy 2002
D. rotundata	71.5	74.8	80.5	~19.8	~1:2.3	2-100°C, 10°C/min	Farhat *et al.*, 1999
D. rotundata	63.5	66	71	-	-	Kofler hot stage microscopy	Emolia & Delarosa 1981
D. dumetorum	81.7	-	-	16.7	1:5	25-160°C, 10°C/min	Amani *et al.*, 2004
D. dumetorum	78.1	81.3	86.4	18.6	~1:2.3	2-100°C, 10°C/min	Farhat *et al.*, 1999
D. dumetorum	65.5	68	72.5	-	-	Kofler hot stage microscopy	Emolia & Delarosa 1981
D. cayenensis	66-69	72.9	76.7	-	Not available	Not available	Moorthy 2002
D. cayenensis	69.4	72.9	76.7	~19.8	~1:2.3	2-100°C, 10°C/min	Farhat *et al.*, 1999
D. cayenensis	68	72	74.5	-	-	Kofler hot stage microscopy	Emolia & Delarosa 1981
D. opposita	~74-75	~81-82	~85-87	10.8-11.4	1:2	20-120°C, 10°C/min	Shujun *et al.*, 2006b
D. opposita	~74-75	~79-81	83-87	8.4-12.4	1:2	20-120°C, 10°C/min	Shujun *et al.*, 2006c
D. opposita *	~73-74	~78-80	82-86	6.5-12.1	1:2	20-120°C, 10°C/min	Shujun *et al.*, 2004

Table 2-8 Gelatinization parameters and scanning conditions of various tuber starches (cont.,)

Starch source	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	Starch:H_2O	Scanning conditions (DSC/Kofler)	Reference
D. abysinica	64.2	68.2	74.8	19.2	1:2	40-90°C, 10°C/min	Gebre-Mariam & Schimidt 98"
Dioscorea	64.6	70.9	77.8	13.3	1:3	25-100°C, 10°C/min	McPherson & Jane 1999
Canna edulis	65.4	70.1	74.8	14.2	1:3	25-100°C, 10°C/min	Peroni *et al.*, 2006
Canna edulis	67.4	70.0	78.9	18.7	1:2	15-120°C, 2°C/min	Srichuwong *et al.*, 2005a
Canna edulis *	~66-67	~68-69	~70-72	~17-18	1:2	30-150°C, 5°C/min	Thitipraphunkul *et al.*, 2003
Canna edulis	65	-	70	-	-	Kofler hot stage microscopy	Soni *et al.*, 1990
C. maxima *	~35-37	52-55	64-65	7.8-8.9	1:3	10-120°C, 10°C/min	Stevenson *et al.*, 2005
Cucurbita foetidissima	61.2	-	68.8	-	-	Kofler hot stage microscopy	Dreher & Berry 1983
S. tuberosum *	~59-62	~62-65	~70-74	~14.3-17.4	Not available	30-100°C, 5°C/min	Karim *et al.*, 2007
S. tuberosum *	~60-63	~64-67	~70-72	~12.8-15.8	1:2	20-100°C, 10°C/min	Singh *et al.*, 2006
Solanum tuberosum	61.4	65.0	77.7	19.8	1:2	15-120°C, 2°C/min	Srichuwong *et al.*, 2005a
S. tuberosum *	~65-68	~70-75	-	16-18	~1:3	5-180°C, 10°C/min	Liu *et al.*, 2003
Solanum tuberosum	59.8	64.3	69.3	17.9	~1:2.3	2-100°C, 10°C/min	Farhat *et al.*, 1999
S. tuberosum- waxy	62.5	66.6	70.2	18.2	1:3	25-100°C, 10°C/min	McPherson & Jane 1999
Manihot esculenta	63	70	79	13.5	1:9	30-120°C, 10°C/min	Karam *et al.*, 2006
Manihot esculenta	61.6	66.7	72.9	10.4	1:3	25-100°C, 10°C/min	Peroni *et al.*, 2006
Manihot esculenta	59.3	65.7	79.6	17.6	1:2	15-120°C, 2°C/min	Srichuwong *et al.*, 2005a
Manihot esculenta	51.5	71.0	63.5	6.5	1:2	Not available, 5°C/min	Freitas *et al.*, 2004
Manihot esculenta	65.5	69.7	80.6	18.1	1:2	30-150°C, 5°C/min	Thitipraphunkul *et al.*, 2003
Manihot esculenta	58.5	-	70.0	-	-	Kofler hot stage microscopy	Srivastava *et al.*, 1970
Manihot esculenta	64.1	69.0	76.4	-	~1:2.3	2-100°C, 10°C/min	Farhat *et al.*, 1999
M. arundinacea	73.5	75.9	86.8	17.5	1:2	15-120°C, 2°C/min	Srichuwong *et al.*, 2005a
M. sagu (sago)	65.4	70.4	81.9	17.0	1:2	15-120°C, 2°C/min	Srichuwong *et al.*, 2005a
Ipomea batatas	62.9	70.6	77.9	12.9	1:3	25-100°C, 10°C/min	Peroni *et al.*, 2006
Ipomea batatas	66.7	74.0	86.6	18.4	1:2	15-120°C, 2°C/min	Srichuwong *et al.*, 2005a
S. rotundifolius *	~74-77	~79-83	~87-91	17.8-18.7	1:3	25-130°C, 10°C/min	Jayakody *et al.*, 2005
Caryota urines	74.1	77.5	83.2	12.6	1:3	25-130°C, 10°C/min	Jayakody *et al.*, 2007b
Borassus flabellifer	75.6	81.4	88.8	14.9	1:3	25-130°C, 10°C/min	Jayakody *et al.*, 2007b
X. sagitifolium	74.3	77.2	87.3	13.7	1:2	15-120°C, 2°C/min	Srichuwong *et al.*, 2005a
X. sagitifolium	71.5	77.2	85.4	13.1	1:3	20-120°C, 10°C/min	Gunaratne & Hoover 2002
Colocasia esculenta	74.2	77.4	86.4	16.2	1:2	15-120°C, 2°C/min	Srichuwong *et al.*, 2005a
Colocasia esculenta	76.8	83.0	95.2	14.5	1:3	20-120°C, 10°C/min	Gunaratne & Hoover 2002
Pueraria lobata	68.4	74.8	88.2	17.4	1:2	15-120°C, 2°C/min	Srichuwong *et al.*, 2005a
A.heterophyllus	60.1	66.1	74.2	12.8	1:3	25-130°C, 10°C/min	Jayakody *et al.*, 2007b
Zingiber officinale	82.4	87.4	92.5	15.9	1:3	25-100°C, 10°C/min	Peroni *et al.*, 2006

* Study on varieties Scanning conditions: canning range (°C), heating rate (°C/min)

postulated that gelatinization parameters are influenced by the molecular architecture of the crystalline region, which corresponds to the distribution of amylopectin short chains (DP 6-11) and not by amylose: amylopectin ratio. X-ray scattering studies have shown that a rapid drop in crystallinity occurs between the onset (T_o) and maximum or peak (T_p) temperature in the DSC endotherm. After the conclusion temperature (T_c), all amylopectin double helices are dissociated (Tester & Debon 2000). It is believed that the high peak temperature (T_p) is a reflection of perfection (registration) of crystallites (Jacobson & BeMiller 1998). Cooke and Gidley (1992) have suggested that the gelatinization enthalpy (ΔH) primarily reflects loss of double-helical (molecular) order rather than loss of crystalline register (perfection). However, Tester and Morrison (1990b) have suggested that ΔH reflects crystallite perfection.

The endothermic enthalpy values of native starches are in the range ~10-21 J/g **[Table 2-8]**. In general, T_o, T_p, T_c and ΔH are higher for *Dioscorea* starches than normal maize, rice, and oat starches (Jayakody 2001). T_o values for *Dioscorea* starches have been shown to be higher than those of potato, sweet potato, cassava, and canna starches **[Table 2-8]**. This indicates existence of the stronger bonding forces between *Dioscorea* glucan chains than in the other starch sources.

2.4.1.3 Factors influencing gelatinization characteristics

Gelatinization parameters of starches are influenced by: botanical source (Peroni *et al.*, 2006, Srichuwong *et al.*, 2005a, Matsuki *et al.*, 2003, Vandeputte *et al.*,

2003a, Wong *et al.*, 2003, Nakamura *et al.*, 2002, Noda *et al.*, 1998), method of starch extraction (Waigh *et al.*, 2000b), granule size (Shujun *et al.*, 2006c, Singh *et al.*, 2006, Jayakody *et al.*, 2005, Yusuph *et al.,* 2003, Karlsson & Eliasson 2003, Liu *et al.*, 2003, Stevens & Elton 1971, Geddes *et al.*, 1965), growth temperature (Tester *et al.*, 1999, Debon *et al.*, 1988, Cottrell *et al.*, 1995, Myllärinen *et al.*, 1998b, MacLeod & Duffus 1998, Tester 1997, Lu *et al.*, 1996, Tester *et al.*, 1995, Shi *et al.*, 1994, Tester *et al.*, 1991, Morrison & Azudin 1987, Asaoka *et al.*, 1985a,b, & 1984, Wiegand & Cuellar 1981), planting and harvesting period (Sriroth *et al.*, 1999, Defloor *et al.*, 1998, Noda *et al.*, 1997, Campbell *et al.*, 1994), development stage of tuber/roots (Noda *et al.*, 1997), maturity of starch granule (Karlsson & Eliasson 2003), granule morphology (Stevens & Elton 1971), starch: water ratio (Farhat *et al.*, 1999, Cottrell *et al.*, 1995, Ziegler *et al.*, 1993, Barichello *et al.*, 1991, Blanshard 1987, French 1984, Donovan 1979), damaged starch content (Waduge *et al.*, 2005, Karlsson and Eliasson 2003, Tester *et al.*, 1998, Hoover & Manuel 1996, Kulp 1972), amylose content (Peroni *et al.*, 2006, Shujun *et al.*, 2006c, Sandhu *et al.*, (2004), Baldwin 2001, Hizukuri 1996, Cottrell *et al.*, 1995, Hizukuri 1969), lipid content (Russell 1987b, Biliaderis *et al.*, 1986b, Evans 1986, Biliaderis *et al.*, 1985, Kugimiya & Donovan 1981), phosphorus content (Singh *et al.*, 2006, Yuan 1993), crystallite size (Singh *et al.*, 2006), degree of crystallinity (Tester *et al.*, 1991, Tester & Morrison 1990b), crystallite perfection (Perera *et al.*, 2001, Tester *et al.*, 1991), double helical order/molecular order (Cooke & Gidley 1992), length of the double helices (Jacobs *et al.*, 1998a), amylopectin chain length (Wang *et al.*, 2006,

Srichuwong *et al.*, 2005a, Matsuki *et al.*, 2003, Vandeputte *et al.*, 2003a, Wong *et al.*, 2003, Nakamura *et al.*, 2002, Noda *et al.*, 1998, Cooke & Gidley 1992), degree of amylopectin chain branching (Leszkowiat *et al.*, 1990, Hoover & Sosulski 1985), starch structure stability (Singh *et al.*, 2006, Biliaderis *et al.*, 1986a, Hoover & Sosulski 1985, Maurice *et al.*, 1985), stability of amorphous region (Biliaderis *et al.*, 1990, Leszkowiat *et al.*, 1990, Tester & Morrison 1990b), intermolecular attractions (Moorthy 2002), granule swelling (Emiola & Delarosa 1981), thermal history (Waigh *et al.*, 2000a,b), heating rate (Freitas *et al.*, 2004, Ziegler *et al.*, 1993, Liu & Leliévre 1991a, Dodd & Tonge 1987, Biliaderis *et al.*, 1986b, Donovan 1979), moisture equilibration period (Jayakody 2001, Gebre-Mariam & Schmidt 1998, Barichello *et al.*, 1990, Hoover & Sosulski 1984), and possibly location and quantity of starch granule-associated proteins and lipids (Hamaker & Griffin 1993, Hamaker & Griffin 1990, Mañingat & Juliano 1980).

2.4.1.3.1 Effect of water to starch ratio

The relationship between the type of endotherm (e.g. single peak or double peak) and water to starch ratio was first demonstrated by Donovan (1979). The single peak endotherm (G) is associated with a high water [**Figure 2-9-A**]: starch ratio (>65%) and the second endotherm (M_1) occurs at a medium water content (< 50%) (Cottrell *et al.*, 1995, Barichello *et al.*, 1991, Tester & Morrison 1990a) [**Figure 2-9-B**]. At extremely low water content, the first endotherm disappears and gelatinization occurs entirely by melting of the crystallites (Blanshard 1987, French 1984, Donovan *et al.*, 1983) [**Figure 2-9-C**]. However, no apparent

Figure 2-9 Gelatinization mechanism at different starch: water ratios

Adapted from Waigh *et al.*, (2000a), Jayakody & Hoover (2004)

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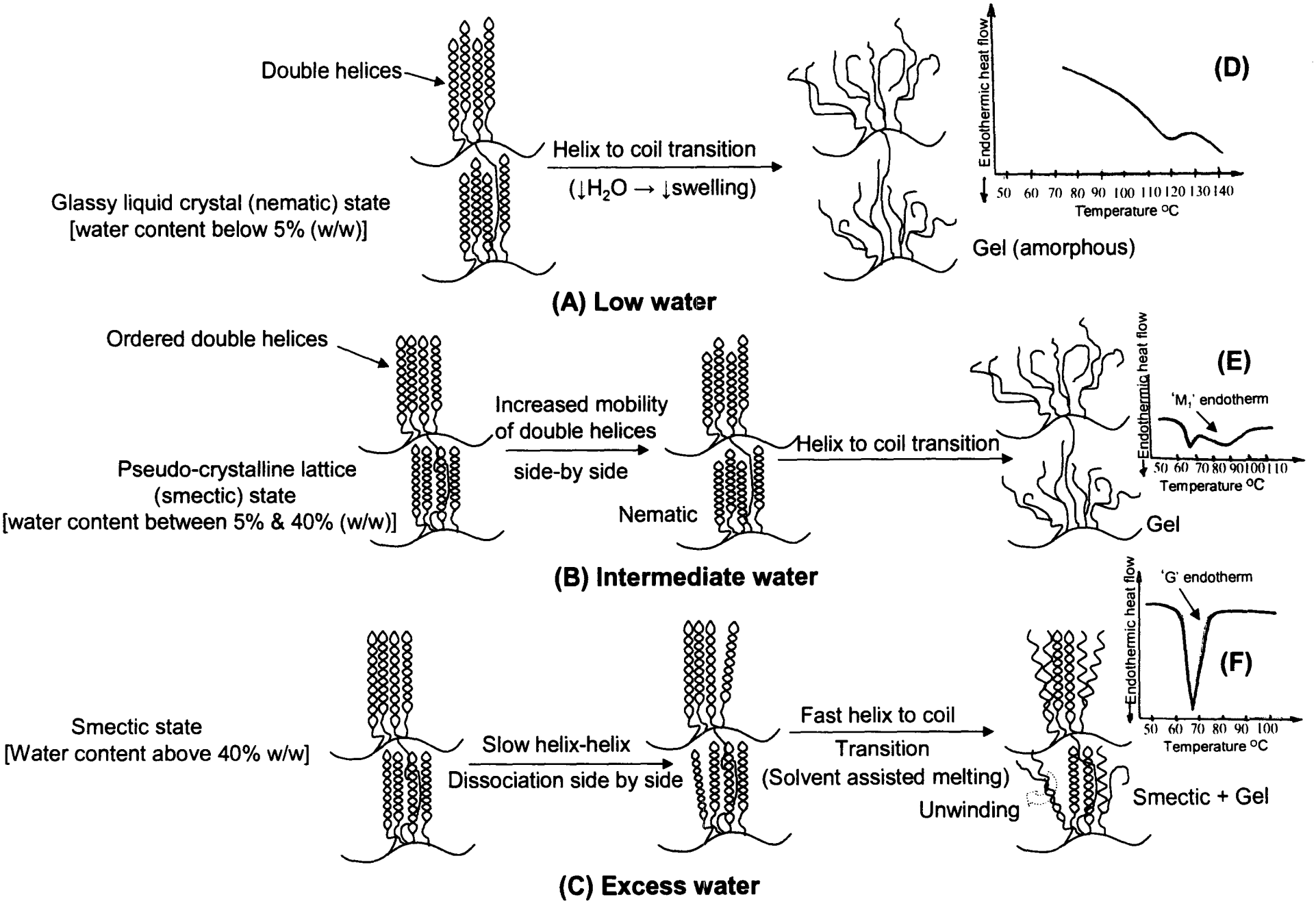
A-C indicates the helix to coil transformation in presence of excess water **(A)**, intermediate water **(B)** and low water **(C)** contents

D-F indicates the DSC endotherm in presence of excess water **(D)**, intermediate water **(E)** and low water **(F)** contents


Double helices
Helix to coil transition
(↓H_2O → ↓swelling)
Glassy liquid crystal (nematic) state
[water content below 5% (w/w)]
Gel (amorphous)
Endothermic heat flow
50 60 70 80 90 100 110 120 130 140
Temperature °C
(D)
(A) Low water
Ordered double helices
Increased mobility
of double helices
side-by side
Pseudo-crystalline lattice
(smectic) state
[water content between 5% & 40% (w/w)]
Nematic
Helix to coil transition
Gel
'M_1' endotherm
(E)
50 60 70 80 90 100 110
(B) Intermediate water
Smectic state
[Water content above 40% w/w]
Slow helix-helix
Dissociation side by side
Fast helix to coil
Transition
(Solvent assisted melting)
Unwinding
Smectic + Gel
'G' endotherm
(F)
50 60 70 80 90 100
(C) Excess water

endothermic transition is observed when starch concentration is at or below 30% (Ziegler *et al.*, 1993).

2.4.1.3.2 Effect of heating rate and gelatinization parameters

In general, the height of the endotherm is proportional to the heating rate. Therefore, a reasonably fast heating rate is needed to obtain peaks in the thermograms with a good signal-to-noise ratio. However, Dodd and Tong (1987) have shown that increasing the heating rate will often decrease the resolution between two adjacent peaks. Therefore, a median heating rate of 10°C/min is normally used. The upper temperature limit is generally below 180°C, since starch thermal decomposition occurs at 180°C (Puddington 1948). It has been observed that an increase in the heating rate increases peak temperature (T_p) but does not significantly influence the onset temperature (T_o) (Freitas *et al.*, 2004, Ziegler *et al.*, 1993, Liu & Leliévre 1991a, Biliaderis *et al.*, 1986b). Freitas *et al.*, (2004) have shown that increasing the heating rate slightly increases T_c but it does not significantly affect the enthalpy values (ΔH) of yam (*D. alata*) starches.

2.4.1.3.3 Effect of phosphorous content

Singh *et al.*, (2006) and Yuan *et al.*, (1993) have shown a positive correlation between lower gelatinization transition temperatures and higher amount of phosphorus in starches. The phosphate groups may destabilize the crystalline structures in the amylopectin regions of the starch granules, leading to lowering of the gelatinization and melting temperatures of the starches (Wischmann *et al.*,

2005). Wang *et al.*, (2006) suggested that phosphorus reduces crystallite perfection and decreases gelatinization temperatures.

2.4.2 Granular swelling

Overview

The initial absorption of water and rapid radial swelling occur primarily within the amorphous growth ring. When starch is heated in excess water, the crystalline structure is disrupted (due to dissociation of hydrogen bonds) and water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of the starch components (Liu *et al.*, 1999, Tester & Karkalas 1996, Lee & Osman 1991). This leads to increase in granule swelling and amylose leaching (Tester & Morrison 1990a, Blanshard 1987). Tester and Morrison (1990a) have hypothesized that the swelling is a property of amylopectin, and amylose acts as a diluent. In general, legume, tuber and root starches exhibit single stage swelling and solubilization patterns (Hoover 2001, Hoover & Sosulski 1991, Richard *et al.*, 1991, Kawabata *et al.*, 1984), whereas, cereal starches show a two stage swelling and solubilization (Langton & Hermansson 1989, Doublier *et al.*, 1987b, Soni *et al.*, 1985, Leach *et al.*, 1959). A single stage swelling pattern indicates relaxation of bonding forces within starch granules over one temperature and not at multiple temperature ranges because bonding forces are more uniform and stronger. Two-stage swelling indicates that there are two types of forces within granule structure which requires different energy inputs to weaken glucan chain interactions (Soni & Agarwal 1983, Leach 1965, Leach *et al.*, 1959). Several authors have shown that *Dioscorea* starches swell to a much

lower extent than potato starch (Karam *et al.*, 2006, Brunnschweiler *et al.*, 2005, Srichuwong *et al.,* 2005c, Emiola & Delarosa 1981). However, among different starches, the extent of granular swelling follows the order: waxy>normal>high amylose (Debet & Gidley 2006, Waduge *et al.*, 2005, Jayakody & Hoover 2002). Debet and Gidley (2006) have shown that although the temperatures of structural disorganization (as monitored by loss of birefringence or by DSC) are relatively similar, yet swelling profiles show major differences. This illustrates the fact that starch swelling rates and the extent of swelling cannot be predicted directly from a knowledge of the thermally induced loss of granular order.

Gravimetry (Leach *et al.*, 1959), colorimetry (Tester & Morrison 1990a), and laser light scattering (Ziegler *et al.*, 1993) have been used to determine the extent of granular swelling. The extent of granular swelling has been expressed as swelling power [SP] (Kite *et al.*, 1957) and as swelling factor [SF] (Tester & Morrison 1990a) [**Table 2-9**]. The SF is defined as the ratio of the swollen volume to the initial volume of air dried starch and has no units (Tester & Morrison 1990a). The measurement of SF was based on the observation that blue dextran dye (molecular weight $2x10^6$ Da) will dissolve in the supernatant and interstitial water but not in the intragranular water. Hence, SF measures only the water that enters the granule and hence contributes to volume expansion on heating. The SP is defined as weight of a sedimented starch gel, relative to its dry weight, obtained after gelatinizing starch in excess water at a given temperature for a specified time followed by centrifugation (Crosbie 1991, Swinkels 1985a, Leach *et al.*, 1959, Kite *et al.*, 1957).

Table 2-9 Swelling factor (SF), swelling power (SP), solubility and amylose leaching (AML) of various starches

Starch source	SF	SP [g/g]	Solubility (%)	AML (%)	Reference
D. alata (2 varieties)	-	14.5-19.5 [95°C]	-	-	Wang *et al.*, 2006
D. alata (different varieties)	-	13.8-16.0 [90°C]	-	-	Amani *et al.*, 2004
D. alata	-	~2 [70°C]	0.0 [70°C]	-	Srichuwong *et al.*, 2005c
D. alata	-	~15 [80°C]	~9 [80°C]	-	Srichuwong *et al.*, 2005c
D. alata	-	~23 [90°C]	~10 [90°C]	-	Srichuwong *et al.*, 2005c
D. alata	5.3 [70°C]	-	-	1.1 [70°C]	Gunaratne & Hoover 2002
D. alata	26.0 [80°C]	-	-	13.0 [80°C]	Gunaratne & Hoover 2002
D. alata	33.0 [90°C]	-	-	20.1 [90°C]	Gunaratne & Hoover 2002
D. alata	-	20.5 [95°C]	7.8 [95°C]	-	Emolia & Delarosa 1981
D. esculenta	-	~2 [60°C]	~0 [60°C]	~0 [60°C]	Srichuwong *et al.*, 2005c
D. esculenta	-	~8 [70°C]	~5 [70°C]	~4 [70°C]	Srichuwong *et al.*, 2005c
D. esculenta	-	~21 [80°C]	~8 [80°C]	~7 [80°C]	Srichuwong *et al.*, 2005c
D. esculenta	-	~34 [90°C]	~15 [90°C]	~9 [90°C]	Srichuwong *et al.*, 2005c
D. esculenta	-				
D. esculenta (different varieties)	-	13.9-14.8 [90°C]	-	-	Amani *et al.*, 2004
D. rotundata (different varieties)	-	10.8-16.4 [90°C]	-	-	Amani *et al.*, 2004
D. rotundata	-	21.5 [95°C]	11.9 [95°C]	-	Emolia & Delarosa 1981
D. dumetorum (different varieties)	-	13.7 [95°C]	-	-	Amani *et al.*, 2004
D. dumetorum	-	18.6 [95°C]	16.8 [95°C]	-	Emolia & Delarosa 1981
D. opposita (different varieties)	-	10.9-12.4 [NA]	10.6-11.3 [NA]	-	Shujun *et al.*, 2006a
D. opposita (4 varieties)	-	10.5-11.8 [NA]	10.2-11.7 [NA]	-	Shujun *et al.*, 2006c
D. abyssinica	-	10 [65°C]	3.5 [65°C]	-	Gebre-Mariam & Schmidt 1998
D. abyssinica	-	17 [75°C]	5.5 [75°C]	-	Gebre-Mariam & Schmidt 1998
D. abyssinica	-	23 [85°C]	11.0 [85°C]	-	Gebre-Mariam & Schmidt 1998
D. hispida Dennst	-	15.6 [90°C]	-	15.8 [90°C]	Tattiyakul *et al.*, 2006
D. batata (2 varieties)	-	10.6-11.1 [95°C]	-	-	Wang *et al.*, 2006

Table 2-9 Swelling factor (SF), swelling power (SP), solubility and amylose leaching (AML) of various starches (cont.,)

Starch source	SF	SP [g/g]	Solubility (%)	AML (%)	Reference
Solenostemon rotundifolius - 2 verities	~2.47 [60°C]	-	-	0.0 [60°C]	Jayakody *et al.*, 2005
Solenostemon rotundifolius - 2 verities	~1.4-2.9 [70°C]	-	-	0.0 [70°C]	Jayakody *et al.*, 2005
Solenostemon rotundifolius - 2 verities	~17-23 [80°C]	-	-	~3.4-1.6 [80°C]	Jayakody *et al.*, 2005
Solenostemon rotundifolius - 2 verities	~34-27 [90°C]	-	-	~3.8-10.3 [90°C]	Jayakody *et al.*, 2005
Coleus parvilorus	-	~35 [95°C]	~35 [95°C]	-	Abraham & Mathew 1985
Curcubita foetidisima	-	14.6-26.5 [80°C]	14-15.6 [80°C]	-	Dreher & Berry 1983
Pueraria tuberosa	-	23 [95°C]	22 [95°C]	-	Soni & Agrawal 1983
Solanum tuberosum	-	31.3-48.9 [80°C]	3.3-8.9 [80°C]	-	Karim *et al.*, 2007
S. tuberosum (different varieties)	94-146 [80°C]	-	-	-	Yusuph *et al.*, 2003
Solanum tuberosum	37.6 [60°C]	-	-	4.5 [60°C]	Gunaratne & Hoover 2002
Solanum tuberosum	57.4 [70°C]	-	-	18.1 [70°C]	Gunaratne & Hoover 2002
Solanum tuberosum	60.0 [80°C]	-	-	22.0 [80°C]	Gunaratne & Hoover 2002
Solanum tuberosum	54.0 [90°C]	-	-	22.2 [90°C]	Gunaratne & Hoover 2002
Artocapuc artilis – breadfruit -native	-	6.40 [80°C]	93.5 [80°C]	-	Adebowale *et al.*, 2005
Artocapuc artilis – annealed starch	-	5.57 [80°C]	88.47 [80°C]	-	Adebowale *et al.*, 2005
Artocapuc artilis – HMT starch	-	5.44 [80°C]	49.93 [80°C]	-	Adebowale *et al.*, 2005
Manihot esculenta	4.6 [60°C]			7.0 [60°C]	Gunaratne & Hoover 2002
Manihot esculenta	31.0 [70°C]	-	-	15.0 [70°C]	Gunaratne & Hoover 2002
Manihot esculenta	43.0 [80°C]	-	-	16.6 [80°C]	Gunaratne & Hoover 2002
Manihot esculenta	36.5 [90°C]	-	-	17.2 [90°C]	Gunaratne & Hoover 2002
Manihot esculenta	-	51 [95°C]	26 [95°C]	-	Tian *et al.*, 1991
Colocasia esculenta	2.5 [60°C]	-	-	0.3 [60°C]	Gunaratne & Hoover 2002
Colocasia esculenta	8.5 [70°C]	-	-	2.3 [70°C]	Gunaratne & Hoover 2002
Colocasia esculenta	36.0 [80°C]	-	-	22.1 [80°C]	Gunaratne & Hoover 2002
Colocasia esculenta	34.2 [90°C]	-	-	23.0 [90°C]	Gunaratne & Hoover 2002
Canna edulis	-	~38 [90°C]	~21 [90°C]	~17 [90°C]	Srichuwong *et al.*, 2005c
Canna edulis	-	19 [95°C]	17 [95°C]	-	Rickard *et al.*, 1991
Xanthosoma sagitifolium	5.0 [60°C]			0.1 [60°C]	Gunaratne & Hoover 2002
Xanthosoma sagitifolium	10.6 [70°C]	-	-	0.6 [70°C]	Gunaratne & Hoover 2002
Xanthosoma sagitifolium	18.0 [80°C]	-	-	2.9 [80°C]	Gunaratne & Hoover 2002
Xanthosoma sagitifolium	22.0 [90°C]	-	-	5.4 [90°C]	Gunaratne & Hoover 2002
Xanthosoma sagitifolium	-	NA	189 [100°C]	-	Louzon *et al.*, 1995
Ipomea batatas	-	80 [90°C]	68 [90°C]	-	Sego *et al.*, 1987

SF, SP, AML and solubility determined temperatures are shown in parentheses HMT: heat moisture treated NA: Data not available

SP is expressed on a weight basis (g/g). The SP measures both the intergranular and intragranular water. The SF and SP of various starches are represented in **Table 2-9**.

2.4.2.1 Factors influencing granular swelling

Differences in granular swelling among starches are influenced by the interplay of several factors: botanical source (Debet & Gidley 2006, Peroni *et al.*, 2006, Brunnschweiler *et al.*, 2005, Hoover & Sosulski 1991, Hoover & Sosulski 1985, Swinkeles 1985a, Emiola & Delarosa 1981), granule size (Vasanthan *et al.*, 1999, Vasanthan & Bhatty 1996, Wong & Leliévre 1982), granule integrity (Sandhya Rani & Bhattacharya 1989), crystallinity (Jayakody *et al.*, 2007a & 2005, Jayakody & Hoover 2002, Vasanthan *et al.*, 1999, Tester & Morrison 1990a,b), amylose content (Waduge *et al.*, 2006, Tester *et al.*, 2000, Sasaki & Matsuki 1998, Morrison *et al.*, 1993a,b), amylose-lipid complex (Waduge *et al.*, 2006, Jayakody *et al.*, 2005, Moorthy 2002, Hoover & Manuel 1995,Tester *et al.*, 1993, Tester & Morrison 1990a,b, Swinkels 1985a, Lorenz & Kulp 1983, Mañingat & Juliano 1980), amylopectin structure (Sasaki & Matsuki 1998, Tester *et al.*, 1993, Shi & Seib 1992), amylopectin unit-chain length distribution (Srichuwong *et al.*, 2005c), glucan chain interactions (Jayakody *et al.*, 2007a, Waduge *et al.*, 2006, Tester *et al.*, 2000, Hoover & Manuel 1996, Hoover & Vasanthan 1994 a,c), phosphorous content (Karim *et al.*, 2007, Jayakody *et al.*, 2005, Singh *et al.*, 2003, Vasanthan *et al.*, 1999, Galliard & Bowler 1987) extent of starch damage (Tester *et al.*, 1998, Kulp 1972), hydrothermal modifications (Hoover & Vasanthan 1994a,b), chemical modifications (Landerito & Wang

2005), surface proteins and lipids (Debet & Gidley 2006), growth temperature (Myllärinen *et al.*, 1998b, Shi *et al.*, 1994, Tester *et al.*, 1991, Hizukuri 1969, Nikuni *et al.*, 1969). Starches having a high phosphate content have been shown to exhibit a high extent of granule swelling (Gérad *et al.*, 2001c). This has been attributed to increased accessibility of water molecules to the amorphous lamealle resulting from repulsion between negatively charged phosphate groups present on starch molecules (Karim *et al* 2007, Wang *et al.*, 2006, Singh *et al.*, 2006, Gérad *et al.*, 2001c, Vasanthan *et al.*, 1999, Kim *et al.*, 1996).

2.4.3 Amylose leaching (AML)

Studies on amylose leaching is important because the soluble fraction provides information on the extent of interaction between amylose-amylose and/or amylose-amylopectin in the granule interior. The extent of AML has been shown to be influenced by: heating temperature (Rolland-Sabaté *et al.*, 2003, Roger & Colonna 1986, Ring *et al.*, 1985), total amylose content (Nakazawa & Wang 2003, Rolland-Sabaté *et al.*, 2003), strength of interaction between amylose-amylose and amylose-amylopectin chains within granules of native starches (Waduge *et al.*, 2006, Jayakody *et al.*, 2005, Hoover & Vasanthan 1994a, Hoover & Hadziyev 1981), molecular size of amylose (Nakazawa & Wang 2003), amount of lipid complexed amylose chains (Jayakody & Hoover 2002, Hoover & Vasanthan 1994a, Tester & Morrison 1990a), and extent of glucan chain interactions within the amorphous domains (Waduge *et al.*, 2006). Data on mylose leaching at various temperatures are presented in **Table 2-9**.

Molecular properties of leached amylose have been shown to be influenced by the duration and extent of heating (Rolland-Sabaté *et al.*, 2003, Roger & Colonna 1986, Ring *et al.*, 1985). Several researchers (Tester & Morriosn 1990, Svegmark & Hermannson 1991, Ellis *et al.*, 1988, Doublier 1981) have shown that the material leached out during heating (50-70°C) of potato and most cereal starch granules in water is mainly amylose. In these starches, most of the amylose is solubilized before leaching of amylopectin begins (Doublier 1981). However, co-leaching of amylose and amylopectin has been shown to occur in the temperature range 70-90°C in oat starches (Hoover *et al.*, 1992, Doublier 1987b). Ellis *et al.*, (1988) have shown that not all of the amylose present within the native granule leaches out during heating. For instance, at 90°C, pea, wheat and maize starches were found to contain, 16%, 8.3% and 8.0% amylose, respectively. Colonna and Mercier (1985) showed that the amount of leached amylose corresponds to only 6 to 9% of the total starch in high amylose maize and pea starches but 60 to 76% in normal maize starch. It has been shown that the extent of amylose leaching of high amylose and the high lipid containing starches (e.g. amylomaize V, VII and oat) are much lower than normal and low lipid containing starches due to strong glucan chain interactions and high amount of amylose complexed with lipids (Jayakody & Hoover 2002). Both these factors hinder amylose leaching due to low mobility of glucan chains and reduced granular hydration. Amylose-lipid complexes decrease the extent of amylose leaching. Lipid does not leach out with amylose, since the AM-lipid complex does not dissociate until the temperature reaches 94-104°C (Tester & Morrison

1990a). Rolland-Sabaté *et al.*, (2003) reported that the leached fraction may contain different sizes of soluble components (e.g. amylose of different chain lengths). The above authors also showed that even at 90°C, densely branched fractions remain inside the granule. The leaching of amylose is necessary for gel formation. However, in the production of pasta or dehydrated potato flakes, leached amylose causes stickiness (Hoover & Hadziyev 1981). This problem has been overcome by adding monoglycerides during manufacture of dehydrated potato granules (Hoover & Hadziyev 1981).

2.4.5 Pasting characteristics

Overview

A paste is defined as a viscous mass consisting of a continuous phase (a molecular dispersion) of solubilized amylose and/or amylopectin and a discontinous phase of granule ghosts and fragments (Whistler & BeMiller 1977). The changes that occur during gelatinization and pasting greatly affect the rheological properties of the starch suspension (Jacobs & Delcour 1988). Thus, pasting characteristics are usually studied by observing changes in viscosity during heating of a starch suspension. Pasting characteristics of starch were first demonstrated by Caesar (1932) using a 'consistometer'. Subsequently, several other measuring devices have been introduced, such as the Brabender amylograph in the 1930s (Brabender 1965), corn industries' viscometer (Kesler & Bechtel 1947), the Ottawa starch viscometer (Voisey *et al.*, 1977), the Haake Rotovisko (Čeh & Stropnik 1976), and rapid visco analyzer [RVA] (Ross *et al.*, 1987). Many researchers have used the terms gelatinization and pasting

interchangeably. However, it should be borne in mind that gelatinization refers specifically to the disruption of the molecular order of the starch polymer and occurs first, whereas, pasting refers to the evidence of disruption of molecular order, such as an increase in viscosity when a starch paste is subjected to shear (Atwell *et al.*, 1988). As a starch-water system cools, glucan chain-water interactions (i.e. AM-H_2O and/or AMP-H_2O) are replaced mainly with starch-glucan chains interactions, and a gel network is formed (Zeng *et al.*, 1997). Use of starch in the food, paper and textile industries depends on the viscosity of the starch paste. Viscosity stability during heating and shear is important in foods that are subjected to high temperature processing.

2.4.5.1 Factors influencing pasting properties

The factors that influence viscosity development on heating and cooling of a starch suspenstion has been attributed to the interplay of several factors: botanical source (Otegbayo *et al.*, 2006, Akissoé *et al.*, 2003, Alves *et al.*, 2002, Jacobs & Delcour 1998, Liu *et al.*, 1997), granule size (Singh *et al.*, 2006, Jayakody *et al.*, 2005, Zheng & Sosulski 1997, Goering & DeHass 1972), starch concentration (Jacobs *et al.*, 1995, Standsted & Abbot 1961), amylopectin content (Peroni *et al.*, 2006, Singh *et al.*, 2006 & 2005, Zeng *et al.*, 1997), granule swelling (Hamaker & Griffin 1993, Doublier *et al.*, 1987a , Lineback & Rasper 1988), phosphorus content (Karim *et al.*, 2007, Moorthy 2002), leaching of macromolecules [e.g. amylose] (Ziegler *et al.*, 1993, Olkku and Rha 1978) that form an entangled network (Miller *et al.,* 1973), formation of a tightly packed array of swollen, deformable granules (Evans & Haisman 1979, Schoch 1969),

amylose-lipid complex formation (Olkku & Rha 1978), the resistance of the swollen granules to dissolution by heat or fragmentation (Schoch & Maywald 1968), friction between swollen granules (Singh *et al.*, 2006), heating and cooling rate (Jacobs and Delcour 1998), rotational speed of spindle (Deffernbaugh & Walker 1989, Goto & Yokoo 1969), competition between leached amylose & remaining ungelatinized granules for free water (Olkku & Rha 1978), harvesting period (Liu *et al.*, 2003, Madsen & Christensen 1996), and growth temperature (Hizukuri *et al.*, 1969, Nikuni *et al.*, 1969). The pasting conditions are presented in [**Table 2-10**].

It has been shown that higher proportions of large granules contain fewer granule remnants in their pastes (Singh *et al.*, 2006), thus allowing for greater light transmittance. This feature is important in the production of clear gels or films. The viscosity properties of *Dioscorea* and other starches have been extensively investigated. *Dioscorea* starches show lower peak viscosity than potato starch (Brunnschweiler *et al.*, 2006, Frarhat *et al.*, 1999). Otegbayo *et al.*, (2006) have shown that starch pastes of *D. rotundata* exhibit a higher viscosity breakdown, set back and final viscosity than pastes from *D. alata*. Moorthy (2002) reported that *D. alata* starch does not exhibit a sharp peak viscosity, whereas, a peak viscosity occurs in *D. esculenta* starch. However, both *D. alata* (Brunnschweiler *et al.*, 2005) and *D. esculenta* (Moorthy 2002) starches are resistant to viscosity breakdown during heating and shear. Singh *et al.*, (2006) have shown that the presence of higher amounts of large granules increase peak viscosity.

Table 2-10 Pasting profiles of various starches determined by Brabender (units BU) and Rapid visco analyzer (units RVU)

Botanical source	Pasting temp. (°C)	Peak time (min)	Peak viscosity RVU/BU	Viscosity breakdown RVU/BU	Final viscosity RVU/BU	Setback RVU/BU	Conditions Starch concentration % (w/w), rotational speed (rpm), heating range (°C), heating rate °C/min	Reference
D. alata	76.6	-	172 RVU	28 RVU	304 RVU	160 RVU	9%,160 rpm, 50-90°C, 6°C/min	Peroni *et al.*, 2006
D. alata -variety TN2	NA	NA	613 RVU	NA	342 RVU	40 RVU	NA	Wang *et al.*, 2006
D. alata –fresh paste	82.6	4.62	231 RVU	71 RVU	202 RVU	42 RVU	NA	Otegbago *et al.*, 2006
D. alata	83.2	11.1	394 RVU	17.7 RVU	556.3 RVU	179.4 RVU	8%,160 rpm, 50-90°C, 6°C/min	Srichuwong *et al.*, 2005c
D. alata- varieties	82.9	-	23.7 RVU	-	32.9 RVU	-	4%,160 rpm, 30-90°C, 6°C/min	Amani *et al.*, 2004
D. alata	79.8	5.70	3230 mPa	NA	3034 mPa	5826 mPa	10%, NA, 50-95°C, 12°C/min	Farhat *et al.*, 1999
D. alata	83-97	-	400 BU	300 BU	-	600 BU	5%, NA	Moorthy 1991
D. esculenta	75.7	8.2	219 RVU	118.7 RVU	246.9 RVU	73.5 RVU	8%,160 rpm, 50-90°C, 6°C/min	Srichuwong *et al.*, 2005c
D. esculenta	78.7	-	9.1 RVU	-	15.3 RVU	-	4%,160 rpm, 30-90°C, 6°C/min	Amani *et al.*, 2004
D. esculenta	79-97	-	580 BU	560 BU	-	620 BU	6%, NA	Moorthy 1991
D. cayenensis	75.0	4.67	3893 mPa	NA	3073 mPa	3965 mPa	10%, NA, 50-95°C, 12°C/min	Farhat *et al.*, 1999
D. dumetorum	87.5	-	2.5 RVU	-	5.0 RVU	-	4%, 160 rpm, 30-90°C, 6°C/min	Amani *et al.*, 2004
D. dumetorum	83.1	4.67	2028 mPa	NA	1593 m Pa	2278 m Pa	10%, NA, 50-95°C, 12°C/min	Farhat *et al.*, 1999
D. rotundata- fresh paste	79.5	4.62	375 RVU	172 RVU	397 RVU	195 RVU	NA	Otegbago *et al.*, 2006
D. rotundata	78.2	4.93	3273 mPa	NA	2779 mPa	4227 mPa	10%, NA, 50-95°C, 12°C/min	Farhat *et al.*, 1999
D. rotundata	83-97	-	480 BU	400 BU	-	420 BU	5%, NA	Moorthy 1991
D. hispida Dennst	78.3	NA	361 RVU	285 RVU	377 RVU	92 RVU	12%, NA, 50-95°C, 12°C/min	Tattiyakul *et al.*, 2006
Ipomea batatas	74.2	-	281 RVU	148 RVU	206 RVU	73 RVU	9%, 160 rpm, 50-90°C, 6°C/min	Peroni *et al.*, 2006
Ipomea batatas	75.2	7.0	265 RVU	151 RVU	187 RVU	73 RVU	8%, 160 rpm, 50-90°C, 6°C/min	Srichuwong *et al.*, 2005c
Ipomea batatas	81-84		340 BU	340 BU	-	360 BU	6%, NA	Moorthy 1991
S. tuberosum- verities	NA	NA	338 RVU	119 RVU	218 RVU	31 RVU	7.4%,160 rpm,50-90°C, 12°C/min	Singhe *et al.*, 2006
S. tuberosum- verities	NA	NA	489 RVU	256 RVU	266 RVU	37 RVU	7.4%,160 rpm,50-90°C, 12°C/min	Singhe *et al.*, 2006
Solanum tuberosum	67.3	5.1	79 RVU	563 RVU	287 RVU	59 RVU	8%, 160 rpm, 50-90°C, 6°C /min	Srichuwong *et al.*, 2005c
Solanum tuberosum	65.5	2.87	8944 mPa	NA	1933 mPa	2968 mPa	10%, NA, 50-95°C, 12°C/min	Farhat *et al.*, 1999
Pueraria lobata	76.0	7.3	231 RVU	109 RVU	188 RVU	66 RVU	8%, 160 rpm, 50-90°C, 6°C/min	Srichuwong *et al.*, 2005c

Table 2-10 Pasting profiles of various starches determined by Brabender (units BU) & Rapid visco analyzer (units RVU) (cont.,)

Botanical source	Pasting temp. (°C)	Peak time (min)	Peak viscosity	Viscosity breakdown	Final viscosity	Setback	Conditions Starch concentration % (w/w), rotational speed (rpm), heating range (°C): heating rate °C/min	Reference
S. rotundifolius- (Bola)	78.0	9.2	122 RVU	15 RVU	166 RVU	60 RVU	7%,160 rpm, 50-90°C, 6°C/min	Jayakody *et al.*, 2005
S. rotundifolius- (Dik)	83.0	8.8	119 RVU	12 RVU	187 RVU	80 RVU	7%,160 rpm, 50-90°C, 6°C/min	Jayakody *et al.*, 2005
M. arundinacea	77.8	6.7	361 RVU	203 RVU	255 RVU	96 RVU	8%, 160 rpm, 50-90°C 6°C/min	Srichuwong *et al.*, 2005c
M. arundinacea	71.7	-	339 RVU	204 RVU	197 RVU	62 RVU	9%, 160 rpm, 50-90°C 6°C/min	Peroni *et al.*, 2006
M. sagu (sago)	72.8	6.3	204 RVU	128 RVU	137 RVU	61 RVU	8%, 160 rpm, 50-90°C 6°C/min	Srichuwong *et al.*, 2005c
Caryota urines	73.8	6.4	134 RVU	60 RVU	130 RVU	55 RVU	7%,160 rpm, 50-90°C, 6°C/min	Jayakody *et al.*, 2007b
Borassus flabellifer	76.7	8.2	92 RVU	33 RVU	97 RVU	38 RVU	7%,160 rpm, 50-90°C, 6°C/min	Jayakody *et al.*, 2007b
A.heterophyllus -flesh	65.4	8.8	130 RVU	36 RVU	182 RVU	88 RVU	7%,160 rpm, 50-90°C, 6°C/min	Jayakody *et al.*, 2007b
A.heterophyllus -seed	83.0	8.3	41 RVU	16 RVU	37 RVU	11 RVU	7%,160 rpm, 50-90°C, 6°C/min	Jayakody *et al.*, 2007b
Canna edulis	71.2	-	413 RVU	198 RVU	335 RVU	120 RVU	9%,160 rpm, 50-90°C, 6°C/min	Peroni *et al.*, 2006
Canna edulis	72.4	7.5	397 RVU	140 RVU	381 RVU	124 RVU	8%,160 rpm, 50-90°C 6°C/min	Srichuwong *et al.*, 2005c
X. sagitifolium	73.6	7.3	248 RVU	98 RVU	269 RVU	120 RVU	8%, 160 rpm, 50-90°C 6°C/min	Srichuwong *et al.*, 2005c
X. sagitifolium	81-87	-	470 BU	470 BU	-	550 BU	6%, NA	Moorthy 1991
Colocasia esculenta	78	7.7	251 RVU	112 RVU	249 RVU	110 RVU	8%, 160 rpm, 50-90°C 6°C/min	Srichuwong *et al.*, 2005c
Colocasia esculenta	79-88	-	420 BU	400 BU	-	400 BU	6%, NA	Moorthy 1991
Artocapuc artilis –native	64.6	-	467 RVU	379 RVU	641 RVU	174 RVU	NA	Adebowale *et al.*, 2005
A. artilis – annealed	63.6	-	407 RVU	321 RVU	443 RVU	36 RVU	NA	Adebowale *et al.*, 2005
A. artilis- HMT	65.0	-	43.8 RVU	20.3 RVU	47.2 RVU	3 RVU	NA	Adebowale *et al.*, 2005
Manihot esculenta	67.4	-	263 RVU	160 RVU	164 RVU	61 RVU	9%,160 rpm, 50-90°C, 6°C/min	Peroni *et al.*, 2006
Manihot esculenta	67.4	6.1	188 RVU	121 RVU	113 RVU	47 RVU	8%,160 rpm, 50-90°C, 6°C/min	Srichuwong *et al.*, 2005c
Manihot esculenta	68.6	3.73	3134 mPa	NA	1316 mPa	2381 mPa	10%, NA, 50-95°C, 0.2°C/s	Farhat *et al.*, 1999
Manihot esculenta	70-90	-	480 BU	420 BU	-	380 BU	5%, NA	Moorthy 1991
Zingiber officinale	>95	-	78 RVU	0.2 RVU	119 RVU	41 RVU	9%, 160 rpm,50-90°C, 6°C/min	Peroni *et al.*, 2006
Lathyrus sativus-(lath)	74	8.8	268 RVU	41 RVU	417 RVU	189 RVU	7%,160 rpm, 50-90°C, 6°C/min	Jayakody *et al.*, 2007a

NA: data not available some authors have reported viscosity (using the RVA) as mili Pascal (mPa) HMT: Heat moisture treated

Large granules (e.g. potato starch) swell to a greater extent and occupy more volume. Consequently, they enhance viscosity (Singh *et al.*, 2006). Studies on *Colocasia* starches showed small granules have a lower pasting temperature than large granules (Zheng & Sosulski 1997, Goering & DeHass 1972). Amani *et al.*, (2004) reported that viscosity variables positively correlate with amylose content, granule size and swelling power of *Dioscorea* starches. However, in general, starches with lower amylose contents (i.e. apparent or total) are generally associated with higher peak viscosities (Moorthy 2002, Dengate 1984), greater viscosity (e.g. corn starch) breakdown, lower final viscosity and low setback (Zeng *et al.*, 1997). Zeng *et al.*, (1997) have observed that a 1% reduction in apparent or total amylose content corresponds to an increase in peak viscosity of about 22 to 25 Rapid Visco Analyzer units (RVU) at 12% starch concentration (Zeng *et al.*, 1997). Hamaker and Griffin (1993) have shown that proteins with intact disulfide bonds make the swollen granules less susceptible to breakdown under shear, either by giving extra strength to the swollen granules or by decreasing granular swelling. Phosphorus has been shown to have a negative effect on setback (Karim *et al.*, 2007). In *Dioscorea* starches, phosphorus content was found to correlate positively with peak viscosity, but was negatively correlated with final viscosity (Wang *et al.,* 2006). Studies on *D. alata*, *D. esculenta* and *D. rotundata* starches revealed that pasting properties do not significantly change with the maturity stage of the tubers (Moorthy 2002). The RVA pasting profile data of *Dioscorea* starches are presented in [**Table 2-10**].

2.4.5.2 Pasting curves

The changes in viscosity or pasting characteristics of a starch suspension during heating and cooling under shear stress has been monitored using the Brabender and the rapid visco analyzer (RVA). In the RVA, viscosity is often expressed in Rapid visco analyser units (RVU) which could also be expressed as standard units (1 centipoise [cP] = 12 RVU), whereas, in the Brabender, viscosity is expressed in Brabender units (BU). The major disadvantage of the Brabender viscoamylogram are the units (BU) in which viscosity is expressed (this unit does not match with SI units), and the large sample size. In contrast, the RVA has several significant differences over the conventional Brabender amylograph such as: (1) smaller sample size/run [~25g vs 500g] (Perera & Hoover 1999, Jacobs *et al.*, 1996), (2) shorter analysis time [~20 min vs ~120 min], (3) out-put in standard viscosity units (cP vs BU), (4) higher heating and cooling rates [6.34°C/min vs 1.5°C/min] (AACC 2000, Jacobs *et al.*, 1996), (5) higher start and end temperatures [50°C vs 35°C/min] (Jacobs *et al.*, 1996), (6) geometry of the stirring device [stirring paddle vs stationary vertical pins and moving bowl], (Jacobs *et al.*, 1996), (7) higher stirring speed [160 rpm vs 75 rpm] (Perera & Hoover 1999, Jacobs *et al.*, 1996), (8) greater simplicity (Deffenbaugh & Walker 1989), and (9) higher shear rate (Jacobs *et al.*, 1995 & 1996). In addition, Dengate (1984) reported that the results of Brabender are not reproducible unless certain critical points are considered such as: time and temperature regime, starch concentration, model type, bowl speed, volume of slurry, torsion spring settings in use, exact slurry concentration, method of slurry preparation,

total weight of volume, and holding time. However, Jacobs *et al.*, (1996) have reported that pasting properties mainly depend on the heating/cooling rate and /or the start/end temperature of the measurement rather than on the measuring geometry, sample size or shear rate conditions. Several researchers have shown that compared to the Brabender, the RVA has a higher shear rate and therefore enhances greater granule swelling which leads to a more significant breakdown of the granules (Jacobs *et al.*, 1996, Deffenbaugh & Walker 1989).

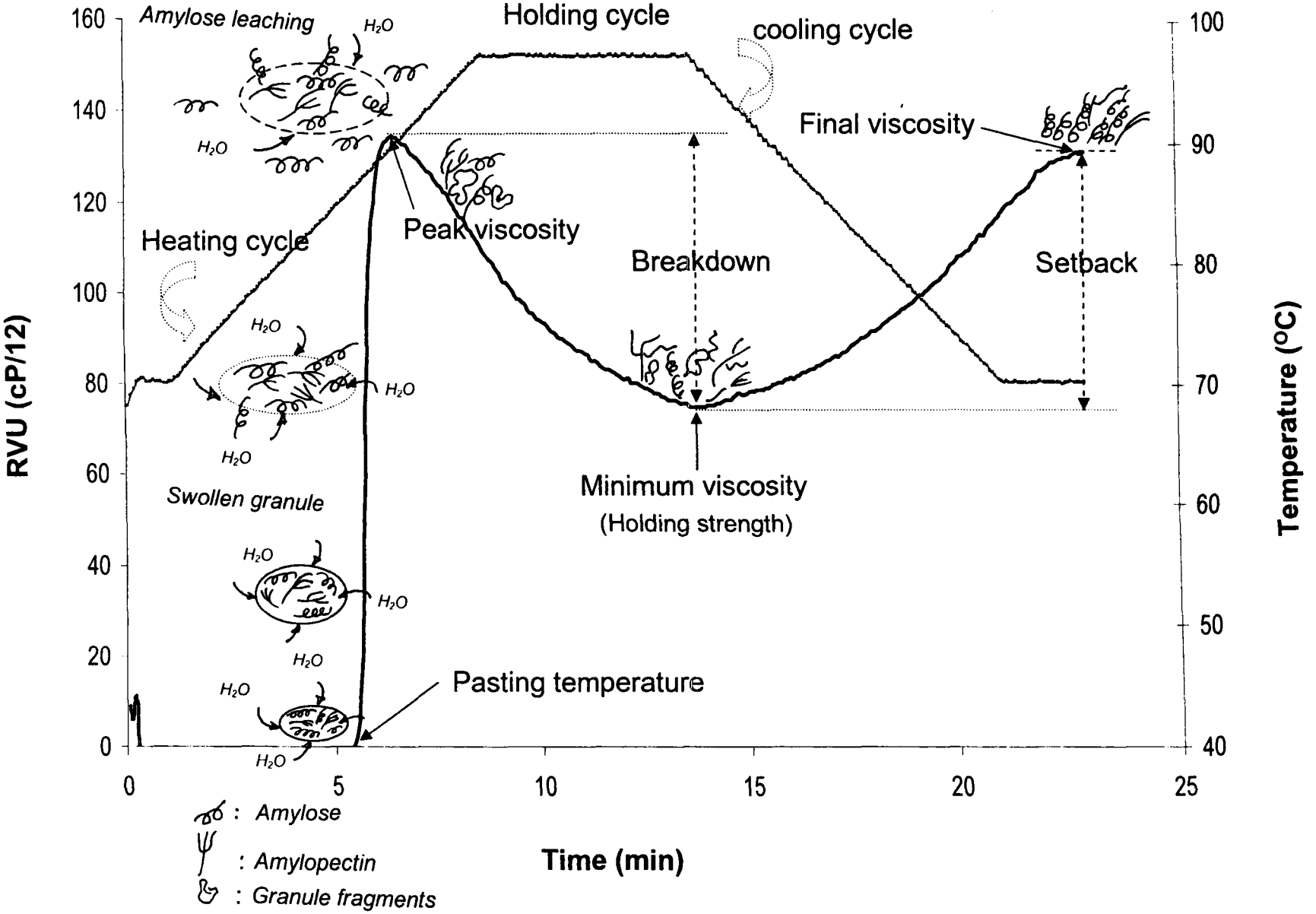
The viscosity profile of a starch suspension subjected to controlled heating and cooling in the RVA provides the following information [**Figure 2-10**]: (1) pasting temperature (indicates the initial increase in viscosity), (2) the peak viscosity (maximum viscosity attained during the heating cycle), (3) peak time (time to reach the peak viscosity) (4) break down viscosity or paste stability (difference between the peak viscosity and minimum viscosity during holding cycle), (4) set-back or cold paste viscosity (difference between the maximum viscosity during cooling and the minimum viscosity during holding cycle), (5) final viscosity or stability of the cooked paste (viscosity at the end of the RVA run) (Dengate 1984).

During the holding cycle, the granule disintegration appears to reach equilibrium. Breakdown viscosity is considered as a measure of the degree of disintegration of the swollen granule (Mazurs *et al.*, 1957). The more swollen the starch granules, the more shear-sensitive the paste. Trough or minimum viscosities have been shown to be lower in *Dioscorea* starches than in potato or

Figure 2-10 RVA pasting curve showing changes to granule size and integrity

Adapted from Jayakody & Hoover (2004) and information from: Jayakody *et al.*, (2007a,b), Thomas & Atwell (1999), Zhou *et al.*, (1998), Dengate (1984), Zeng *et al.*, (1977)

(cP and RVU indicate centipoises and rapid visco analyzer units, respectively)


Amylose leaching
H_2O
Holding cycle
cooling cycle
Final viscosity
Peak viscosity
Heating cycle
Breakdown
Setback
Swollen granule
Minimum viscosity
(Holding strength)
Pasting temperature
RVU (cP/12)
Temperature (°C)
Time (min)
: Amylose
: Amylopectin
: Granule fragments

cassava starches (Frarhat *et al.*, 1999). This indicates that the granules of *Dioscorea* starches are more stable to shear. Hot paste viscosity has been reported to decrease in the order: *D. alata* > *D. rotundata* > *D. esculenta* >*D. dumetorum* (Amani *et al.*, 2004, Farhat *et al.*, 1999). Setback viscosity is generally considered as a measure of gelling capacity or 'retrogradation' tendency of a starch paste (Mazurs *et al.*, 1957). Amylose content has been positively correlated with set-back (Singh *et al.*, 2006, Singh *et al.*, 2005, Lie *et al.*, 2003, Leelavathi *et al.*, 1987) and negatively correlated with viscosity breakdown (Singh *et al.*, 2006). During the cooling cycle, the solubilized amylose fraction retrogrades. The extent of the retrogrdation is measured by the difference between the final viscosity and the viscosity at the end of the holding period. In general, *Dioscorea* starches show positive setback during cooling (Moorthy 2002).

2.4.6 Acid hydrolysis

Overview

Acid hydrolysis has been used for over a century to modify starch granule structure and produce 'soluble' starch (Kirchoff 1811). Prolonged acid hydrolysis is commonly performed with either sulfuric (15.3%, 25°C) or hydrochloric acid (2.2M, 30-40°C) to produce Nägeli (1874) or Lintner (1886) amylodextrins, respectively (Jayakody 2001). In general, acidic and enzymatic attack on starch are similar since they both involve hydrolysis of the bonds in the starch polymers. In more precise terms however, significant differences exist between enzyme and acid hydrolysis with respect to hydrolytic cleavage. α-Amylase hydrolysis the

amorphous and crystalline regions simultaneously, the mode of attack being concentrated only on the α-D-(1→4) linkages, whereas acids hydrolysis the amorphous regions initially and then the crystalline region. Acid hydrolysis both α-D-(1→4) and α-D-(1→6) linkages. Furthermore, the extent of starch polymer degradation has been shown to be higher during enzyme hydrolysis (Zhou *et al.*, 2004, Jayakody 2001, Zherebtsov *et al.*, 1995).

The rate and extent of acid hydrolysis has been shown to be influenced by: starch source (Srichuwong *et al.*, 2005a, Hoover 2001, Hoover & Vasanthan 1994a), granule size (Jayakody *et al.*, 2005, Jayakody & Hoover, 2002, Jayakody 2001, Vasanthan & Bhatty 1996, Biliaderis *et al.*, 1981), type of unit cell (Jane 2006), proportion of B-type crystallites (Srichuwong *et al.*, 2005b), presence of pores on the granule surface (Jayakody & Hoover 2002), amylopectin structure (Srichuwong *et al.*, 2005b, Jacobs *et al.*, 1998a), crystallinity (Jayakody *et al.*, 2005), amylopectin unit chain-length distribution (Srichuwong *et al.*, 2005b, Tester *et al.*, 2005), characteristics of amorphous lamellae (Srichuwong *et al.*, 2005b), amylose content (Jayakody & Hoover 2002, Jayakody 2001), lipid complexed amylose chains (Waduge *et al.,* 2006, Jayakody & Hoover 2002, Jayakody 2001, Hoover 2000, Morrison *et al.*, 1994), phosphorus content (Jayakody *et al.*, 2005, Hoover 2000), granular swelling (Jayakody *et al.*, 2005) and extent of starch damage (Tester *et al.*, 1998).

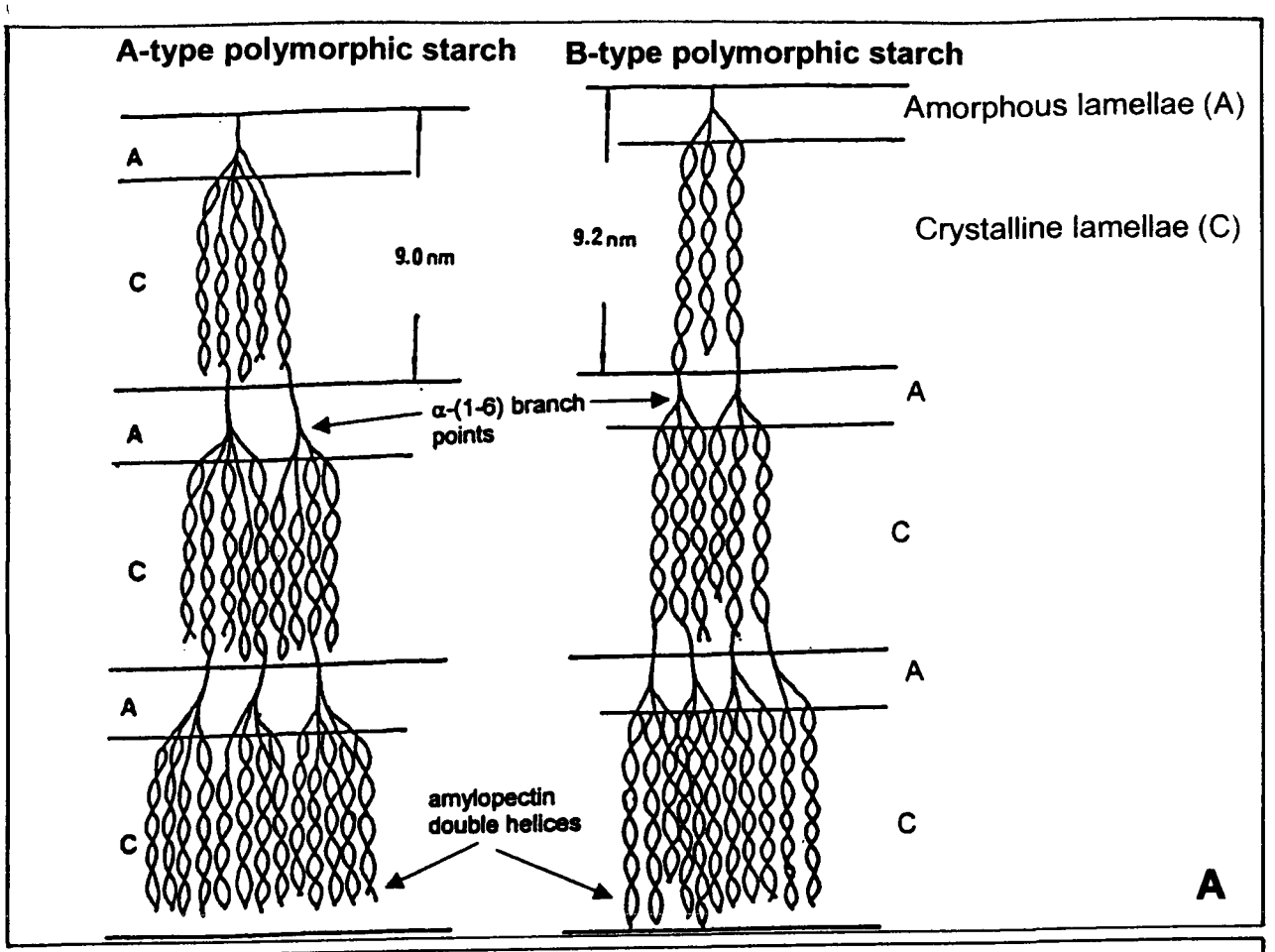
Two mechanisms have been recently proposed to explain how branch chain linkages are protected from acid hydrolysis (Jane 2006). The A-type starches

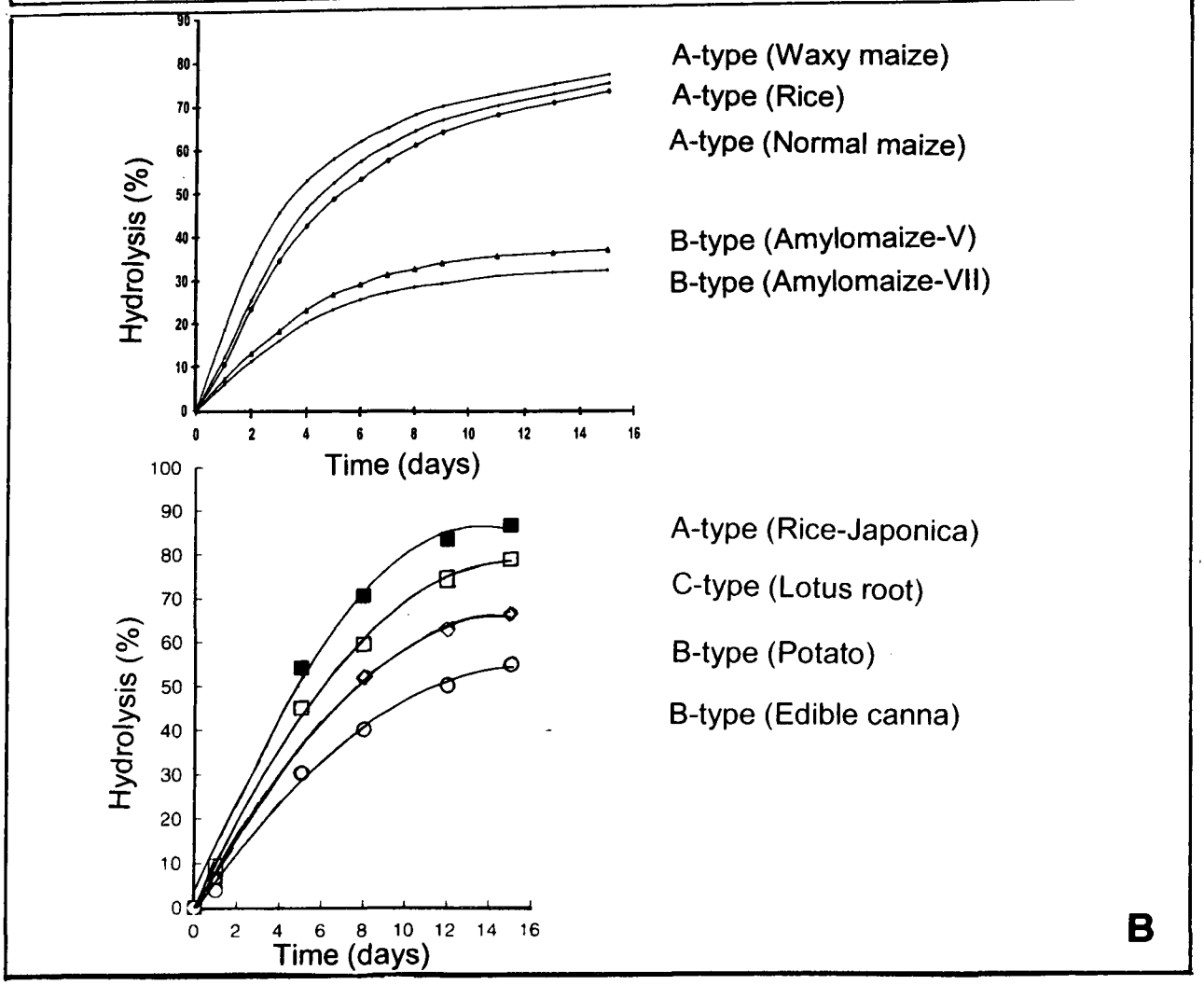
contain more shorter chains than B-type starches and most of their α-D-(1→6) branch points are present in the crystalline regions [**Figure 2-11-A**]. The α-D-(1→6) branch linkages that are scattered in the amorphous region are readily susceptible to acid hydrolysis, whereas branch points located in the crystalline regions are protected from acid attack [**Figure 2-11-A**]. However, B-type starches contain a larger proportion of long B chains with most of the branch linkages in the amorphous region, which are, therefore, more susceptible to acid hydrolysis (Jane *et al.*, 1997).

The second mechanism is based on the type of unit cell present in the A- and B-type of starches. The A- and B-types of polymorphic starches have a monoclinic and hexagonal unit cell, respectively. The α-D-(1→6) branch points of A-type starches are tightly packed within the monoclinic unit cell, consequently, they are not easily accessible to attack by H_3O^+. However, the α-D-(1→6) branch points of B-type starches are loosely packed within the hexagonal unit cell and are thus more accessible to hydolysis by H_3O^+. The postulates of Jane (2006) are not in agreement with the results obtained by Srichuwong *et al.*, (2005b), Vermeylen *et al.*, (2004), Jayakody & Hoover (2002), and Jayakody (2001). The above researches have shown that B-type starches are more resistant to acid hydrolysis than A-type starches [**Figure 2-11-B**]. It has also been shown that long B chains (DP~30) (low branch density of B-type starches) are more acid resistant than the short chains (high branch density) of A-type starches (Vermeylen *et al.*, 2004). Resistance of B-type crystallites to acid hydrolysis has also been shown to be due to their higher stability and three dimensional size

Figure 2-11 (A) Schematic representation of amylopectin branch chains and branch point distribution between A- and B-type starches **(B)** Acid hydrolysis patterns of A-, B & C-type starches

Adapted from Srichuwong *et al.,* (2005b), Jayakody (2001) Jane *et al.*, (1997) with permission from Elsevier


A-type polymorphic starch
B-type polymorphic starch
Amorphous lamellae (A)
Crystalline lamellae (C)
A
C
9.0 nm
9.2 nm
α-(1-6) branch points
amylopectin double helices
A
B


Hydrolysis (%)
Time (days)
A-type (Waxy maize)
A-type (Rice)
A-type (Normal maize)
B-type (Amylomaize-V)
B-type (Amylomaize-VII)
A-type (Rice-Japonica)
C-type (Lotus root)
B-type (Potato)
B-type (Edible canna)
B

(Vermeylen *et al.*, 2004). Srichuwong *et al.*, (2005b) have shown by studies on cereal and tuber starches, that although the characteristics of the amorphous lamella are a critical factor influencing hydrolysis rates, the amylopectin chain length distribution also plays a significant role in influencing the extent of acid hydrolysis. These authors also showed that very short chains (DP 6-8) of amylopectin are readily hydrolyzed together with amorphous material by acid, since they are not long enough to form stable double helices.

2.4.6.1 Susceptibility of amorphous and crystalline domains towards acid hydrolysis

Two distinct phases are observed during acid hydrolysis. The first phase is attributed to the relatively fast hydrolysis within the amorphous lamellae, followed by slow hydrolysis within the crystalline lamellae (Jayakody *et al.*, 2005, Jayakody & Hoover 2001, Hoover 2000, Manelius *et al.*, 2000, Jacobs *et al.*, 1998a,b, Lineback 1984, Biliaderis *et al.*, 1981b, Robin *et al.*, 1974, Buttrose 1963, Cowie & Greenwood 1957, Alsberg 1938). To account for the heterogeneous rates of hydrolysis of the starch granule, two hypotheses have been proposed (Kainuma & French 1971, BeMiller 1967). One suggests that the compact packing of glucan chains within the starch crystallites does not readily permit the penetration of H_3O^+ into these regions (Kainuma & French 1971), whereas, amorphous regions of the starch molecule are penetrable by the H_3O^+ and are thus hydrolyzed much faster. BeMiller (1967) has postulated that acid hydrolysis of a glucosidic bond may require a change in conformation (chair $\rightarrow$ half chair) of the α-D-glucopyranosyl unit. Thus, if the crystalline structure

immobilizes the sugar conformation then the switch from the chair conformation to a half chair conformation would be sterically impossible, hence, slow hydrolysis. Crystallinity has been shown to increase with hydrolysis time (Jayakody & Hoover, 2002, Jayakody 2001, Jenkins & Donald, 1997, Muhr *et al.*, 1984, Biliaderis *et al.*, 1981b, Robin *et al.*, 1974, Kainnuma & French 1971) since acid preferentially attacks the amorphous regions.

2.4.7 Alpha-amylase hydrolysis

Overview

A study of starch digestibility by α-amylase is important for evaluating nutritive value and also suitability for some industrial applications (Moorthy 2002). α-amylases (1→4 α-D-glucanohydrolase, E.C. 3.2.1.1) also known as 'liquefying' enzymes cleave α-D-(1→4) glucosidic bonds but not α-D-(1→6) glucosidic bonds. The products of hydrolysis, which are oligosaccharides of varying chain lengths, have the α-configuration at C_1 of the reducing glucose unit, hence the name α-amylase. α-amylases hydrolyze the bonds located in the inner regions of the substrate resulting in a rapid decrease in the viscosity of the starch solution, as well as a decrease in iodine binding capacity. Granular starches are more resistant towards α-amylolysis than their gelatinized counterparts (Jacobs *et al.*, 1998c). When intact granules are exposed to α-amylase, the first alteration in structure is seen to be pitting at the surface (Zhang *et al.*, 2006). In the case of cereal starches, these small pits become pores with extensive hydrolysis.

2.4.7.1 Amylolysis patterns

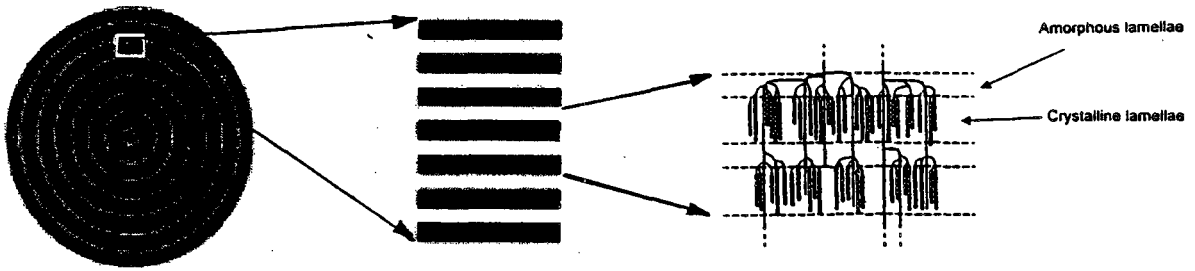
The morphological changes undergone by starches from various botanical sources has been well documented (Li *et al.*, 2004, Hoover & Zhou 2003, Sarikaya *et al.*, 2000, Lauro *et al.*, 1999, Bertoft *et al.*, 1993 & 1992, Gallant *et al.*, 1992, 1982, & 1972, Hoover & Sosulski 1985). Two different types of amylolysis mechanims have been proposed: (1) inside-out digestion [**Figure 2-12-(b)**] (Gallant *et al.*, 1992) and (2) side-by-side digestion [**Figure 2-12-(c)**] (Zhang *et al.*, 2006, Pohu *et al.*, 2004).

(A) *Side-by-side digestion*

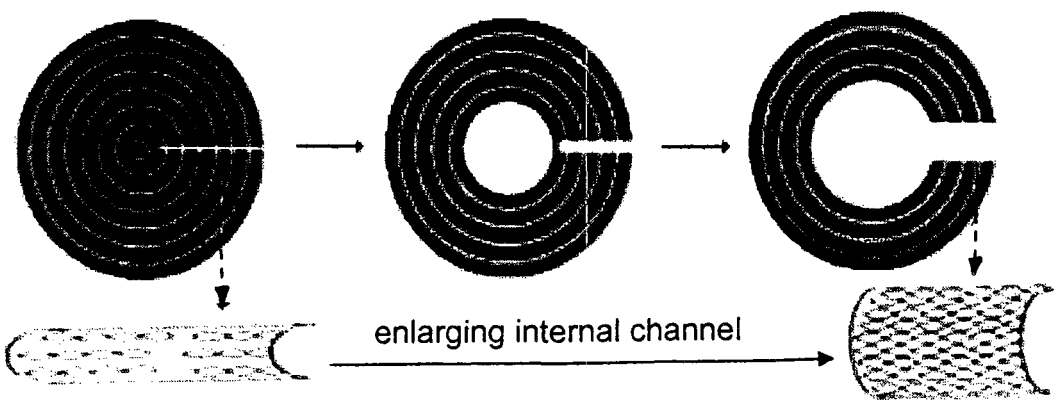
Hydrolysis begins with the diffusion of α-amylase to the starch surface, followed by adsorption and catalysis. The porosity and accessibility of the starch surface has been shown (Oates 1997, Leloup *et al.*, 1991b) to influence the number of adsorption sites on the granule surface. Digestion occurs initially at the granule surface followed by pore formation as the enzyme penetrates into the granule interior. The internal sides of the pores and channels then become active sites for α-amylase action. Since double helices are arranged parallel to each other and perpendicular to the granule surface, the enzyme easily binds with the double helices in a parallel direction or side by side. Hydrolysis from the sides of the crystalline lamellae enlarges the internal channels [**Figure 2-12**] resulting in granule fragmentation. This digestion profile is called side-by-side digestion. According to this digestion mechanism, there is no preferential attack either on the amorphous or crystalline lamellae and both regions are digested evenly. The side-by-side digestion mechanism produces a different hydrolysis pattern known

Figure 2-12 Digestion patterns of amylolysis

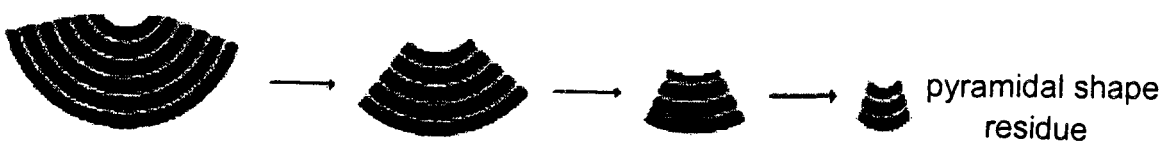
Adapted from Zhang *et al.*, (2006) with permission from American Chemical Society



(a) Native starch granule



(b) Inside-out-layer by layer digestion pattern



(c) Side by side digestion pattern

as 'exo-pitting' (Gallant *et al.*, 1997, 1992).

(B) *Inside-out digestion*

The initiation of hydrolysis from the hilum region towards the outside of the granule is called inside-out digestion. Inside-out digestion occurs when there is a high concentration of amylopectin and tightly packed chains on the granule surface. The starch granule then presents a resistant surface to α-amylase (Oates 1997) and consequently, the granule surface is digested slowly, whereas, digestion proceeds rapidly within the granule interior. The above pattern of hydrolysis is seen mainly in A-type (mainly cereal) starches, since pores and channels present in these starches provide a gateway for the entry of α-amylase into the granule interior (Lynn & Stark 1992). Zhang *et al.*, (2006) believe that inside-out digestion is a different projection of the side-by-side digestion.

2.4.7.2 Factors influencing α-amylase hydrolysis

Starch hydrolysis by α-amylases is known to be controlled by numerous factors such as: botanical source (Srichuwong *et al.*, 2005a, Jacobs *et al.*, 1998c, Ring *et al.*, 1988, Gudmundsson & Eliasson 1993, Gallant & Bouchet 1986, Snow & O'Dea 1981, Fuwa *et al.*, 1979, Rašper *et al.*, 1974, Gallant *et al.*, 1972), amylase source (Liakopoulou-Kyriakides *et al.*, 2001, Wang *et al.*, 1995, Valetudie *et al.*, 1993, Colonna & Buléon 1992a, Gallant *et al.*, 1992 & 1973, Robyt & Whelan 1968), granule morphology (Planchot *et al.*, 1997b, Valetudie *et al.*, 1993, Fujita *et al.*, 1989, Colonna *et al.*, 1988), granuler size (Noda *et al.*, 2005, Snow & Glover 1997, Cottrell *et al.*, 1995, Valetudie *et al.*, 1993, Ring *et al.*, 1988, Snow & O'Dea, 1981, Leach & Schoch 1961), granule size distribution

(Zhang *et al.*, 2006, Vasanthan & Bhatty 1996, Ring *et al.*, 1988, Knutson *et al.*, 1982), compound granules (Snow & O'Dea 1981), surface area: volume ratio (Tester *et al.*, 2006, Kong *et al.*, 2003, Yook & Robyt 2002, Guraya *et al.*, 2001, Leloup *et al.*, 1992, Knutson *et al.*, 1982), surface pores (Zhang *et al.*, 2006, Jane *et al.*, 1997, Fannon *et al.*, 1993 & 1992), presence of channels and blocklets (Fannon *et al.*, 2004, Huber & BeMiller 1997, Gallant *et al.*, 1997, Helbert *et al.*, 1996), amylose: amylopectin ratio (Li *et al.*, 2004, Noda, *et al.*, 2002, Vasanthan & Bhatty 1996, Barichello *et al.*, 1991, Gallant *et al.*, 1992, Leloup *et al.*, 1990, Fujita *et al.*, 1989, Ring *et al.*, 1988, Sievert & Pomeranz 1989, Hoover & Sosulski 1985, Knutson *et al.*, 1982, Fuwa *et al.*, 1977), extent of packing of amylopectin and amylose at the granule surface (Jane 2006, Zhang *et al.*, 2006), glucan chain interactions in granule interior (Dreher, *et al.*, 1984), double helical content (Tester *et al.*, 2004, Gérard *et al.*, 2001a, Tester & Sommerville 2000, Zhang & Oates 1999, Karkalas *et al.*, 1992, Gallant *et al.*, 1992), accessibility of enzyme to substrate (Colonna *et al.*, 1998), structural inhomogeneities (Leloup *et al.*, 1992), crystallinity (Jayakody *et al.*, 2005, Srichuwong *et al.*, 2005a, Planchot *et al.*, 1997a, Colonna *et al.*, 1992b, Jane *et al.,* 1992a, Gallant *et al.*, 1992, Hoover & Sosulski 1985, Knutson *et al.*, 1982), crystal size (Planchot *et al.*, 1997b), extent of crystallite perfection (Zhang *et al.*, 2006), amount of crystallites at the granule surface (Colonna & Buléon 1992a), polymorphic form (Zhang *et al.*, 2006, Srichwong *et al.*, 2005a, Gérard *et al.*, 2001a, Jane *et al.*, 1997, Jacobs *et al.*, 1998c, Planchot *et al.*, 1997b, Valetudie *et al.*, 1993, Gallant *et al.*, 1992, Williamson *et al.*, 1992), B-type crystallites

content and distribution (Jane 2006, Zhang *et al.*, 2006, Srichuwong *et al.*, 2005a, Planchot *et al.*, 1997b, Gérard *et al.*, 2001a,b, Bertoff *et al.*, 1993), amylopectin chain length distribution (Srichuwong *et al.*, 2005a, Gallant *et al.*, 1992), extent of distribution of α-(1→6) branch points between the amorphous and crystalline region of amylopectin (Jane *et al.*, 1992b), degree of gelatinization (Sievert & Pomeranz 1989, Siljeström *et al.*, 1989), cooked starch (Holm *et al.*, 1985), lipid complexed amylose chains (Jayakody *et al.*, 2005, Cui & Oates 1999, Lauro *et al.*, 1999, Perera & Hoover 1998, Anger *et al.*, 1994, Seneviratne & Biliaderis 1991, Colonna *et al.*, 1988, Holm *et al.*, 1983, Hanna & Leliévre, 1975), starch protein interactions (Holm & Björck 1988, Jenkins *et al.*, 1987, Valetudie *et al.*, 1993, Dreher *et al.*, 1984), phosphate content (Slaughter *et al.*, 2001, Sitohy & Ramadon 2001, Gallant *et al.*, 1973), enzyme adsorption at granule surface (Leloup *et al.*, 1992, Svensson 1988), ability of the enzyme to diffuse into the granule interior (Colonna & Buléon 1992a, Leloup *et al.*, 1992, Colonna *et al.*, 1988), composition and concentration of hydrolyzed products (Leloup *et al.*, 1991b, Franco *et al.*, 1987), antinutrients (Thorne *et al.*, 1983), food processing (Slaughter *et al.*, 2001, Oates 1997), physical (Hoover & Vasanthan 1994a,b, Lauro *et al.*, 1993) & chemical (Wolf *et al.*, 1999, Tharanathan & Ramadas Bhat 1988) modification, and extent of starch damage (Leliévre 1974).

Scanning and transmission electron microscopy studies on the susceptibility of starches towards α-amylases (e.g. bacterial, fungal, and porcine pancreatic) have shown the presence of successive strong and weak radial internal layers (Zhou *et al.*, 2004, Planchot *et al.*, 1995, Bertoft *et al.*, 1993, Gallant *et al.*, 1973).

Valetudie *et al.*, (1993) have shown that the hydrolysis rate is higher for potato, sweet potato, and cassava starches with porcine pancreatic amylase than with bacterial amylase [*Bacillus subtilis*]. Hydrolysis of native *Dioscorea* [e.g. *D. alata*] (Valetudie *et al.*, 1993), potato (Leach & Schoch 1961), sago [*Metroxylon* sp.] (Wang *et al.,* 1995) and wheat (Colonna *et al.*, 1988, Jacobs *et al.*, 1998c) starches with bacterial α-amylase has shown that hydrolysis occurs granule by granule. In contrast acid hydrolysis occurs throught the entire granular population with preferential attack on the amorphous parts (Robin *et al.*, 1974, Kainuma & French 1971). *Bacillus licheniformis* α-amylase is one of the most efficient enzymes among bacterial α-amylases (Liakopoulou-Kyriakides *et al.*, 2001). Valetudie *et al.*, (1993) have suggested that proteins on the granule surface of yam starches greatly decrease the extent of hydrolysis. However, purification of the granule surface by proteolysis has been shown to increase hydrolysis.

A-type starches are more susceptible to α-amylase activity than B-type starches (Zhang *et al.*, 2006, Srichuwong *et al.*, 2005a, Planchot *et al.*, 1997b). The digestibility of C-type starches is intermediate between that of A- and B-type starches (Jacobs *et al.*, 1998c, Valetudie *et al.*, 1993). Colonna and Buléon (1992a) proposed that the low susceptibility of B-type starches is due to the presence of a larger number of crystallites at the surface of the granules. Suceptibility towards α-amylase has been shown to be influenced by granule size [e.g. small > large granules] (Cottrell *et al.*, 1995, Snow & Glover 1997, Noda *et al.*, 1992) and shape [truncated>polyhedral>spherical granule shapes] (Valetudie *et al.*, 1993). Srichuwong *et al.*, (2005a) have observed that starch digestibility by

α-amylase has a positive correlation with shorter chains (DP 8 to 12) and a negative correlation with longer chains (DP 16 to 26). Gallant *et al.*, (1992) have shown that long amylopectin side chains and the thickness of the growth rings influence α-amylase hydrolysis. Several researchers (Zhang *et al.*, 2006, Gérad *et al.*, 2001b, Leach & Schoch 1961, Colonna *et al.*, 1988, Lauro *et al.*, 1999) have shown that α-amylases can simultaneously solubilize both amorphous and crystalline regions of starch granules. This is evident by the unchanged granule crystallinity after α-amylolysis, whereas acid hydrolysis occurs throughout the entire granular population with preferential attack on the amorphous regions. Amylose-lipid complexes in native cereal starch granules (Morrison *et al.*, 1993 a,b) have been shown to be fairly resistant to α-amylolysis (Anger *et al.*, 1994 Seneviratne & Biliaderis 1991, Holm *et al.*, 1983, Hanna & Leliévre 1975).

2.4.7 Retrogradation

Overview

Retrogradation means, in very simple terms, 'return to the granular state' (Miles *et al* 1985c). Retrogradation is of great interest to food scientists, technologists or food processors, since it profoundly affects quality, consumer acceptability and shelf-life of starch containing products (Biliaderis 1991). Retrogradation is a term that was first used to describe a group of events that occurs when a starch paste ages (e.g. reformation of H-bonds within or between aqueous amylose, and formation of a precipitate at low starch concentration (>2%) (Atwell 1988, Collison 1968b). However, this definition is not valid for gels and food systems in which the starch: water ratio is high. Starch retrogradation is now defined as a

process that occurs when the starch components in gelatinized starch reassociate in an ordered structure. In its initial phase, two or more glucan chains may form a simple juncture point which then may develop into more extensively ordered regions. Ultimately, under favorable conditions, a crystalline order appears (Atwell, *et al.*, 1988). Retrogradation or set-back results in an increase in viscosity that occurs on cooling of a gelatinized starch paste or during aging of products containing starch as an ingrendient (Swinkels 1985a).

2.4.7.1 Advantages, disadvantages and consequences of retrogradation

The effects of retrogradation in starch-based products are often considered as undesirable. For instance, staling or undesirable firming of bread and other bakery products (Seow & Thevamalar 1988, D'Appolonia & Morad 1981, Kulp & Ponte 1981, Knightly 1977, Marga 1975, Willhoft 1973), weeping or syneresis (Swinkels 1985a), turbidity/opacity formation (Swinkels 1985a), high tendency to form stiff gels (Swinkels 1985a), and decreased starch digestibility (Okuda *et al.*, 2006). However, retrogradation has been shown to be beneficial in the production of parboiled rice, breakfast cereals (Colonna *et al.*, 1992b) and dehydrated mashed potatoes since it results in reduced stickiness and improved graininess of the products (Ooraikul *et al.*, 1974). Weeping or syneresis (expulsion of water) is a visible direct consequence of retrogradation.

2.4.7.2 Techniques of measuring extent of retrogradation

Retrogradation has been monitored using a wide variety of techniques. These methods are mainly based on time-dependent changes in structure, sensory or

digestibility of gelatinized starch. The commonly used techniques in the study of starch retrogradation are: DSC [measures enthalpy change in reformed crystallites e.g. gel and paste] (Jayakody *et al.*, 2005, Fredriksson *et al.*, 1998, Atwell, *et al.*, 1988, Russell 1987b), freeze thaw stability [measures extent of syneresis e.g. gel] (Yuan & Thompson 1998, Zheng & Sosulski 1998, Hoover *et al.*, 1991), turbidity [measures precipitation of insoluble aggregates e.g. 0.2-5% aqueous starch solutions] (Jacobson *et al.*, 1997, Gidley & Bulpin 1989, Ring *et al.*, 1987, Swinkels 1985a, Miles *et al.*, 1985a,b), light-scattering [measures low molecular weight amylose chains e.g. stored amylose solutions] (Kadama & Noda 1978, Pfannemüller *et al.*, 1971), X-ray diffraction [measures transformation of polymorphic pattern and crystallinity e.g. gels, bread crumb] (l'Anson *et al.*, 1988, Katz & van Itallie 1930), elastic modulus measurement [measures extent of crystallization e.g. bread] (Colwell *et al.*, 1969, Axford *et al.*, 1968, McIver *et al.*, 1968), texture profile analysis (TPA) [measures compressibility of sample e.g. bread, starch gels] (Karim *et al.*, 2000), rheology [measures changes in viscoelastic properties (i.e. viscous and solid like) e.g. cooked noodles] (Gudmundsson 1994, Mita 1992, l'Anson *et al.*, 1988), light microscopy on stained paste [measures loss of network nature of amylose on retrogradate paste] (Jacobson *et al* 1997), Fourier transform infared spectroscopy (FTIR) [measures extent of molecular order by band narrowing e.g. bread crumbs] (van Soest *et al.*, 1995, Wilson *et al.*, 1991), ^{1}H NMR [measures changes in molecular mobility e.g. bound water content in starch gel] (Karim *et*

al., 2000, Wu & Eads 1993) and acid or enzyme hydrolysis [measures resistant of starch to hydrolysis e.g. gels] (Sievert *et al.*, 1991, Ring *et al.*, 1988).

The differential thermal analysis (DTA) for retrogradation studies was first used by Axford and Colwell (1967). DSC (Russell 1987b, Fredriksson *et al* 1998) and X-ray diffraction (I'Anson *et al* 1988) are the most widely employed methods, for the study of rate and extent of starch retrogradation. Katz and van Itallie (1930) were the first to observe transformation of A-type pattern into to B-type on aging by X-ray diffraction. The intensity of the B polymorph increases slowly with time (Gudmundsson 1994, Zoble 1973). However, starch retrogradation is a complex process affected by various factors. Therefore, it is unlikely that any single technique would be able to give a comprehensive picture of the retrogradation at the macroscopic and supramolecular levels. Independent evidence derived from two or more methods would allow cross comparisons that can provide more reliable and realistic information.

2.4.7.3 Mechanism of retrogradation

The relation between crystallization of gelatinized starch and retrogradation upon storage has been known for a long time (Cornford *et al.*, 1964). During retrogradation, gelatinized starch transforms from a solvated, dispersed or amorphous state to an insoluble, aggregated or crystalline condition (Swinkels 1985b). This phenomenon has been understood as a non-equilibrium thermo-reversible re-crystallization process (Thygesen *et al.*, 2003). Polymer crystal growth theory states that there are three sequential steps to polymer

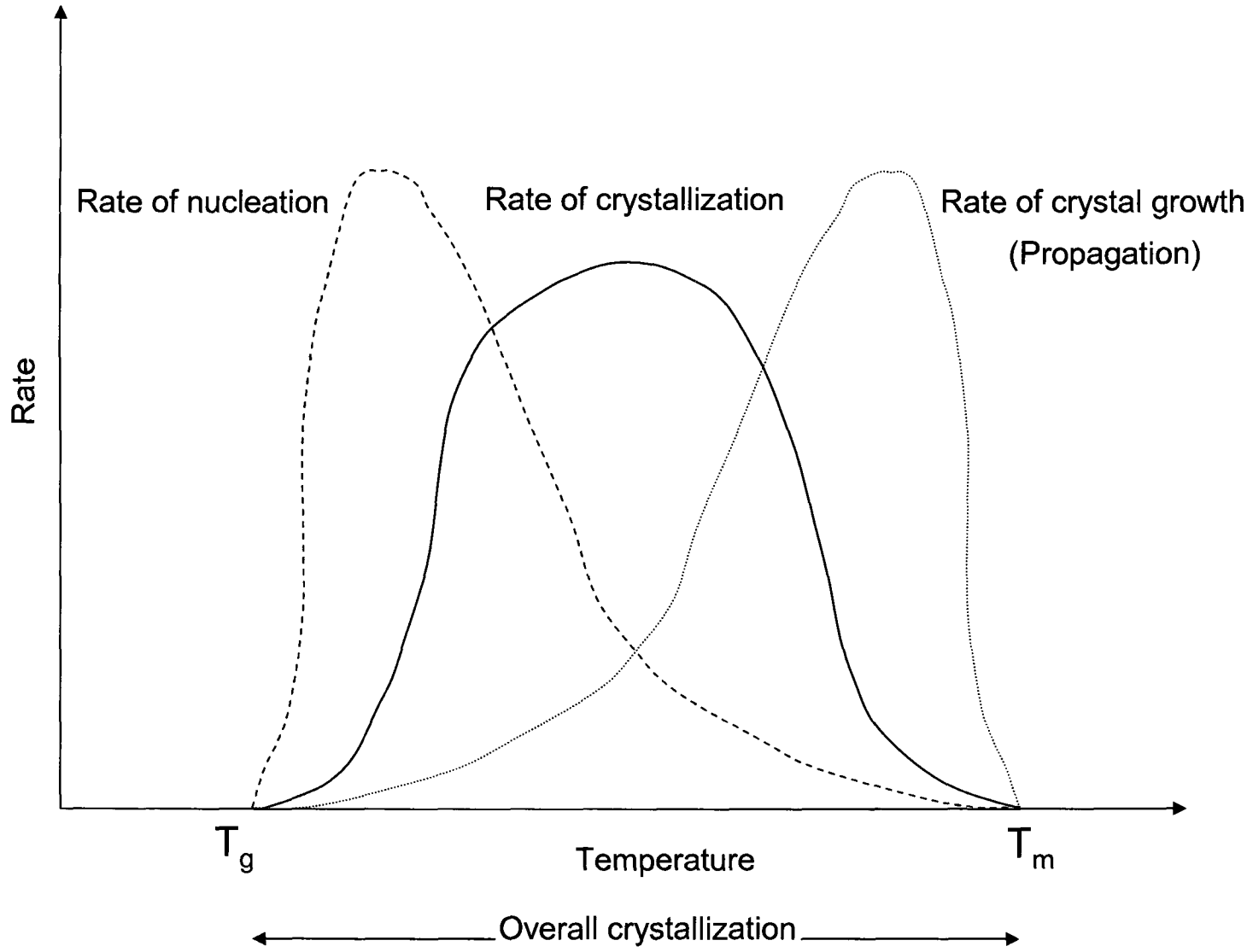
crystallization such as nucleation, propagation (crystal growth) and maturation (crystal perfection) (Slade & Levine 1987). March and Blanshard (1988) and Slade and Levine (1987) have shown that amylopectin crystallization is a nucleation-limiting process that occurs above the glass transition temperature (T_g) (~-5°C) but below the melting temperature (T_m) of amylopectin (~60°C) (Jacobson & BeMiller 1998). It has been shown that the rate of retrogradation of starch paste (50% w/w) is optimum at around 5°C (March & Blanshard 1988, Slade & Levine 1987). X-ray diffraction studies have shown that crystallization occurs during gelation and storage of starch gels (Banks & Greenwood 1975, Hellman *et al.*, 1954). However, the mechanism involved on a molecular level is still not completely understood.

2.4.7.3.1 Step 1 - Nucleation

Amylopectin re-crystallization is a nucleation-controlled process [**Figure 2-13**] (Slade & Levine 1987, Miles *et al.*, 1985c). It has been proposed that nucleation occurs at junction points of two or more glucan chains (Slade & Levine 1987). Crystal growth requires that molecules are able to diffuse to the surface of the growing nuclei. The rate of the process is sensitive to temperature (e.g. refrigeration, frozen), cooling rate, and impurities. Some degree of supercooling below the crystallization temperature is necessary in order to form crystallites. The greater the degree of supercooling the more rapidly the crystallites (less symmetrically perfect) form. During storage at low temperatures (e.g. 4°C), gelatinized starch molecules re-associate, but in a less ordered and hence a less

Figure 2-13 Schematic representation of the effect temperature on rate of nucleation, crystal growth and crystallization on retrogradation

Adapted from Slade & Levine (1987)


Rate of nucleation
Rate of crystallization
Rate of crystal growth
(Propagation)
Rate
T_g
Temperature
T_m
Overall crystallization

perfect or stable form than their native counterparts (Gidley 1987, Nakazawa *et al.*, 1985). Nucleation of amylose crystals in a high-moisture environment is significantly high near -5°C (Slade & Levine 1987). These crystals have a lower melting temperature than those formed at higher storage temperatures (Gudmundsson 1994). It has been shown that the temperature location of the endotherm associated with melting of recrystallized amylopectin also depends upon the storage temperature (Gudmundsson 1994). For instance, less perfect crystallites are produced at low storage temperatures. The melting temperature range indicates the quality and heterogeneity of the recrystalized amylopectin. Thus, a wider melting range (T_o-T_c) might imply a large number of crystals of varying stabilities (formed during aging), whereas a narrow range implies that crystals are more homogenous in quality and are nearly of the same order of stability (Karim *et al.*, 2000). Nucleation rate increases exponentially with decreasing temperature down to T_g (Slade & Levine 1987). However, storage of gels below T_g significantly inhibits the nucleation process (Eliasson 1985, Colwell *et al.*, 1969). Slade and Levine (1987) reported that homogeneous nucleation of new amylose-lipid crystals occurs above room temperature (near 33°C). Several studies have shown that the rate limiting step during re-crystallization is nucleation (enhanced at low temperature) rather than propagation (enhanced at high temperature) (Slade & Levine 1987, Jankowski & Rha 1986, Fearn & Russell 1982). Therefore, maximizing the nucleation step (both temperature and duration) is very critical for the study of starch retrogradation. Small angle X-ray scattering (SAXS) studies have shown that nucleation and limited growth of rod-

shaped crystals occur during network formation of amylose and nucleation is the factor limiting the overall levels of crystallization (Morris 1990). Gelatinization parameters of retrograded starches and the conditions used in their study are summarized in **Table 2-11**.

2.4.7.3.2 Step 2 - Propagation (crystal growth)

Jankowski and Rha (1986) have shown that an increase in propagation temperature (30-40°C) has a positive correlation with increased onset (T_o) temperature of retrogradation, but a negative correlation with the melting temperature range (T_c-T_o) of the crystallites (Silverio *et al.*, 2000, Jang & Pyun 1997, Jankowski & Rha 1986, Eliasson 1985, Nakazawa *et al.*, 1985). DSC studies have shown that starch crystals appear more symmetrically perfect and have increased stability at higher temperatures than at prolonged low temperature storage (Longton & LeGrys 1981). Silverio *et al.*, (2000) have postulated that a narrowing of the melting temperature range reflects formation of homogeneous crystallites at 40°C. Slade and Levine (1987) have reported that no propagation occurs below Tg, because propagation is a diffusion-controlled process and requires the liquid state. However, propagation rate increases exponentially with increasing temperature up to crystalline melting temperature (T_m) [**Figure 2-13**]. Consequently, at temperatures above T_g the rate of crystal growth also goes to zero, since crystals can neither nucleate nor propagate (i.e. crystals melt instantaneously). Slade and Levine (1987) have shown that the optimum temperature for nucleation and propagation is 4°C and 40°C, respectively, for maximum rate of re-crystallization (Slade & Levine 1987). The

Table 2-11 Gelatinization parameters of retrograded starches and the conditions used in their study

Starch source	AMP (%)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_R (J/g)	Starch:H_2O	Incubation Temp/time	Propagation Temp/time	Range (°C) Speed (°C/min)	Reference
D. alata	67.4	42.48	60.65	74.76	10.6	1:3	4°C/7days	**	25-100°C, 10°C/min	Peroni *et al.*, 2006
D. alata	79.2	55.8	66.6	77.9	11.3	1:2	4°C/2days	37°C/7days	15-120°C, 2°C/min	Srichuwong *et al.*, 2005a
D. esculenta	85.8	54.0	64.6	73.5	11.1	1:2	4°C/2days	37°C/7days	15-120°C, 2°C/min	Srichuwong *et al.*, 2005a
Dioscorea (spp.NA)	70.8	39.2	51.9	61.5	5.0	1:3	4°C/7days	**	25-100°C, 10°C/min	McPherson & Jane 1999
Canna edulis	68.3	42.8	61.1	74.5	7.9	1:3	4°C/7days	**	25-100°C, 10°C/min	Peroni *et al.*, 2006
Canna edulis	72.7	55.2	65.8	76.2	8.7	1:2	4°C/2days	37°C/7days	15-120°C, 2°C/min	Srichuwong *et al.*, 2005a
*S. tuberosum**	81.3-76.1	~42-46	~56-59	~67-75	3.8-10	NA	4°C/7days	-	30-100°C, 5°C/min	Karim *et al.*, 2007
S. tuberosum	82	54.4	65.1	75.0	9.1	1:2	4°C/2days	37°C/7days	15-120°C, 2°C/min	Srichuwong *et al.*, 2005a
*S. tuberosum**	71.7-68.6	~46-48	~64-70	-	~8.4-10	~1:3	4°C/14days	**	5-180°C, 10°C/min	Liu *et al.*, 2003
S. tuberosum	62.2	41.1	56.1	66.4	6.9	1:3	4°C/7days	**	25-100°C, 10°C/min	McPherson & Jane 1999
S. tuberosum-waxy	19.2	38.6	56.0	65.2	7.8	1:3	4°C/7days	**	25-100°C, 10°C/min	McPherson & Jane 1999
M. arundinacea	80.0	53.6	63.3	72.8	8.5	1:2	4°C/2days	37°C/7days	15-120°C, 2°C/min	Srichuwong *et al.*, 2005a
M. arundinacea	79.2	41.2	54.4	65.9	4.9	1:3	4°C/7days	**	25-100°C, 10°C/min	Peroni *et al.*, 2006
M. sagu (sago)	78.1	52.5	61.5	69.5	7.8	1:2	4°C/2days	37°C/7days	15-120°C, 2°C/min	Srichuwong *et al.*, 2005a
Manihot esculenta	80.2	42.7	64.6	62.0	2.7	1:3	4°C/7days	**	25-100°C, 10°C/min	Peroni *et al.*, 2006
Manihot esculenta	82.1	53.1	61.5	67.1	3.1	1:2	4°C/2days	37°C/7days	15-120°C, 2°C/min	Srichuwong *et al.*, 2005a
Ipomea batatas	80.2	53.9	63.3	70.2	7.5	1:2	4°C/2days	37°C/7days	15-120°C, 2°C/min	Srichuwong *et al.*, 2005a
Ipomea batatas	77.4	42.0	55.4	66.3	6.4	1:3	4°C/7days	**	25-100°C, 10°C/min	Peroni *et al.*, 2006
Ipomea batatas	66.9	39.9	52.7	63.2	6.1	1:3	4°C/7days	**	25-100°C, 10°C/min	McPherson & Jane 1999
*S. rotundifolius**	83.7-77.0	57-62	69-72	80-84	~9-12	1:2	4°C/1 day	40°C/1-7days	15-120°C, 2°C/min	Jayakody *et al.*, 2005
X. sagitifolium	77.5	53.0	62.1	70.5	9.2	1:2	4°C/2days	37°C/7days	15-120°C, 2°C/min	Srichuwong *et al.*, 2005a
C. esculenta	83.7	53.6	62.5	69.5	7.7	1:2	4°C/2days	37°C/7days	15-120°C, 2°C/min	Srichuwong *et al.*, 2005a
Pueraria lobata	80.4	53.7	63.3	70.8	8.1	1:2	4°C/2days	37°C/7days	15-120°C, 2°C/min	Srichuwong *et al.*, 2005a
Zingiber officinale	73.5	45.3	60.9	74.0	10.9	1:3	4°C/7days	**	25-100°C, 10°C/min	Peroni *et al.*, 2006

AMP: Amylopectin content (%) *: Indicates different varieties **: Indicates propagation step was not involved ΔH_R - Retrogradation enthalpy

NA: Information not reported/specified Range: DSC scanning range (°C) Speed: DSC scanning speed (°C/min)

onset (T_o) temperature of melting of retrograded crystallites and the enthalpy of retrogradation is usually 10-26°C (Srichuwong *et al.*, 2005a, Baker & Rayas-Duarte 1998, Yuan *et al.*, 1993, White *et al.*, 1989) and 60-80% lower (Karim *et al.*, 2000), respectively, than that of their native counterparts. The lower DSC parameters [**Table 2-11**] of retrograded starches indicate improper alignment of the glucan chains during re-association (Srichuwong *et al.*, 2005a, Jane *et al.*, 1999, Kalichevsky *et al.*, 1990).

2.4.7.3.3 Step 3 - Maturation (crystal perfection)

Maturation is further crystal growth and/or perfection (Wunderlich 1976). At the maturation stage, microcrystallites become perfect crystallites via Oswald ripening (Levine & Slade 1986). During crystal perfection the radius of the spherulitic superstructure gradually increases by merging neighbouring spherulites (Wunderlich 1976). The rate of maturation process increases with increasing temperature up to a maximum crystalline melting temperature of most mature crystals (Slade & Levine 1987).

2.4.7.4 Factors affecting starch retrogradation

Many factors such as botanical source (Jacobson & BeMiller 1998, Jacobson *et al.*, 1997, Russell 1987b, Orford *et al.*, 1987), storage temperature (Slade & Levine 1987, Jankowski & Rha 1986), water content (Zeleznak & Hoseney 1986, Longton & LeGrys 1981), starch concentration (Liu & Thompson 1998, Orford *et al.*, 1987, Longton & Legrays 1981), initial heating temperature (Liu & Thompson 1998), rate of freezing (Volz & Ramstad 1952), chain length distribution of

amylopectin (Thygesen *et al.*, 2003, Yao *et al.*, 2002, Gudmundsoon 1994, Liu & Thompson 1998, Shi & Seib 1995, Yuan *et al.*,1993), amylose content (Sing *et al.*, 2006, Thygesen *et al.*, 2003, Ishiguro *et al.*, 2000, Fan & Marks 1998, Silverio *et al.*, 1996), molecular size of amylose (Lu *et al.*, 1997a), molar ratio and structure of amylose and amylopectin (Jacobson & BeMiller 1998, Morris 1990), lipids (Keetels *et al.*, 1996, Hoover *et al.*, 1994, Ward *et al.*, 1994, Biliaderis & Tonogai 1991), protein (Escarpa 1997), salts (Ward *et al.*, 1994), sugars (Seow *et al.*, 1996, Biliaderis & Prokopowich 1994), shear force (Jacobson & BeMiller 1998), time of adding ingredients (Jacobson & BeMiller 1998), physical modification (Orford *et al.*, 1993), and chemical modification (Perera & Hoover 1998) have been shown to influence starch retrogradation. However, in general, it is quite difficult to compare data between studies due to differences in starch concentration, preparation techniques, different instrumental techniques, variable temperatures of storage and other conditions [**Table 2-11**].

2.4.7.4.1 Water content and starch concentration

Several studies have shown that the extent of retrogradation is very sensitive to the water content of starch gels (Fredriksson 1998, Longton & LeGrys 1981). To study the retrogradation mechanism by DSC, a starch concentration of >20% (w/w) is required (Karim *et al.*, 2000). A gelatinized starch gel is completely amorphous and its water is uniformly distributed. The re-crystallization process depends on the T_g of the amorphous gel as the mobility of the chains determines their rate of aggregation (Gudmundsson 1994). Since water is a plasticizer, it influences the T_g of the amorphous gel. At very low water content, T_g is above

room temperature consequently, the amorphous gel is glassy and effectively hinders chain mobility. However, the rate and extent of re-crystallization increases with increase in water content (T_g is depressed below room temperature, and hence glucan chains have greater mobility). DSC studies have shown that re-crystallization increases with increase in water content (up to 40-60%, w/v). However, with further increase of water content up to 90% [10% starch] (Thygesen *et al.*, 2003, Léon *et al.*, 1997, Leloup *et al.*, 1991a, Zeleznak & Hoseney 1986, Eliasson 1983, Longton & LeGrys 1981) re-crystallization does not occur due to excess dilution of the components with the potential to crystallize (Slade & Levine 1987). Highly concentrated (80%) gels also suppress re-crystallization (Longton & LeGrys 1981). Retrogradation is only dependent on the water content during ageing, but not during gelatinization (Zeleznak & Hoseney 1986). **Table 2-11** shows starch to water ratios of retrograded gels.

2.4.7.4.2 Effect of amylose and amylopectin

2.4.7.4.2.1 Role of amylose in retrogradation

The exact role of amylose on starch retrogradation is still unclear. However, amylose itself retrogrades rapidly within a few hours after cooking, forming an ordered (on the molecular level) matrix which is not necessarily highly crystalline (Ring *et al* 1987, Miles *et al* 1985a,c). Amylose can form inter chain associations with other amylose molecules. Miles *et al.*, (1985c) reported that some of these amylose crystallites remain ('residual crystallinity') even after heating to 100°C. It is believed this interaction accounts for the greater stability of amylose crystallites. Jacobson *et al.*, (1997) reported that the initial turbidity formation of a

potato starch slurry (2% w/w) corresponds to aggregation of the amylose, this is deduced from the reduction in the blue color of amylose iodine complex. Singh *et al.*, (2006), Gidley and Bulpin (1989) studied different varieties of potato starches and concluded that amylose has a positive correlation with retrogradation. Gidley and Bulpin (1989) reported that the rate of aggregation of amylose is strongly dependent on chain length. For instance, amylose aggregation is initially slow (for DP<50), increases rapidly to a maximum rate (at DP~80), and then becomes steadily slower until at DP> 2000, only slow and limited aggregation is observed in dilute amylose solution. However, extensive amylose-amylose chain interactions occur at ~DP 50 (Roulet *et al.*, 1988). Therefore, a DP in the range ~50-80 is critical for amylose retrogradation. Jane and Robyt (1984) have postulated that a DP in the range 50-80 may be responsible for the initial retrogradation of starch gels. *Dioscorea* starches show a high initial rate and extent of retrogradation, which implies rapid amylose re-organization (Brunnschweiler *et al.*, 2005). Amylose retrogradation basically involves a gelation-via-crystallization process which gives rise to a B-type X-ray diffraction pattern (Gidely 1989, Marsh & Blanshard 1988, Galliard & Bowler 1987, Blanshard 1987, Miles *et al.*, 1985b, Miles *et al.*, 1985c). The extent of amylose retrogradation is known to be an inhibiting factor in the enzymic digestion of starch (Morris 1990).

2.4.7.4.2.2 Role of amylopectin in retrogradation

It was first suggested by Schoch and French (1947) that staling of bread essentially involves the retrogradation of the amylopectin (AMP) but not the

amylose fraction. Amylopectin (AMP) is more stable in solution and retrogrades very slowly (Thygesen *et al.*, 2003). Retrogradation of AMP extends over a long-term, possibly taking several days or weeks to attain maximum crystallinity after starch gelatinization (Ring *et al.,* 1987, Miles *et al.*, 1985c). Schoch (1965) reported that gradual re-association of the amylopectin fraction is mainly responsible for long term changes in bread or starch gels during storage. He further reported that the amylose fraction does not retrograde immediately on cooling. Much evidence suggests that changes in amylopectin are the main cause for retrogradation because they are responsible for all long-term rheological and structural changes. However, amylose is responsible for the short-term changes (Gudmundsson 1994). Amylopectin from A-type starches such as cereals, cassava and sweet potato have been shown to retrograde to a lesser extent than potato, *Dioscorea*, pea, and canna amylopectin (Srichuwong *et al.*, 2005a,c, Kalichevsky *et al.*, 1990, Orford *et al.*, 1987). The association of amylopectin chains in a starch gel is extensive and is thermoreversible at temperatures below 100°C (Ring *et al.*, 1987, Miles *et al.*, 1985c). However, amylose gels are thermally irreversible at this temperature (Gudmundsson 1994). The stability of amylopectin crystallites has been shown to be lower than that of amylose crystallites. Recrystallized amylopectin melts in the temperature range 40-100°C (Srichuwong *et al.*, 2005a, Karim *et al.*, 2000, Russell 1987b). However, amylose crystallites melt at a much higher temperatures (120-170°C) (Eerlingen *et al.*, 1994, Sievert & Pomeranz 1989).

2.4.7.4.2.3 Interactions between amylose and amylopectin on retrogradation

Yao *et al.*, (2002) suggested, from studies on 18 different rice cultivars, that amylose may interact with the external chains of amylopectin during retrogradation. Russell (1987b), postulated that the amylose fraction has synergistic effects on amylopectin retrogradation. Vanderputte *et al.*, (2003) have suggested that amylose squeezes itself in between amylopectin chains and hence may restrict the possibility of amylopectin chains partially restoring a crystalline polymer structure and/or amylose chains could co-crystallize with amylopectin chains, resulting in a less perfectly regained crystalline polymer system (reflected by a lower enthalpy of retrogradation ΔH_R of amylopectin).

2.4.7.4.3 Role of chain length distribution

The minimum requirement for the aggregation of amylose and amylopectin chains has been reported to be 10 (Gidley & Bulpin 1987) and 15 (Ring *et al.*, 1987, Robin *et al.*, 1974) glucose units, respectively. The relative proportion of amylopectin short chains has been shown to inhibit or retard the rate of retrogradation (Thygesen *et al.*, 2003). It has been reported that short [DP 6] (Vandeputte *et al.*, 2003b), medium chains [DP 12-22] (Vandeputte *et al.*, 2003b), [DP 14-24] (Levine & Slade 1986, Shi & Seib 1992), [DP 14-18] (Karim *et al.*, 2000), [DP 15-18] (Yao *et al.*, 2002), [DP 18-19] (Vandeputte *et al.*, 2003b) and long chains > [DP 40] (Silverio *et al.,* 2000) increase amylopectin retrogradation. However, the influence of AMP unit chain length on the extent of retrogradation is in dispute. For instance, very short chains [DP 6-9] (Gudmundsson 1994, Shi & Seib 1992), [DP 8-11] (Silverio *et al.,* 2000), and

long chains [DP 22-34] (Silverio *et al.*, 2000), [> DP 25] (Srichuwong *et al.*, 2005a, Vandeputte *et al.*, 2003b) inhibit or retard retrogradation. However, DSC studies showed that T_o, T_p and T_c of retrograded starch gels (e.g. potato and edible canna) was negatively correlated with shorter chains (DP 8 to 12) and positively correlated with longer chains (DP 16 to 26) (Srichuwong *et al.*, 2005a). Yao *et al.*, (2002) also showed that DP ~15-18 facilitates the formation of double helices which results in a higher rate of retrogradation. Several studies have shown that longer branch-chains increase the extent of retrogradation (Wang *et al.*, 2006, Srichuwong *et al.*, 2005a, Vandeputte *et al.*, 2003b, Silverio *et al.*, 2000, Shi & Seib 1995 & 1992, Kalichevsky *et al.*, 1990). For instance, Wang *et al.*, (2006) have suggested that long chain fractions of amylopectin facilitate retrogradation in *Dioscorea* starch gels. It is clear from the above studies, that the influence of chain length on the extent of retrogradation is different for different starch sources. However, there is no conclusive agreement between unit chain length distribution and rate of retrogradation [**Table 2-12**]. Chain length is not the sole factor which determines the retrogradation rate because various other factors also influence the extent of retrogradation.

2.4.7.4.4 Effect of storage temperature and freezing rate

The first study of the effect of temperature on the rate of retrogradation was reported by Maquenne (1904). Several studies (Slade & Levine 1987, Jankowski & Rha 1986, Fearn & Russell 1982) have shown that although the rate limiting step for starch retrogradation is nucleation, the physicochemical properties of a retrograded starch gel depends on the storage temperature (Jankowski & Rha

Table 2-12 Amylopectin unit branch chain length distribution of various starches

Starch source	Very short (%)	Short (%)	Medium (%)	Medium long (%)	Average (%)	Reference
D. alata –variety- TN 2	-	-	-	41.7 (DP <40)	25.5-28.5	Wang *et al.*, 2006
D. alata –variety- Chinalong	-	-	-	48.0 (DP <40)	18.8-19.3	Wang *et al.*, 2006
D. alata	3.9 (DP 6-8)	18.9 (DP 9-12)	67.5 (DP 13-24)	9.7 (DP 25-30)	-	Srichuwong *et al.*, 2005a
D. esculenta	11.6 (DP 6-8)	24.9 (DP 9-12)	56.2 (DP 13-24)	7.3 (DP 25-30)	-	Srichuwong *et al.*, 2005a
Yam (spp. not specified)	19.1(DP 6-12)	44.8 (DP13-24)	14.3 (DP 25-36)	21.8 (DP>37)	25.8	McPherson & Jane 1999
Solanum tuberosum normal	10.2 (DP 6-8)	23.5 (DP 9-12)	58.9 (DP 13-24)	7.4 (DP 25-30)	-	Srichuwong *et al.*, 2005a
Solanum tuberosum normal	13.1 (DP 6-12)	44.4 (DP13-24)	14.0 (DP 25-36)	28.5 (DP>37)	28.6	McPherson & Jane 1999
Solanum tuberosum waxy	14.8 (DP 6-12)	48.4 (DP13-24)	14.4 (DP 25-36)	22.4 (DP>37)	25.8	McPherson & Jane 1999
Ipomea batatas	11.0 (DP 6-8)	27.9 (DP 9-12)	54.1 (DP 13-24)	7.0 (DP 25-30)	-	Srichuwong *et al.*, 2005a
Ipomea batatas	17.1 (DP 6-12)	48.1 (DP13-24)	13.6 (DP 25-36)	23.4 (DP>37)	26.3	McPherson & Jane 1999
Metrozylon sagu	9.0 (DP 6-8)	28.1 (DP 9-12)	56.2 (DP 13-24)	6.7 (DP 25-30)	-	Srichuwong *et al.*, 2005a
Cana edulis	7.2 (DP 6-8)	21.5 (DP 9-12)	63.4 (DP 13-24)	7.9 (DP 25-30)	-	Srichuwong *et al.*, 2005a
Xanthosoma sagitifolium	7.4 (DP 6-8)	27.3 (DP 6-8)	58.7 (DP 6-8)	6.6 (DP 6-8)	-	Srichuwong *et al.*, 2005a
Colocasia esculenta (taro)	7.4 (DP 6-8)	28.9 (DP 6-8)	57.3 (DP 6-8)	6.4 (DP 6-8)	-	Srichuwong *et al.*, 2005a
Maranta arundinacea	4.0 (DP 6-8)	27.7 (DP 6-8)	58.4 (DP 6-8)	9.9 (DP 6-8)	-	Srichuwong *et al.*, 2005a
Manihot esculenta	9.9 (DP 6-8)	36.3 (DP 6-8)	48.3 (DP 6-8)	5.5 (DP 6-8)	-	Srichuwong *et al.*, 2005a

Note: This table shows that there is no consensus with regard to classifying the amylopectin unit branch length into: very short, short, medium and medium long. Degree of polymerization (DP) is indicated in parenthesis.

1986) and storage period (Colwell *et al.*, 1969). The nucleation rate increases with a decrease in temperature and the propagation rate increases with increasing temperature (Wunderlich 1976). Thygesen *et al.*, (2003) have shown that rate of retrogradation increases when the temperature is lowered from 25 to 5°C. It has been shown that fast or slow cooling has no effect on rate of crystallization (McIver *et al.*, 1968).

2.4.7.4.5 Effect of phosphorous content

The effect of covalently linked phosphate on retrogradation is less clear. It is known that amylose readily retrogrades. However, amylose retrogradation is decreased in the presence of high levels of starch phosphate monoesters (Thygesen *et al.*, 2003). In waxy starches, retrogradation is dramatically suppressed, but in this situation phosphorus plays a less significant role (Thygesen *et al.*, 2003).

2.4.7.4.6 Lipid

It is known that amylose complexes with lipids during gelatinization (Moorthy 2006, Kugimiya *et al.*, 1980, Zoble 1973). It is believed that only the outer branches of the amylopectin could complex with lipids (Eliasson & Ljunger 1988, Batres & White 1986, Evans 1986). Yao *et al.*, (2002) hypothesized that amylose-lipid interactions decrease the amount of amylose available for interaction with the external chains of amylopectin, thus leading to a decrease in co-crystallization of amylose and amylopectin. Various theories have been put forward to explain the role of lipids on starch retrogradation such as : (1)

formation of a V-amylose-lipid complex retards chain mobility, thereby decreasing the extent of retrogradation [Gudmundsson1992, Gudmundsson & Eliasson 1990, Eliasson & Lunger 1988, Slade & Levine 1987, Evans 1986, Batres & White 1986], (2) the V-amylose-lipids complex changes the water distribution around the helical complex thereby retarding retrogradation [D'Appolnia & Morad 1981], and (3) lipids reduce retrogradation by mechanically covering parts of the starch granule, reducing the ability of starch molecules to absorb water [Germani *et al.*,1983].

2.4.8 Annealing

Overview

Gough and Pybus (1971) showed that elevated gelatinization temperatures and a sharply narrowed gelatinization temperature range occurs when wheat starch is treated with water at 50°C for 72h. This was similar to that observed in annealed starches. However, the above authors never used the term annealing for their treatment. Ahmed and Leliévere (1978) were the first to define the term annealing as an increase in order of crystalline material. A general definition of annealing in the context of polymer science was coined by Wunderlich (1976). In polymer science, the word annealing (from the old English *anael>onaelan→ heat*) is used to describe the improvement of crystallization by heating to temperatures below the melting point of crystallites which results in growth of crystalline areas, perfection of crystals and a change to a more stable crystalline structure. Annealing of starch (a semicrystalline polymer) is a physical treatment of starch granules in the presence of heat and excess water. The effect of

moisture content on *in vitro* annealing of wheat starch (Tester *et al.*, 1998) has shown (by the use of differential scanning calorimetry [DSC]) that annealing can be initiated at room temperature when the moisture content exceeds 22% on a total weight basis, but is restricted (in terms of its effect on increasing the gelatinization temperature) unless it exceeds 60% by weight. Annealing is a process whereby starch granules in excess (>60% [w/w]) or at intermediate water content (40% [w/w]) is held at a temperature above the glass transition temperature (T_g) but below the onset (T_o) temperature of gelatinization for a set period of time (Tester & Debon 2000, Jacobs & Delcour 1998). T_g refers to the temperature at which the amorphous domains of the starch granule are transformed from a rigid glassy to a mobile rubbery state when heated in the presence of solvents such as water or glycerol. These solvents are refered to as plasticizers (Tester & Debon 2000). The plasticizing effect of water increases glucan chain mobility within the amorphous lamellar regions of the semicrystalline growth ring (Perry & Donald 2000). Several authors (Tester & Debon 2000, Jacobs & Delcour 1998, Muhrbeck & Svensson 1996, Seow & Teo 1993, Larsson & Eliasson 1991, Tester & Morrison 1990b, Slade & Levine 1987, Lorenz *et al.*, 1984) have described starch annealing as a crystal growth/perfection, diffusion controlled non-equilibrium process.

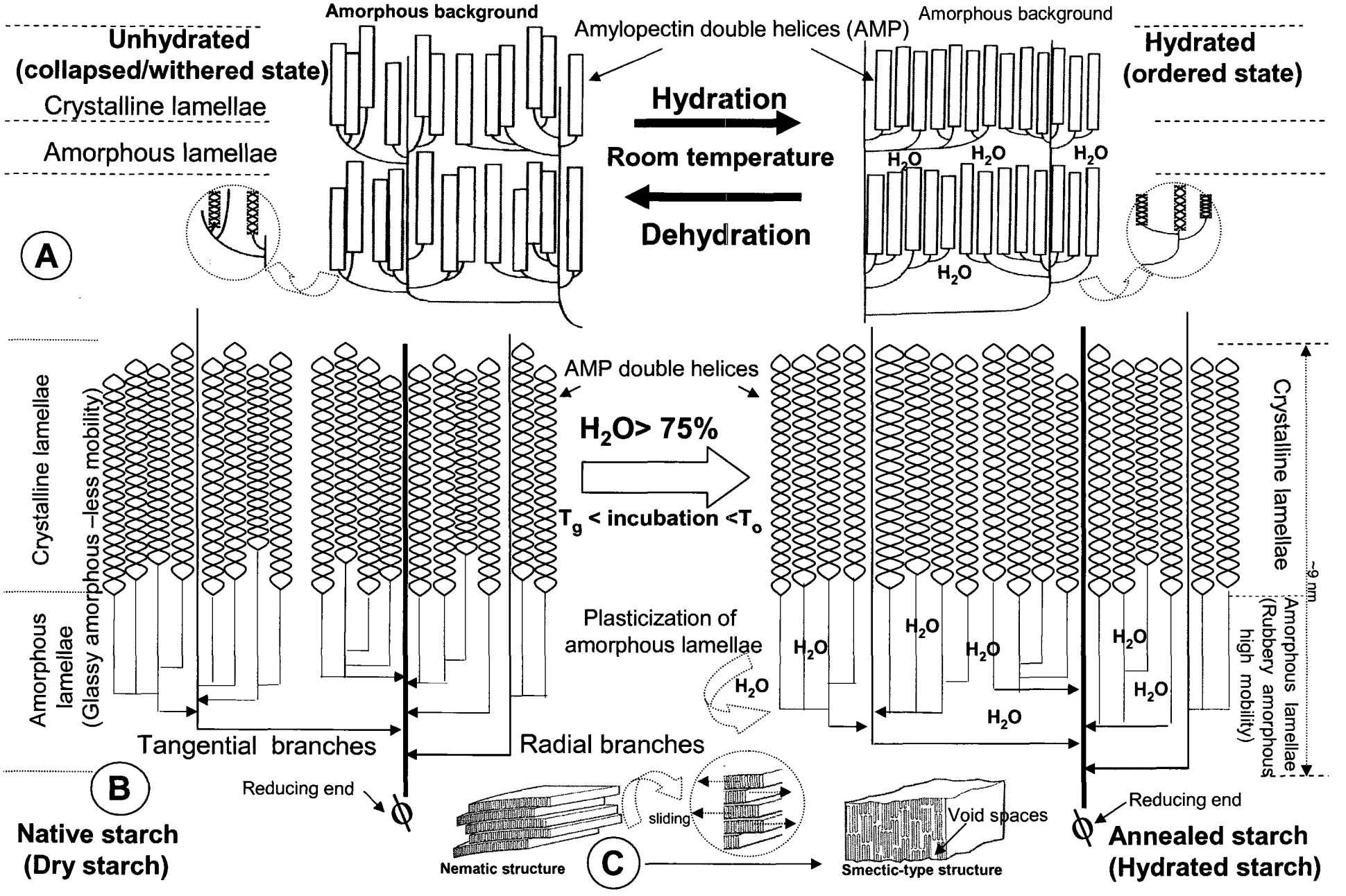
2.4.8.1 Mechanism of annealing

In semicrystalline polymers, annealing has been interpreted as a: (1) 'sliding diffusion', which entails the movement of complete molecular sequences within a crystalline lattice (this mechanism being favoured by high mobility of the chains in

the crystals), and/or (2) a 'complete or partial fusion' of crystals and subsequent re-crystallization of the melted materials at the annealing temperature. Experimental evidence supports both mechanisms (Martuscelli & Pracella 1974). According to the side-chain liquid crystalline analogy of Waigh (1997), the rigid amylopectin double helices are attached to an amorphous backbone [**Figure 2-14-A**]. Waigh *et al.*, (1996) and Perry and Donald (2000) have proposed that double helices of the unhydrated form of starch are intact, but are not arranged regularly side by side [**Figure 2-14-A**], due to the differing lengths of radial and tangential branches [**Figure 2-14-B**] (Waigh *et al.*, 1996). This state is called a nematic, collapsed or a 'withered state' [**Figure 2-14-A**]. The amorphous region of the granule is the area most vulnerable to the initial water absorption and plasticization. Before hydration the amorphous area is more glassy and immobile [**Figure 2-14-B**]; hydration of the starch granule increases the mobility of the amorphous regions. This induces vibrational movement of tangential and radial chains in both amorphous and crystalline domains [**Figure 2-14-B**]. Simultaneously hydration causes limited but reversible granule swelling, allowing mobility of crystalline domains [**Figure 2-14-A**]. An increase in annealing temperature ($> T_g$ but $< T_o$) and excess water accelerates the rate of hydration and increases glucan chain mobility [**Figure 2-14-B**]. This dynamic nature allows limited side by side movement of the double helices resulting in the formation of a smectic-type structure [**Figure 2-14-C**] (Waigh 1997, Perry & Donald 2000). An increase in the incubation temperature enhances, initially, the order of the amorphous lamellae and, subsequently, the order of double helices of

Figure 2-14 Schematic representation of mechanism of annealing **(A)** Hydration of starch at room temperature, **(B)** Crystalline perfection during annealing, **(C)** Movement of crystalline lattices during annealing

Adapted from Tester & Debon (2000), Waigh *et al.,*(1996) and Martuscelli & Pracella (1974) with permission of The Royal Society of Chemistry Elsevier BV, and Elsevier


Amorphous background
Amylopectin double helices (AMP)
Amorphous background
Unhydrated
(collapsed/withered state)
Crystalline lamellae
Amorphous lamellae
Hydration
Room temperature
Dehydration
Hydrated
(ordered state)
H2O
H2O
H2O
H2O
A
Crystalline lamellae
Amorphous lamellae
(Glassy amorphous –less mobility)
AMP double helices
H2O> 75%
Tg < incubation <To
Plasticization of
amorphous lamellae
H2O
H2O
H2O
H2O
H2O
H2O
H2O
Crystalline lamellae
~9 nm
Amorphous lamellae
(Rubbery amorphous
high mobility)
Tangential branches
Radial branches
B
Native starch
(Dry starch)
Reducing end
sliding
Nematic structure
C
Smectic-type structure
Void spaces
Reducing end
Annealed starch
(Hydrated starch)

amylopectin (Tester *et al.*, 1999). At this stage, molecules are closely aligned in a distinct series of layers, with their axes lying perpendicular to the plane of the layers [**Figure 2-14-C**]. With the progress of annealing the initially weaker or imperfect crystallites gradually disappear, while the rest of the crystallites become more perfect due to fusion and re-crystallization. The crystallite perfection on annealing was first suggested by Lorenz *et al.*, (1980). Stute (1992) postulated that crystallite perfection may also occur due to: (1) larger crystal formation from smaller crystals, (2) a change of crystal shape, (3) a change in direction of crystal growth, (4) orientation of crystallites, (5) interactions between crystallites, and (6) changes within amorphous regions. This clearly indicates that crystalline perfection does not necessarily correlate with an increase in crystallinity. The native starch (*in vivo*) contains crystallites of varying stabilities. However, annealing decreases the variations in crystalline stabilities resulting in more homogenous crystallites (Tester & Debon 2000, Tester *et al.*, 1998, Jacobs *et al.*, 1998b, Hoover & Vasanthan 1994a, Larsson & Eliasson 1991, Paredes-Lopez & Hernández-Löpez 1991, Tester & Morrison 1990b, Yost & Hoseney 1986). Jacobs *et al.*, (1998a) and Hoover & Vasanthan (1994a) postulated that amylose chain mobility could increase on annealing, resulting in the formation of double helices arising from interactions between amylose-amylose and/or amylose-amylopectin chains.

2.4.8.2 Single, double and multi-step annealing

Studies on annealing have been mainly conducted as a single cycle event [single step annealing] (Tukomane *et al.*, 2007, Kohyama & Sasaki 2006, Waduge *et al.*,

2006, Vermeylen *et al.*, 2006, Qi *et al.*, 2005, Tester *et al.*, 2005, Kiseleva *et al.*, 2005 & 2004, Genkina *et al.*, 2004a,d, Ozcan & Jackson 2003, Muhrbeck & Wischmann 1998, Hoover & Manuel 1996, Muhrbeck & Svensson 1996, Jacobs *et al.*, 1996 & 1995, Hoover & Vasanthan 1994a, Seow & Vasanti-Nair 1994, Seow & Teo 1993, Stute 1992, Larsson & Eliasson 1991, Krueger *et al.*, 1987b). Whereas, double (Jacobs *et al.*, 1998a,b, & c) and multi-step (Nakazawa & Wang 2004 & 2003, Knutson 1990) annealing have been carried out to a limited extent. **[Table 2-13]**. The multi-step annealing involves initial annealing below T_o of the native starch, then re-annealing below T_o of the annealed starch. Since the first annealing increases T_o, the sample could be held at a higher annealing temperature, that is just below the new T_o, without triggering gelatinization. This process could be repeated until no further increase in T_o of the annealed starch.

2.4.8.3 Starch to water ratio on annealing treatment

Annealing of starches has been studied at various starch: water ratios (1:1, 1:3, 1:5) and at temperatures ranging from 40 to 75°C (Kozlov *et al.*, 2007, Tukomane *et al.*, 2007, Kohyama & Sasaki, 2006, Vermeylen *et al.*, 2006, Kiseleva *et al.*, 2005, 2004, Lawal 2005, Genkina *et al.*, 2004a, Genkina *et al.*,2004b, Gomez *et al.*,2004, Kiseleva *et al.*, 2004, Nakazawa and Wang, 2004, Qi *et al.,* 2004, Nakazawa & Wang, 2003, Ozcan & Jackson, 2003, Atichokudomchai *et al.*,2002, Tester *et al.*, 2000, Andreev, *et al.*,1999, 1998a, Jacobs *et al.*,1998b, Jacobs *et al.*, 1998c, Tester *et al.*, 1998, Wang *et al.*, 1997, Hoover & Manuel, 1996, Muhrbeck & Svensson, 1996, Jacobs *et al.*,1995, Seow & Vasanti-Nair 1994, Hoover & Vasanthan 1994a,b, Seow & Teo 1993, Stute 1992, Cameron &

Table 2-13 Annealing conditions for various starches

Starch source	Determinative factor	Annealing conditions: temperature (°C), container method of incubation	No. of steps	Time	H_2O: starch ratio	Reference
Potato, cassava, corn	*	40, 45, 50°C**[a]	Multi	24h	3:1	Nakazawa & Wang 2004
Potato, wheat, cassava, mung	*	50°C**[a]	Multi	24h	3:1	Nakazawa & Wang 2003
Potato, wheat, and pea	*	55-70°C**	Multi	2-72h	3:1	Knutson 1990
Potato, wheat and pea	3-4%below T_P (K)	~45-50°C, in H_2O bath[a]	1 & 2	24-48h	2:1	Jacobs *et al.*,1998a
Potato and wheat	3-4%below T_P (K)	48-55°C, in H_2O bath[a]	1 & 2	24h	2:1	Jacobs *et al.*,1998b
Potato, wheat, and pea	3-4%below T_P (K)	~45-50°C, in H_2O bath[a]	1 & 2	24h	2:1	Jacobs *et al.*,1998c
Potato, wheat, and corn	*	20-50°C, samples in H_2O bath	Single	72h	10:1	Kohyama & Sasaki 2006
Potato	3.3%below T_P (K)	~44-51°C, in H_2O bath[a]	Single	24h	2:1	Vermeylen *et al.*, 2006
Potato different varieties	*	5-25°C: *in vivo* tuber & 55°C: *in vitro***	Single	7 days	4:1	Tester *et al.*, 2005
Potato	2-3°C below T_O	57-68°C, in H_2O bath[a]	Single	0-10h	97:3	Genkina *et al.*, 2004d
Potato, and waxy corn	5°C below T_O	40-45°C, in DSC pan	Single	2min-48h	4:1	Muhrbeck & Wischmann 1998
Potato, wheat, pea, rice	3-4%below T_P (K)	43-55°C, in H_2O bath[a]	Single	24h	2:1	Jacobs *et al.*, 1996 & 1995
Potato, wheat, lentils, & oat	*	50°C,in an air oven[a]	Single	0.5- 72h	3:1	Hoover & Vasanthan 1994a
Potato	*	52°C, continuous rotation in oil bath	Single	95h	5:1	Stute 1992
Potato, normal & waxy corn	*	25-50°C**	Single	10min 48h	1:1	Larsson & Eliasson 1981
Sweet potato	2-3K below T_O	54-78°C, in thermostat cell[a]	Single	0-10h	97:3	Genkina *et al.*, 2004a
Cassava	*	Spray dried at 60°C	NA	NA	NA	Tukomane *et al.*, 2007
Cassava	4% below T_P (K)	51°C, in H_2O bath[a]	Single	24h	3:1	Atichokudomchai *et al.*, 2002
Sago	*	60°C, 0.1M acetate buffer**	Single	0.5-5h	4:1	Wang *et al.*, 1996
Breadfruit	*	50°C, in H_2O bath[a]	Single	48h	2:1	Adebowale *et al.*, 2005a
Black & pinto beans, lentils, pea	*	55°C**	Single	72h	3:1	Hoover & Manuel 1996
Barley	*	50°C, in H_2O bath[a]	Single	72h	3:1	Waduge *et al.*, 2006
Barley (grains & starch)	2-3 K below T_O	7-20°C:*in situ* & ~30-46°C:*in vitro***	Single	0-10h	2:1, 3:1	Kiseleva *et al.*, 2004
Corn: 9 varieties: normal/waxy	*	50-55°C[a]	Single	3-7 days	96:4	Qi *et al.*, 2005
Corn: normal, waxy, HAM	*	50°C**	Single	48h	NA	Kruger *et al.*, 1987b
Wheat: waxy, normal, HAM	2-3 K below T_O	56-63°C, in H_2O bath[a]	Single	0-10h	97:3	Kiseleva *et al.*, 2005
Wheat	*	27-56°C, in sealed pan**	Single	30 min	1:1	Yost & Hoseney 1986
Sorghum	*	50°C**	NA	24h	NA	Adebowale *et al.*, 2005b

* Determinative factor of annealing was not specified ** Method of incubation was not available NA: Data was not available

1 & 2 indicates single and double step annealing, respectively a: indicates sample was placed in sealed glass container HAM: High amylose

Donald 1992, Larsson & Eliasson 1991, Lopez & Lopez 1991, Knutson 1990, Krueger *et al.*,1987a, Krueger *et al.*,1987b, Yost & Hoseney 1986, Kuge & Kitamura 1985). **Table 2-13** shows annealing conditions (starch: water, temperature, time, steps) for various starches.

2.4.8.4 Probes used in the study of annealing

The effect of annealing on the molecular structure and properties of starches has been probed using XRD [wide angle & small angle] (Tukomane *et al.*, 2007, Waduge *et al.*, 2006, Qi *et al.*, 2005, Jacobs *et al.*, 1998b, Muhrbeck & Wischmann 1998, Hoover & Manuel 1996, Cameron & Donald 1992, Stute 1992, Lorenz *et al.*, 1980, Gough & Pybus 1971), DSC (Waduge, *et al.*, 2006, Qi *et al.*, 2005, Freitas *et al.*, 2004, Kiseleva *et al.*, 2004, Nakazawa & Wang 2003, Ozcan & Jackson 2003, Tester *et al.*, 2000, Jacobs & Delcour 1998, Tester *et al.*, 1998, Jacobs *et al.*, 1998b,c & 1995, Muhrbeck & Svensson 1996, Wang *et al.*, 1997, Hoover & Vasanthan 1994a, Ziegler *et al.*, 1993, Stute 1992, Larsson & Eliasson 1991, Muhrbeck & Eliasson 1991, Liu & Leliévre 1991b, Paredes-Lopez & Hernández-Löpez 1991, Knutson 1990, Tester & Morrison 1990b, Krueger *et al.*, 1987a,b, Slade & Levine 1987, Yost & Hoseney 1986, Zeleznak & Hoseney 1987, Kuge & Kitamura 1985, Lorenz *et al.*, 1984, Lorenz *et al.*, 1980, Lorenz & Kulp 1978a), ^{13}C cross polarization magic angle spinning/NMR [^{13}C-CP MAS/NMR] (Tester *et al.*, 2000 & 1998, Jacobs *et al.*, 1998c), microscopy (Waduge *et al.*, 2006, Kiseleva & *et al.*, 2005, Jacobs *et al.*, 1998c, Hoover & Vasanthan 1994a, Stute 1992, Gough and Pybus 1971, Wiegel 1933), susceptibility to acids (Waduge *et al.*, 2006, Hoover & Vasanthan 1994a, Jacobs

et al., 1998a, Tester *et al.*, 1998, Nakazawa & Wang 2003), and enzymes (Jacobs *et al.*, 1998c, Wang *et al.*, 1997, Hoover & Manuel 1996, Lauro & *et al.*, 1993, Hoover & Vasanthan 1994a, Kuge & Kitamura 1985, Lorenz *et al.*, 1980, Gough & Pybus 1971), granule swelling (Waduge *et al.*, 2006, Adebowale *et al.*, 2005a, Nakazawa & Wang 2004, Tester *et al.*, 1998, Hoover & Vasanthan 1994a), and amylose leaching (Waduge *et al.*, 2006, Jacobs *et al.*, 1998b, Eliasson & Gudmundsson 1996, Hoover & Vasanathan 1994a, Knutson 1990, Krueger *et al.*, 1987a, Lorenz *et al.*, 1984) and pasting (Jacobs *et al.*, 1995, Hoover & Vasanthan 1994a, Stute 1992). Annealing conditions for various botanical sources are presented in **Table 2-13**.

2.4.8.5 Effect of annealing on structural changes

The following changes have been shown to occur on annealing: a polymorphic transformation of the A+B pattern to the A-pattern (Waduge *et al.*, 2006, Genkina *et al.*, 2004c), increase in granule stability (Hoover and Vasanthan 1994a), crystallite growth and perfection/optimization (Tester & Debon 2000, Tester *et al.*, 1998, Jacobs *et al.*, 1998b, Hoover & Vasanthan 1994a, Larsson & Eliasson 1991, Paredes-Lopez & Hernández-Löpez 1991, Tester & Morrison 1990b, Yost & Hoseney 1986), increase in granule rigidity (Jacobs *et al.*, 1995), twisting of unordered ends of double helices (Tester *et al.*, 1999, Tester *et al.*, 1998), glucan chain interactions within the amorphous and crystalline domains of the granule (Jacobs & Delcour 1998, Hoover and Vasanthan 1994a, Stute 1992), increase in order within the amorphous domain without increase in crystallinity (Tester & Debon 2000, Jacobs & Delcour 1998), development of crystallinity in

the amorphous regions of the granule (Münzing 1989, Krueger *et al.*, 1987a,b), formation of double helices and compartmentalization of AM-AM, AMP-AMP and AM-AMP helices (Atichokudomchai *et al.*, 2002, Tester *et al.*, 2000, Jacobs *et al.*, 1998a,b, Shi *et al.*, 1998, Hoover and Vasanthan 1994a, Seow & Vasanti-Nair 1994, Morrison *et al.*, 1993a, Knutson 1990), induce AM-lipid interactions (Jacobs *et al.*, 1998b), extra reinforcing of α-D-(1→6) linkages (Jacobs *et al.*, 1998a), polymer chain realignment within granules and partial crystallite melting (Marchant & Blanshard 1980), mobility differences in amorphous or crystalline regions (Stute 1992, Nakazawa *et al.*, 1984), reorientation of the crystallites (e.g. amylose) within the amorphous matrix (Stute 1992), increase binding (coupling) forces between crystallites and the amorphous matrix (Stute 1992), and increase in glassy nature (more rigid and less mobile) of amorphous material (Tester & Debon 2000). However, annealing has been shown to have no influence on the wide angle X-ray diffraction pattern (Tukomane *et al.*, 2007, Waduge *et al.*, 2006, Qi *et al.*, 2005, Muhrbeck & Wischmann 1998, Hoover & Manuel 1996, Stute 1992, Gough & Pybus 1971), peak position tansformation (Muhrbeck & Svensson 1996) and d-spacing (Muhrbeck & Svensson 1996, Hoover and Vasanthan 1994a). Vermeylen *et al.*, (2006) have shown by small angle X-ray scattering studies on potato starch, that the 9 nm scattering intensity increases post-annealing. For instance, the 9 nm scattering intensity was more pronounced at 51°C (close to T_o) than at 44 or 47°C. The enhanced intensity was attributed to more efficient packing (increases density of the crystalline lamellae) of the double helices. The enhanced density contrast between the

crystalline and amorphous lamellae was also observed by Kiseleva *et al.*, (2005) and Jacobs *et al.*, (1998b).

2.4.8.6 Effect of annealing on granule morphology

Granule morphology, granule size distribution and surface characteristics play an important role in many food and non-food applications of starch, it was surprising to find that there is a dearth of information (especially for tuber & root starches) on the effect of annealing on the above parameters. Several authors (Waduge *et al.*, 2006, Jacobs *et al.*, 1998c, Hoover & Vasanthan 1994a, Stute 1992, Wiegel 1933) have found no changes to granule morphology on annealing of wheat, oat, lentil, barley (certain cultivars) and potato starches. However, Kiseleva *et al.*, (2005) observed that the lens shaped granules of high amylose and waxy wheat starches were slightly deformed on annealing. The extent of this deformation being greater in the latter. Gough and Pybus (1971) observed a granule size increase (5 μm) on annealing of normal wheat starch. Wang *et al.*, (1997) have postulated that annealing could create pores or fissures. Waduge *et al.*, (2006) reported that in some cultivars of barley, pore size increased slightly on annealing. Kiseleva *et al.*, (2003) reported that the Maltese-cross and concentric growth rings remain unchanged on annealing. However, concentric growth rings were much denser after annealing.

2.4.8.7 Effect of annealing on gelatinization characteristics

Annealing has been shown to increase the gelatinization temperatures (T_o, T_p, T_c) and decrease the gelatinization temperature range (T_c-T_o) in all starches

(Kohyama & Sasaki 2006, Vermeylen *et al.*, 2006, Waduge, *et al.*, 2006, Kiseleva *et al.*, 2004, Tester *et al.*, 2005, Tester *et al.*, 2000, Jacobs & Delcour 1998, Tester *et al.*, 1998, Jacobs *et al.*, 1998c & 1995, Muhrbeck & Svensson 1996, Wang *et al.*, 1997, Hoover & Vasanthan 1994a, Stute 1992, Larsson & Eliasson 1991, Muhrbeck & Eliasson 1991, Liu & Leliévre 1991b, Paredes-Lopez & Hernández-Löpez 1991, Knutson 1990, Tester & Morrison 1990b, Krueger et al., 1987a,b, Slade & Levine 1987, Yost & Hoseney 1986, Kuge & Kitamura 1985, Lorenz *et* al., 1984, Lorenz *et al.*, 1980, Lorenz & Kulp 1978a). However, gelatinization enthalpies (ΔH) have been reported to increase (Waduge, *et al.*, 2006, Kiseleva *et al.*, 2005, Kiseleva *et al.*, 2004, Genkina *et al.*, 2004b, Nakazawa & Wang 2003 & 2004, Atichokudomchai *et al.*, 2002, Jacobs *et al.*, 1998b,c, Hoover & Manuel 1996, Muhrbeck & Svensson 1996, Jacobs *et al.*,1995, Hoover & Vasanthan 1994a, Larsson & Eliasson 1991, Knutson 1990, Krueger *et al.*, 1987a,b, Slade & Levine 1987), remain unchanged (Waduge, *et al.*, 2006, Qi *et al.*, 2005, Jacobs *et al.*, 1998c, Muhrbeck & Wischmann 1998, Eerlingen *et al.*, 1996, Wang *et al.*, 1997, Seow & Teo 1993, Shi & Seib 1992, Stute 1992, Larsson & Eliasson 1991, Yost & Hoseney 1986) and decrease (Kohyama & Sasaki 2006, Larsson & Eliasson 1991) on annealing [**Table 2-14**]. The increase in gelatinization temperature has been shown to be most pronounced for T_o and least for T_c [**Table 2-14**]. Annealing has a greater influence on T_o, since T_o represents melting of the weakest crystallites (Nakazawa & Wang 2003, Wang *et al.*, 1997, Larsson & Eliasson 1991). These crystallites are more susceptible to crystallite perfection on annealing than

Tables 2-14 Gelatinization parameters and annealing conditions of various starches

Starch source	T_o (°C)	T_p (°C)	T_c (°C)	T_c-T_o (°C)	ΔH (J/g)	Annealing*: H_2O content (%), incubation period (°C) DSC scanning**: range (°C), rate (°C/min)	Reference
Potato- native	60.4	64.4	69.7	9.3	16.9	20-140°C, 10°C/min**	Nakazawa & Wang 2004
Potato- *annealed*	71.4	74.5	78.5	7.1	18.5	75%, 50°C-24h, multi-step* ; 20-140°C, 10°C/min**	Nakazawa & Wang 2004
Potato- native	58.4	67.3	73.5	15.1	16.5	20-120°C, 10°C/min**	Nakazawa & Wang 2003
Potato- *annealed*	69.9	73.3	78.1	8.2	18.5	75%, 50°C-24h, multi-step ; 20-120°C, 10°C/min**	Nakazawa & Wang 2003
Potato- native	59.1	61.9	66.8	7.7	18.3	20-130°C, 4°C/min**	Vermeylen *et al.*, 2006
Potato- *annealed*	61.5	63.6	68.0	6.5	18.4	66%, 44°C-24h,1 step* ; 20-130°C, 4°C/min**	Vermeylen *et al.*, 2006
Potato- *annealed*	63.1	65.0	60.2	6.2	18.4	66%, 47°C-24h, 1 step* ; 20-130°C, 4°C/min**	Vermeylen *et al.*, 2006
Potato- *annealed*	64.9	66.8	71.1	6.2	18.3	66%, 51°C-24h,1 step* ; 20-130°C, 4°C/min**	Vermeylen *et al.*, 2006
Potato- native	NA	NA	NA	NA	NA	25-130°C, 1°C/min**	Kohyama & Sasaki 2006
Potato- *annealed*	54.3	58.1	70.4	16.1	19.1	90%, 20°C-72h,1 step* ; 25-130°C, 1°C/min**	Kohyama & Sasaki 2006
Potato- *annealed*	61.1	63.1	70.7	9.6	18.0	90%, 50°C-72h, 1 step* ; 25-130°C, 1°C/min**	Kohyama & Sasaki 2006
Potato- native	59.1	63.2	70.3	11.1	18.6	5-100°C, 10°C/min**	Tester *et al.*, 2005
Potato- *annealed*	70.3	72.8	78.4	8.3	20.6	80%, 55°C-7days, 1 step* ; 5-100°C, 10°C/min**	Tester *et al.*, 2005
Potato- native	58.0	62.5	70.5	12.5	18.7	5-150°C, 4°C/min**	Jacobs *et al.*, 1998a
Potato- *annealed*	64.7	67.5	72.7	8.0	20.0	66%, ~50°C/24h,1 step* ; 5-150°C, 4°C/min**	Jacobs *et al.*, 1998a & c
Potato- *annealed*	67.3	69.8	74.2	6.9	20.5	66%, 54°C-48h, 2 step* ; 5-150°C, 4°C/min**	Jacobs *et al.*, 1998a & c
Potato- native	57.6	61.3	66.5	8.9	17.5	5-150°C, 2°C/min**	Jacobs *et al.*, 1998b
Potato- *annealed*	63.4	65.5	69.3	5.9	19.1	66%, 50°C-24h,1 step* ; 5-150°C, 2°C/min**	Jacobs *et al.*, 1998b
Potato- *annealed*	65.7	67.8	71.5	5.8	19.1	66%, 55°C-48h,1 step* ; 5-150°C, 2°C/min**	Jacobs *et al.*, 1998b
Potato- native	58.6	63.0	72.2	13.6	19.2	5-150°C 4°C/min**	Jacobs *et al.*, 1996
Potato- *annealed*	64.8	67.7	73.0	8.2	19.9	66%, 50°C-24h,1 step* ; 5-150°C 4°C/min**	Jacobs *et al.*, 1996
Potato- native	58.6	63.0	72.2	13.6	19.2	66%, 5-150°C, 4°C/min**	Jacobs *et al.*, 1995
Potato- *annealed*	64.8	67.7	73.0	8.2	19.9	66%, 50°C-24h,1 step ; 66%, 5-150°C, 4°C/min**	Jacobs *et al.*, 1995
Potato- native	54.0	58.8	64.5	10.5	16.8	40-120°C, 5°C/min**	Hoover & Vasanthan 1994a
Potato- *annealed*	71.2	74.2	78.4	7.2	20.2	75%, 50°C-72h, single step* ; 40-120°C, 5°C/min**	Hoover & Vasanthan 1994a
Cassava- native	63.7	69.6	77.5	13.8	10.7	20-140°C, 10°C/min**	Nakazawa & Wang 2004
Cassava- *annealed*	69.5	73.3	79.5	10.0	13.5	75% 50°C-24h, multi-step* ; 20-140°C, 10°C/min**	Nakazawa & Wang 2004
Cassava- native	61.3	70.1	76.2	14.9	10.6	20-120°C, 10°C/min**	Nakazawa & Wang 2003
Cassava- *annealed*	69.1	73.0	79.4	10.3	13.6	75%, 50°C/24h, multi-step* ; 20-120°C, 10°C/min**	Nakazawa & Wang 2003
Cassava- native	65.4	71.5	81.5	16.1	8.8	20-120°C, 10°C/min**	Atichokudomchai *et al.*,2002
Cassava- *annealed*	70.7	74.0	81.3	10.6	9.4	75%, 2°C-24h, 1 step* ; 20-120°C, 10°C/min**	Atichokudomchai *et al.*,2002

Tables 2-14 Gelatinization parameters and annealing conditions of various starches (cont.,)

Starch source	T_o (°C)	T_p (°C)	T_c (°C)	T_c-T_o (°C)	ΔH (J/g)	Annealing*: H_2O content (%), incubation period (°C) DSC scanning**: range (°C), rate (°C/min)	Reference
Sago-native	63.1	70.1	-	-	15.15	30-110°C, 10°C/min**	Wang *et al.*, 1996
Sago -*annealed*	65.0	70.2	-	-	15.24	80%, 60°C-0.5h, 1 step* ; 30-110°C, 10°C/min**	Wang *et al.*, 1996
Sago -*annealed*	96.6	72.8	-	-	15.16	80%, 60°C-5h,1 step* ; 30-110°C, 10°C/min**	Wang *et al.*, 1996
Wheat-native	NA	NA	NA	NA	NA	25-130°C, 1°C/min**	Kohyama & Sasaki 2006
Wheat-*annealed*	48.2	56.2	68.5	20.3	10.6	90%, 20°C-72h, 1 step* ; 25-130°C, 1°C/min**	Kohyama & Sasaki 2006
Wheat-*annealed*	60.1	62.5	68.1	8.0	9.9	90%, 50°C-72h, 1 step* ; 25-130°C, 1°C/min**	Kohyama & Sasaki 2006
Wheat-native	52.5	58.7	71.5	19.0	10.6	5-100°C, 10°C/min**	Tester *et al.*, 1998
Wheat-*annealed*	54.6	59.4	71.3	16.7	10.2	% NA, 25°C-6days, 1 step* ; 5-100°C, 10°C/min**	Tester *et al.*, 1998
Wheat-*annealed*	59.2	62.2	72.9	13.7	10.4	% NA, 35°C-6days,1 step* ; 5-100°C, 10°C/min**	Tester *et al.*, 1998
Wheat-*annealed*	63.7	66.3	76.3	12.6	10.1	% NA, 45°C-6days,1 step* ; 5-100°C, 10°C/min**	Tester *et al.*, 1998
Wheat-native	52.0	56.8	63.5	11.5	10.3	5-150°C, 2°C/min**	Jacobs *et al.*, 1998b
Wheat-*annealed*	60.2	62.3	65.6	5.4	11.7	66%, 48°C-24h,1 step* ; 5-150°C, 2°C/min**	Jacobs *et al.*, 1998b
Wheat-native	51.5	56.2	61.6	10.1	10.6	5-150°C, 4°C/min**	Jacobs *et al.*, 1996
Wheat-*annealed*	59.7	61.7	64.9	5.2	11.0	66%, 43°C-24h,1 step* ; 5-150°C, 4°C/min**	Jacobs *et al.*, 1996
Wheat-native	54.4	58.7	63.6	9.2	11.8	5-150°C, 4°C/min**	Jacobs *et al.*, 1998a
Wheat-*annealed*	61.6	63.7	66.9	5.3	12.2	66%, ~52°C-24h,1 step* ; 5-150°C, 4°C/min**	Jacobs *et al.*, 1998a & c
Wheat-*annealed*	64.2	66.3	69.7	5.5	12.3	66%, ~52°C-24h,1, 2 step* ; 5-150°C, 4°C/min**	Jacobs *et al.*, 1998a & c
Wheat-native	58.7	65.0	68.5	9.8	10.0	20-120°C, 10°C/min**	Nakazawa & Wang 2003
Wheat-*annealed*	69.1	71.7	75.0	5.9	12.4	75%, 50°C-24h, multi-step* ; 20-120°C, 10°C/min**	Nakazawa & Wang 2003
Normal corn-native	68.1	72.3	77.3	9.2	12.3	20-120°C, 10°C/min**	Nakazawa & Wang 2003
Normal corn-*annealed*	71.3	74.3	78.8	7.5	14.3	75%, 50°C-24h, multi-step* ; 20-120°C, 10°C/min**	Nakazawa & Wang 2003
Waxy corn-native	65.3	72.8	78.2	12.9	14.9	20-120°C, 10°C/min**	Nakazawa & Wang 2003
Waxy corn-*annealed*	71.4	74.4	78.8	7.4	17.5	75%, 50°C-24h, multi-step* ; 20-120°C, 10°C/min**	Nakazawa & Wang 2003
Hylong V-native	68.0	76.2	106.8	38.1	16.5	20-120°C, 10°C/min**	Nakazawa & Wang 2003
Hylong V-*annealed*	71.9	78.5	108.8	36.9	18.8	75%, 50°C/24h, multi-step* ; 20-120°C, 10°C/min**	Nakazawa & Wang 2003
Hylong VII-native	68.9	82.0	110.8	41.9	16.7	20-120°C, 10°C/min**	Nakazawa & Wang 2003
Hylong VII-*annealed*	73.3	85.1	115.0	41.7	20.1	75% 50°C-24h, multi-step* ; 20-120°C, 10°C/min**	Nakazawa & Wang 2003

Annealing conditions: Incubation was carried out in constant temperature in a water bath NA: Data not available

crystallites that have higher stability (represent by T_c) (Jacobs *et al.*, 1998b). The decrease in T_c-T_o on annealing indicates greater homogeneity and cooperative melting of crystallites (Jacobs & Delcour 1998). Increase in starch mobility within the amorphous regions leads to a molecular re-organization which involves interaction between amylose-amylose (AM-AM) and/or amylose-amylopectin (AM-AMP) chains (Atichokudomchai *et al.*, 2002, Tester *et al.*, 2000, Jacobs *et al.*, 1998a,b, Shi *et al.*, 1998, Hoover & Vasanthan 1994a, Seow & Vasanti-Nair 1994, Morrison *et al.*, 1993a, Knutson 1990). This interaction together with crystallite perfection increases T_g. Consequently, this increases T_o, T_p and T_c in the annealed starches. Qi *et al.*, (2005), Kiseleva *et al.*, (2004), Tester *et al.*, (2000) and Tester *et al.*, (1998) have postulated that changes to the gelatinization transition temperataures on annealing could also be due to lengthening of the double helices that were not optimized during biosynthesis. Kiseleva *et al.*, (2004) have postulated that twisting of unordered end of double helices during annealing leads to the formation of additional intrahelical hydrogen bonds. The intertwining being facilitated by the increase in glucan chain mobility that occurs on annealing. Kiseleva *et al.*, (2004) have attributed the increase in melting temperature on annealing to an increase in crystalline lamellae thickness, resulting from the twisting of the unordered ends of the double helices. For such a mechanism to have a significant impact on gelatinization temperatures, the free ends of the double helices should be long enough to intertwine and form strong intrahelcial hydrogen bonds. Similarly, Vermeylen *et al.*, (2006) have postulated

that the increase in gelatinization temperatures on annealing may be due to higher packing density.

Cooke and Gidley (1992) have shown by using ^{13}C cross polarization magic angle spinning/NMR and DSC that ΔH is a reflection of the number of double helices that unravel and melt during gelatinization. Thus, starches in which ΔH remains the same pre- and post-annealing, suggests that the only molecular re-organization that occurs in these starches is crystalline perfection, and that the double helical order (number of double helices and stabilizing hydrogen bonds) is not influenced by annealing. Evidence for the constancy of ΔH pre- and post-annealing in wheat (Tester *et al.*, 1998) and corn (Tester *et al.*, 2000) starch was shown by ^{13}C-CP MAS/NMR. Significant increases in ΔH has been shown to occur mainly in high amylose barley (Waduge *et al.*, 2006) and corn starches (Tester *et al.*, 2000). Waduge *et al.*, (2006), Tester *et al.*, (2000) and Knutson (1990) hypothesized, that when amylose content reaches a certain threshold, amylose chains may be in close proximity to each other and/or with amylopectin chains. Consequently, on annealing interactions could occur between AM-AM and/or AM-AMP chains resulting in the formation of new double helices. Tester *et al.*, (2000) showed using ^{13}C-CP MAS/NMR that the amount of double helices in amylomaize starch (63.1% amylose) increase by 11% on annealing (single step). The corresponding increase in ΔH being ~5%. Waduge *et al.*, (2006) showed by studies on barley starches of varying amylose content (0-55.3%) that a particular cultivar (SB 94893) having the highest amylose content (55.3%) exhibited the largest increase in ΔH (~28%) on annealing. However, its unit

amylopectin chain length distribution was higher (DP 5-17: 56.9%) than the barley cultivar SB 948907 (DP 5-17: 52.6%) [amylose content 43.7%] in which ΔH remained unchanged on annealing. This clearly demonstrates that the increase in ΔH is influenced by the interplay of: (1) amylose content, (2) location of amylose and amylopectin within the starch granule interior, and (3) amylopectin unit chain length distribution.

2.4.8.7.1 Impact of annealing temperature, moisture content and annealing time on gelatinization parameters

Several studies (Tester & Debon 2000, Hoover & Vasanthan 1994a, Larsson & Eliasson 1991, Knutson 1990, Krueger *et al.*, 1987a, Slade & Levine 1987, Lorenz *et al.*, 1984) have shown that the effect of annealing on starch structure is more pronounced if the annealing temperature is set (close) to but below T_o. However, if the annealing temperature is set very close to T_o, then it would trigger starch gelatinization. Therefore, annealing temperatures are generally kept at about 5 to 15°C below T_o (Tester & Debon 2000, Eliasson & Gudmundsson 1996). However, annealing temperatures (15 to 28°C) below T_o has also been shown to have a significant impact on the gelatinization parameters of starches (Nakazawa & Wang 2003, Tester *et al.*, 1998). Kruger *et al.*, (1987a) showed that on annealing T_o and T_p of maize starch increased gradually up to a moisture content of 67% (w/w), after which excess water had no further effect. Hoover and Vasanthan (1994a) reported steep increase in T_o, T_p and T_c at an annealing moisture of 50% in wheat and lentil starches and at 10% and 70%, respectively, in potato and oat starches. For ΔH, a steep increase occurred at moisture

contents of 40 and 50% in potato and wheat starches, respectively. Whereas changes in ΔH for oat and lentil starches were gradual (Hoover & Vasanthan 1994a). Interaction between AM-AM and/or AM-AMP chains allows enthalpically driven assembly of the lamellar structure to be initiated with amylopectin double helices moving into alignment [**Figure 2-14-B**]. This would then explain the increase in gelatinization parameters with increase in moisture content.

The impact of annealing time on T_o, T_p, T_c and ΔH was studied by Kiseleva *et al.*, 2005, Genkina *et al.*, 2004a,b, Jacobs *et al.*, 1998a, Muhrbeck & Wischmann 1998, Hoover & Vasanathan 1994a, Seow & Vasanti-Nair 1994, Seow & Teo 1993, Knutson 1990, Larsson & Eliasson 1991, Krueger *et al.*, 1987a). In general, T_o, T_p and T_c increases with annealing time. The increase being more pronounced in T_o and least in T_c [**Table 2-14**]. Hoover and Vasanthan (1994a) showed that in oat, wheat, potato and lentil starches, increases in T_o, T_p, T_c and ΔH do not begin simultaneously during the time course of annealing (at 50°C). The rates of increase in T_o, T_p, and T_c were gradual in wheat and oat starches, but rapid in potato (during the first 30 min). Annealing beyond 24h, did not significantly increase T_o, T_p, and T_c of oat, potato and lentil starches. However, those of wheat starch became more pronounced as the annealing time exceeded 24h. Increases in ΔH were slower and were evident in wheat, oat, potato and lentil starches only after annealing had been in progress for 48, 6, 2 and 1h, respectively. Genkina *et al.*, (2004a) showed that T_o of sweet potato starches increased rapidly during the first 60 min of annealing (at 45°C). After, that increments were much lower tending towards constant value after 8h. Larsson

and Eliasson (1991) reported that for wheat starch the largest changes in T_o, T_p, T_c and T_c-T_o occurred during the first 4h of annealing (at 50°C). No changes were observed after 6h.

2.4.8.7.2 Impact of phosphorylation on gelatinization parameters

The extent of increase in gelatinization parameters on annealing has been shown to be influenced by the level of negatively charged phosphate groups located on the A-chains and on the inner and outer sections of the B-chains of amylopectin (Muhrbeck & Svensson 1996). Studies on potato starches of varying degrees of phosphorylation (13 to 24 µmol G-6-P/g starch) showed the largest increase in T_p on annealing occurred for those samples with the lowest degree of phosphorylation. Whereas, the largest increase in ΔH was observed for the highly phosphorylated starches (Muhrbeck & Svensson 1996). Muhrbeck *et al.*, (1991) showed that the degree of crystallinity (due mainly to amylopectin) is reduced by a high phosphate level. This was attributed to dislocations in the amylopectin clusters induced by bulky phosphate groups interfering with the building up of the structures during starch biosynthesis. Muhrbeck and Svensson (1996) postulated that reorientation of phosphate groups occur during annealing. Consequently, after annealing, the ΔH would be increased, since the double helices would be better aligned than in the native starch (phosphate groups in the native starch could hinder double helical chain realignment), resulting in stronger interhelical and intrahelical hydrogen bonding. The extent of this increase was found to be more pronounced in the high phosphate level starches, since in their native state ΔH is lower (due to greater extent of crystallite disruption) than in the

low phosphate level starches. The increase in T_p on annealing was found to higher in the low phosphate level starches, due to less crystallite disruption in the native state. Muhrbeck and Wischmann (1998), & Muhrbeck and Svensson (1996) have reported that covalently bound starch-phosphate esters are not hydrolyzed during annealing.

2.4.8.8 Impact of annealing on amylose-lipid complex

Morrison *et al.*, (1993b) have shown by means of ^{13}CCP-MAS NMR, DSC and X-ray studies the presence of amylose-lipid complexes in native starch granules of barley, maize, rice and oat starches. DSC studies have shown that the amylose-lipid complex transition occurs in the range 85-115°C (Slade & Levine 1988, Russell 1987b, Biliaderis *et al.*, 1986b, Biliaderis *et al.*, 1985, Kugimiya & Donovan 1981), 96-125°C (Karkalas *et al.*, 1995) ~110°C (Nakazawa & Wang 2004) and 93.2-96.8°C (Andreev *et al.*, 1999). Tester *et al.*, (2005) postulated that since amylose-lipid complexes are distinct entities and they are unlikely to be formed during annealing (Tester *et al.*, 2005). Several reports have indicated that new amylose-lipid complexes are not formed during single or double step annealing (Kiseleva *et al.*, 2005, Nakazawa & Wang 2004, Jacobs *et al.*, 1998c, Larsson & Eliasson 1991). This was based on the unchanged DSC amylose-lipid complex melting endotherm (Koyama & Sasaki 2006, Kiseleva *et al.*, 2005, Nakazawa & Wang 2004, Wasserman, *et al.*, 2002, Jacobs *et al.*, 1998a & 1995, Larsson & Eliasson 1991), the ^{13}CCP-MAS NMR signal at 31 ppm, which remained unchanged after annealing (Jacobs *et al.*, 1998a), and unchanged apparent amylose content in pre and post annealed [single step, 20 & 50°C/72h]

starches [e.g. potato, wheat & corn] (Koyama & Sasaki 2006). Tester and Debon (2000), Jacobs *et al.*, (1998c), Morrison *et al.*, (1993c), Larsson and Eliasson (1991) have postulated that this may be due to the fact that the annealing temperature (35-50°C) is much lower than the melting temperature range (85-125°C) of the amylose-lipid complex. Andreev *et al.*, (1999) have shown by DSC studies on (maize, wheat, barley, and rye) and high amylose (barley) starches, that only maize starch has the ability to form additional amylose-lipid complexes on annealing. Wasserman, *et al.*, (2002) have postulated that the ability of maize starch to form additional amylose-lipid complexes on annealing may be due to the entry of surface lipids (via the channels on granule surface) into the granule interior. It is likely, that once inside the granule interior, the thermal energy imparted to the fatty acid chain during annealing may increase its mobility, thereby facilitating its interaction with the amylose helix. Waduge *et al.*, (2006), Lorenz *et al.*, (1984) have shown increases in the intensity of V-amylose lipid complex (2θ ~20°) in barley starches on annealing. However, the enthalpy of melting of amylose-lipid complex remained unchanged on annealing [single step] (Waduge *et al.*, 2006). Waduge *et al.*, (2006) postulated that the increased intensity in barley starches was not due to formation of additional amylose-lipid complexes, but to enhanced ordering of lipid molecules that were present as V-amylose-lipid complexes within granules of the native starches.

2.4.8.9 Impact of annealing on X-ray diffraction pattern and crystallinity

Gough and Pybus (1971) were the first to study X-ray diffraction patterns of annealed starches. Muhrbeck and Wischmann (1998) reported that the effect of annealing is more pronounced in B-type starches than on A-type starches. Annealing of potato (Vermeylen *et al.*, 2006, Jacobs *et al.*, 1998a, Hoover & Vasanthan 1994a), cassava (Tukomane *et al.*, 2007), wheat (Qi *et al.*, 2005, Jacobs *et al.*, 1998a, Hoover & Manuel 1996, Hoover & Vasanthan, 1994a, Stute 1992, Gough & Pybus 1971), oat (Hoover & Vasanthan 1994a), pea (Hoover & Manuel 1996), lentil (Hoover & Vasanthan 1994a), maize (Qi *et al.*, 2005, Ozcan & Jackson 2003), and barley (Waduge *et al.*, 2006) starches have shown no effect on their polymorphic pattern. However, in some varieties of barley (Waduge *et al.*, 2006), sweet potato (Genkina *et al.*, 2004c), and cassava (Gomez *et al.*, 2004), the A+B X-ray diffraction pattern changed to a A-type pattern on annealing. The X-ray intensities has been shown to increase slightly on annealing in potato, lentil, oat, wheat (Hoover & Vasanthan 1994a), and barley starches (Waduge *et al.*, 2006, Jacobs *et al.*, 1998b).

X-ray crystallinity has been shown to increase in high amylose barley (Waduge *et al.*, 2006), wheat (Hoover & Vasanthan 1994a), and to decrease in potato (Vermeylen *et al.*, 2006) or remain unchanged in potato (Jacobs & Delcour 1998), wheat (Jacobs & Delcour 1998, Slade & Levine 1987), maize (Ozcan & Jackson 2003), pea (Jacobs & Delcour 1998), and in normal and waxy barley (Waduge *et al.*, 2006) starches on annealing. The increase in crystallinity on annealing was attributed to the interplay of the following factors: (1) amylopectin

content (Waduge *et al.*, 2006), (2) changes in orientation of the starch crystallites (Tester & Debon 2000), (3) crystallite perfection (Tester & Debon 2000, Jacobs & Delcour 1998, Muhrbeck & Svensson 1996, Seow & Teo 1993, Larsson & Eliasson 1991, Tester & Morrison 1990b, Slade & Levine 1987, Lorenz *et al.*, 1984), (4) enhanced ordering of the V-amylose lipid complex (Lorenz *et al.*, 1984) and (5) formation of amylose crystallites (Kruger *et al.*, 1987a,b). The unchanged crystallinity observed in some starches on annealing is indicative that changes in factor 2 to 5 may have been of a low order of magnitude. The slight decrease in crystallinity reported by Vermeylen *et al.*, (2006) may be crystallite disruption or crystallite reorientation. However, the authors have not provided any explanation for this phenomenon.

2.4.8.9.1 Impact of drying method on starch crystallinity

Ahmed and Leliévre (1978) have shown that oven drying (at 40°C), vacuum drying (at 20°C) and freeze drying of wheat starch changes granule crystallinity. However, crystallinity remains unchanged on air drying (at 20°C). Therefore, it is recommended that air-dried starches be used for annealing. In many instances, commercial starches have been used in annealing studies, consequently, it is difficult to ascertain the drying method used after starch isolation. Thus, the annealing properties of a laboratory extracted and air dried starch may not be identical to the same starch that may have been extracted and dried commercially.

2.4.8.10 Impact of annealing on granular swelling

Annealing has been shown to reduce granular swelling in potato (Nakazawa & Wang 2004, Debon & Tester 2000, Hoover & Vasanthan 1994a), cassava (Nakazawa & Wang 2004), breadfruit (Adebowale *et al.*, 2005a), wheat (Tester *et al.*, 1998, Hoover & Vasanthan 1994a, Lorenz & Kulp 1978a), corn (Qi *et al.*, 2005, Nakazawa & Wang 2004), lentil (Hoover & Vasanthan 1994a), oat (Hoover & Vasanthan 1994a), pea (Hoover & Manuel 1996), and barley (Waduge *et al.*, 2006) starches. The decrease in granular swelling has been attributed to the interplay of the following factors: (1) increased crystalline perfection and decreased hydration (Waduge *et al.*, 2006, Tester *et al.*, 1998), (2) AM-AM and/or AMP-AMP interaction (Jacobs *et al.*, 1998b), (3) increased intragranular binding forces and reinforcement of the granule (Hizukuri 1996, Jacobs *et al.*, 1995), and (4) V-amylose-lipid complex formation (Waduge *et al.*, 2006, Jacobs *et al.*, 1998b, Hoover & Vasanthan 1994a).

2.4.8.11 Impact of annealing on amylose leaching (AML)

Annealing [single, two and multi-step] treatments reduce amylose leaching at all temperatures below 100°C in potato (Nakazawa & Wang 2004, Jacobs *et al.*, 1995, Hoover & Vasanthan 1994a, Kuge & Kitamura 1985), wheat (Hoover & Vasanthan 1994a, Lorenz & Kulp 1978a), lentil & oat (Hoover & Vasanthan 1994a), pea & rice (Jacobs *et al.*, 1995), cassava (Gomez *et al.*, 2004), and certain cultivars of barley (Waduge *et al.*, 2006) starches. However, an increase in AML has been reported for wheat (Jacobs *et al.*, 1995) and certain cultivars of barley starches (Waduge *et al.*, 2006) on annealing. The reduction in AML has

been attributed to the interplay of the following: (1) interactions between AM-AM and/or AM-AMP (Waduge *et al.*, 2006, Hoover & Vasanthan 1994a), (2) decrease in granular swelling (Tester *et al.*, 2000), and (3) increase in V-amylose-lipid content (Waduge *et al.*, 2006, Tester *et al.*, 2000) and molecular size of amylose (Waduge *et al.*, 2006).

2.4.8.12 Impact of annealing on pasting properties

The effects of annealing on pasting properties are complex and vary among starches. The information currently available on the pasting properties of annealed starches is for wheat (Jacobs *et al.*, 1995), lentil (Hoover & Vasanthan 1994a), oat (Hoover & Vasanthan 1994a), potato (Jacobs *et al.*, 1995, Hoover & Vasanthan 1994a, Stute 1992), pea (Jacobs *et al.*, 1995), rice (Jacobs *et al.*, 1995), breadfruit [*Artocarpus artilis*] (Adebowale *et al.*, 2005a), sorghum (Adebowale *et al.*, 2005b), and bambarra ground nut [*Voandzeia subterranean*] (Adebowale & Lawal 2002) starches. Generally, annealing has been shown to increase the pasting temperature, thermal stability and decrease peak viscosity and the viscosity at the end of the cooling cycle (Adebowale *et al.*, 2005a, Adebowale & Lawal 2002, Jacobs *et al.*, 1995, Hoover & Vasanthan 1994a, Stute 1992). The exceptions being, rice (Jacobs *et al.*, 1995), wheat (Jacobs *et al.*, 1995, Hoover & Vasanthan 1994a), and pea (Jacobs *et al.*, 1995) starches which exhibit a higher peak viscosity (wheat>pea>rice) on annealing. The RVA profile of annealed [single step] rice starch shows an increase in pasting temperature. However, this parameter remains unchanged in the Brabender viscoamylograph (Jacobs *et al.,* 1995). The reduced viscosity and improved shear stability on

annealing has been attributed to reduced granular swelling and amylose leaching, and increased interaction between glucan chains during annealing (Jacobs *et al.*, 1995, Hoover & Vasanthan 1994a, Stute 1992). The increase in viscosity exhibited by wheat starch on annealing was attributed by Jacobs *et al.*, (1995) to higher rigidity and resistance to shear. Hoover and Vasanthan (1994a) have shown by DSC studies, that on annealing, wheat starch exhibits a higher decrease in T_c-T_o than potato starch. This, indicates, that interaction between double helices (in the crystalline domain) are more extensive in wheat than in potato starch. Thus, although, the extent of granular swelling is reduced (wheat>potato) (Hoover & Vasanthan 1994a) as a result of annealing, the increase in granular stability of wheat starch on annealing is so high that it negates the effect of decreased granular swelling on peak viscosity. This would then explain why the viscosity of wheat starch increases on annealing, whereas that of potato starch decreases.

2.4.8.13 Impact of annealing on acid hydrolysis

The impact of annealing on acid hydrolysis has been shown to be in influenced by the method used for annealing (single step, double step, and multi-step), annealing temperature and starch source (Waduge *et al.*, 2006, Qi *et al.*, 2005, Nakazawa & Wang 2003, Jacobs *et al.*, 1998a, Tester *et al.*, 1998, Hoover & Vasanthan 1994a). Waduge *et al.*, (2006) reported that in starches extracted from different cultivars of barley, the difference in acid hydrolysis [single step, 0.25g starch/10mL 2.2 M HCl, at 35°C/18 days] between native and annealed starches was only marginal. No difference in hydrolysis was observed between

native and annealed wheat and pea starches subjected to double step anenaling [0.167g starch/10mL 2.2 M HCl, at 35°C/20 days] (Jacobs *et al.*, 1998a). However, Nakazawa and Wang (2003) showed by studies on potato, wheat, cassava, maize, waxy maize and high amylose maize starches that annealing increased acid susceptibility [multi-step, 15.3% H_2SO_4, 0.5g starch/10mL, at 38°C/30 days] in all starches, with potato starch showing the greatest and high amylose maize starch showing the smallest change. Tester et al., (1998) reported that during the rapid phase of acid hydrolysis [single step, 0.1g starch/10mL 2M HCl, at 35°C/10 days], annealed wheat starch was more extensively degraded than its native counterpart, while during the slow phase of hydrolysis, there was no difference in the extent of hydrolysis. Hoover and Vasanthan (1994a) reported that in potato, lentil, oat, and wheat the difference in acid hydrolysis [single step, 0.25g starch/10mL 2.2 M HCl, at 35°C/20 days] between native and annealed starches were ~5%.

The decrease in acid hydrolysis on annealing has been attributed to: (1) perfection of starch crystallites (Waduge *et al.*, 2006), (2) formation of double helical structures between amylose chains (Jacobs *et al.*, 1998a), (3) increased embedding of α-(1→6) branch points within the crystalline structure (Jacobs *et al.*, 1998a), and (4) formation of V-amylose lipid complexes (Waduge *et al.*, 2006, Jacobs *et al.*, 1998a, Hoover & Vasanthan 1994a). The increase in acid hydrolysis on annealing has been attributed to: (1) an increase in the concentration of α-glucan in the amorphous region as a consequence of crystalline perfection (Tester *et al.*, 2000) and (2) formation of void spaces in the

crystalline lamellae due to crystalline perfection (Nakazawa & Wang 2003). Similarity in acid hydrolysis between native and annealed starches during the slow phase of hydrolysis has been attributed to: (1) limited crystallite perfection and (2) unchanged double helical content pre- and post-annealing (Nakazawa & Wang 2003, Tester *et al.*, 2000).

In summary, that there is conflicting information with respect to the susceptibility of annealed starches towards acid hydrolysis. For instance, the same type of starch (e.g. potato or corn or wheat) has been shown to behave differently towards H_3O^+ after annealing (Nakazawa & Wang 2003, Tester *et al.*, 2000, Jacobs *et al.*, 1998a, Hoover & Vasanthan 1994a). This could be attributed differences in: (1) variety of the starch source (2) number of steps used in annealing [single vs double vs multi-step], (3) type of acid [HCl vs H_2SO_4], (4) acid concentration [1.7M H_2SO_4 vs 2.2M HCl] (5) starch:acid ratio, (6) temperature of the reaction mixture [35°C vs 38°C] and (7) hydrolysis period.

2.4.8.14 Impact of annealing on α-amylase hydrolysis

There is a dearth of information on the impact of annealing on α-amylase hydrolysis and what is available is often conflicting. It is difficult to find a consensus of the action pattern of α-amylase on native annealed starches reported in the literature (Jacobs *et al.*, 1998c, Wang *et al.*, 1997, Hoover & Manuel 1996, Hoover & Vasanthan 1994a, Gough & Pybus 1971) due to differences in α-amylase sources, enzyme purity, enzyme concentration, time of hydrolysis, varietal differences, annealing temperature, time and the number of

annealing steps. It has been shown that annealing increases the susceptibility of wheat starch towards fungal α-amylase (Lorenz *et al.*, 1980) and bacterial α-amylase [*Bacillus subtilis*] (Gough & Pybus 1971). However, Jacobs *et al.*, (1998c) showed by using pancreatin (a mixture of α-amylase from porcine stomach mucosa, lipids and protease) that during the early stages of hydrolysis (<20h), susceptibility of one step and double step annealed wheat starches is lower than that of its native counterpart. However, during the latter stages (>20h) this trend is reversed. Hoover and Vasanthan (1994a) reported that the susceptibility of annealed (single step) wheat starch was lower than its native counterpart throughout the time course of hydrolysis by porcine pancreatic α-amylase. Both single step (Jacobs *et al.*, 1998c, Hoover & Vasanthan 1994a) and double step (Jacobs *et al.*, 1998c) annealing has been shown to decrease the susceptibility of potato starch towards porcine pancreatic α-amylase (Hoover & Vasanthan 1994a) and pancreatin (Jacobs *et al.*, 1998c). Legume starches such as pinto bean, black bean, lentil and field pea starches have been shown (Hoover & Manuel 1996) to exhibit increased susceptibility towards porcine pancreatic α-amylase on annealing (single step). A similar finding was also reported by Jacobs *et al.*, (1998c) for pancreatin hydrolyzed single and double step annealed pea starch. However, the extent of hydrolysis of the single and double step annealed pea starches were nearly similar. An increase in hydrolysis on annealing (single step) has also been observed in sago (*Metroxylon* sp.) starch (Wang *et al.*, 1997). Lauro *et al.*, (1993) reported that enzyme (*Bacillus licheniformis* and porcine pancreatin α-amylases) hydrolysis of

of annealed [single step] barley starch does not occur below 50°C/3h. The decrease in α-amylase susceptibility on annealing has been attributed to the interplay of the following factors: (1) crystallite perfection and double helical content, (2) crystal type (3) annealing steps, (4) interaction between AM-AM and/or AM-AMP chains and (5) amylose-lipid complex formation on annealing (Jacobs *et al.*, 1998c, Hoover & Vasanthan 1994a). Wang *et al.*, (2004) have speculated that annealing may create pores or fissures which alter the pattern of amylase hydrolysis from surface to internal erosion. If this were to happen, it could negate the effect of glucan chain interaction and crystallite perfection on α-amylase hydrolysis and thereby facilitate the entry of α-amylase into the granule interior. This could then explain the increase in hydrolysis observed in some starches on annealing. However, a clear relationship between pore/fissures development on annealing, and its influence on hydrolysis cannot be made, until a systematic study is carried out on the nature of the granule surface of different starches before and after annealing.

2.4.8.15 Impact of environmental temperature on annealing

The crystalline nature of tuber and root starch has been shown to be influenced by environmental conditions (e.g. soil temperature) during starch biosynthesis (Kiseleva *et al.*, 2004, Genkina *et al.*, 2004d, Protservo *et al.*, 2002). Genkina *et al.*, (2004b) showed that an increase in soil temperature from 10 to 25°C increased gelatinization temperature and enthalpy of potato starch by 17% and 82%, respectively. A similar observation was also reported for sweet potatoes [15 & 33°C] (Genkina *et al.,* 2004a), normal, waxy and high amylose barley [7 &

20°C] (Kiseleva *et al.*, 2004) grown at different temperatures. It was shown that crystallites of starches formed at higher soil temperatures were more perfect than those formed at a lower soil temperature (Genkina *et al.,* 2004a,b, Kiseleva *et al.*, 2004). However, Tester *et al.*, (1999) showed that though the gelatinization temperatures of potato starch increased as growth temperature was increased from 10 to 25°C, ΔH remained constant. Debon and Tester (2000), Tester *et al.*, (1999 & 1998) have shown by studies on starches in potato microtubers and potato starches, that when there is a constant background of amylopectin structure and amylose to amylopectin ratio, the gelatinization and swelling characteristic of starches can be modified during biosynthesis by increasing growth temperature. These observations are analogous to *in vitro* annealing. Both elevated growth temperature (*in vivo* annealing) and heating of starches in excess water (*in vitro* annealing) causes double helical realignment (resulting in crystalline perfection) without the formation of more double helices in crystalline regions (Tester *et al.*, 1999 & 1998). Tester and Debon (2000) have postulated that the major molecular re-organization underlying both *in vivo* and *in vitro* annealing are improved perfection of starch crystallites and improved ordering of glucan chains within the amorphous domains. Waxy starches have been shown to be more responsive to high environmental temperatures than their high amylose counterparts (Debon & Teaster 2000). Thermal energy is the major variable cost associated with annealing. Tester and Debon (2000) have stated that the effects of growth temperature during starch biosynthesis potentially has important industrial consequences such as the energy needed for starch

gelatinization in food products for malting and brewing. Modifying starch structure by *in vivo* and *in vitro* annealing would be a way of reducing the processing cost.

2.4.8.16 Potential uses of annealed starches

Annealing has been shown to improve thermal stability and decrease the extent of set-back (Adebowale *et al.*, 2005a, Jacobs *et al.*, 1995, Hoover & Vasanthan 1994a, Stute 1991), therefore annealed starches could be utilized in the canned and frozen food industries, for their respective advantages. Rice noodles prepared from rice flour are widely consumed in South East Asia. Traditionally, rice noodles are prepared from long-grain rice which has been stored for a period of time. This process limits starch granule swelling and improves the paste or gel quality (Zhou *et al.*, 2003), making the rice flour suitable for preparing good quality noodles. The decrease in granular swelling and amylose leaching, and the increase in heat and shear stability that occur on annealing are all desirable properties for noodle manufacture. Hormdok and Noomhorm (2007) evaluated rice starch (native & annealed), fresh rice flour, aged flour and compound rice flours with 50/100 g native rice starch or annealed rice starch as replacement ingredients for the manufacture of noodles of acceptable quality. The study showed that the textural (adhesiveness, chewiness, tensile strength) quality of the rice noodle prepared using annealed rice starch was comparable to that of commercial noodles.

Lorenz and Kulp (1980 & 1978b) assessed the baked bread quality of wheat starches extracted from wheat grains steeped at various temperatures (25-50°C) for different periods of time (1-3 days). The study showed that crumb softness increased with duration and temperature of grain steeping. The temperature of steeping was found to be more critical in affecting overall bread quality than the actual time of steeping at a given temperature. The authors have postulated that the increase in softness was due to the higher moisture content of the breads made with starches from steeped grains. It is difficult to understand how annealing (which occurs during grain steeping) increases water absorption capacity, since hydration capacity of starch decreases with annealing temperature. It is likely, that the increase in softness may have been due to a decrease in amylose leaching which occurs on annealing. A decrease in amylose leaching would decrease the extent of retrogradation, thereby improving bread texture (e.g. softness).

Lorenz and Kulp (1980) have also shown that cakes of acceptable quality could be produced with starch extracted from wheat grains steeped in the temperature range (25-50°C) for period ranging from 1 to 3 days. The study showed that cakes prepared with starch extracted from grains steeped at 50°C resulted in a complete collapse of the cake during baking, resulting in a course and gummy texture. Whereas, cakes prepared from starches extracted from grains steeped at 25 and 40°C for 3 days produced cakes of acceptable quality. A satisfactory explanation was not provided for the above observations.

CHAPTER 3

Materials and Methods

3.1 Materials

Tubers from *Dioscorea esculenta* (kukulala, java-ala, nattala) and *Dioscorea alata* (raja-ala, hingurala) were grown under the same field conditions in Algama, Sri Lanka. Crystalline porcine pancreatic α-amylase (type 1A, 790 units/mg protein), fungal α-amylase (157 units/mg protein) from *Aspergillus oryzae* were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Isoamylase (68,000 μ/mg protein) from *Pseudomonas amylodermosa* were purchased from Hayashibana Biochemical Laboratories Ltd. (Okayama, Japan). All chemicals and solvents were of ACS certified grade and were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

3.2 Methods

3.2.1 Starch isolation

3.2.1.1 Sampling

For starch isolation, well matured tubers from each variety of *D. esculenta* [**Figure 3-1-A, B, C**] and *D. alata* [**Figure 3-1-D, E**] were harvested from three separate vines in late *Maha* season in 2002.

3.2.1.2 Starch extraction and purification

Starch extraction and purification was performed according to the procedure of Jayakody *et. al.*, (2005). *Dioscorea* tubers were washed, peeled and fibrous roots removed. Immediately after peeling, the tubers were sliced into 2-3 cm cubes,

Figure 3-1 Morphology of *Dioscorea* tubers

Dioscorea esculenta:

(A) kukulala,

(B) java-ala

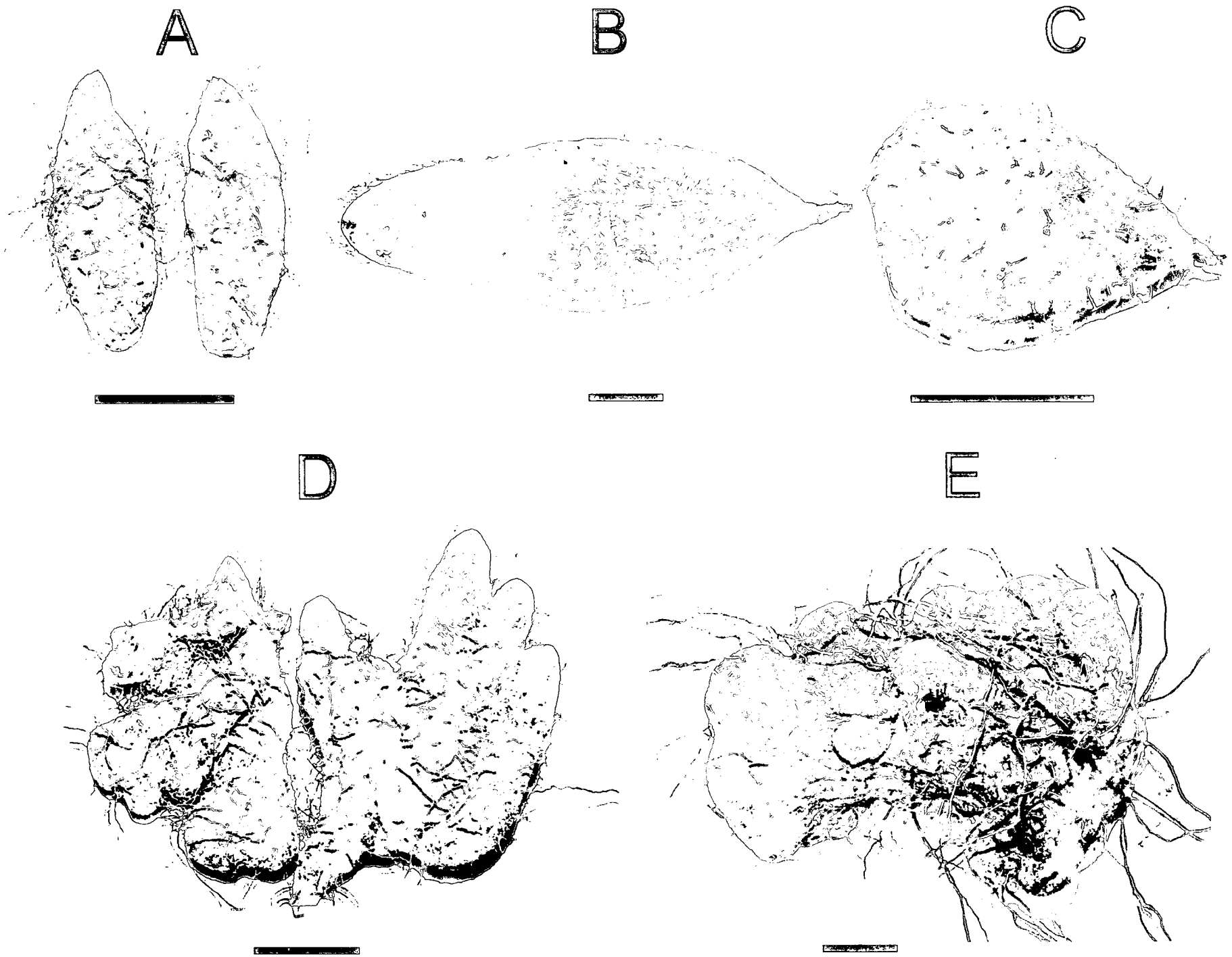
(C) nattala

Dioscorea alata:

(D) hingurala

(E) raja-ala

(The scale bar represents 5 cm)


A
B
C
D
E

soaked in chilled potassium metabisulfite (50 mg/L) solution for 1h, and then shredded (1 part sliced cubes to 3 parts chilled water) in a Waring blender (model 33BL73, New Hartford, CT, USA) for 10s at high speed and then at low speed for 15s. The starch in the slurry was separated from the cell debris by vacuum filtration through a muslin cloth. The filtrate containing the starch was allowed to stand (~ 2h for *D. alata* spp. and ~12 h for *D. esculenta* spp.) at room temperature until a dense firm starch layer was obtained. The supernatant was siphoned and discarded and the precipitate was suspended in excess 0.02% sodium hydroxide. After standing (~ 4h), the supernatant was removed. The washing-sedimentation process with alkali was repeated until the supernatant layer was almost free of color and suspended haze. The final sediment was suspended in deionized water, passed through a 70 µm polypropylene screen (Spectrum Laboratory Products, CA, USA), neutralized to pH 7.0, filtered through a Buchner funnel and thoroughly washed on the filter with deionized water. The starch was air dried at room temperature (25°C) and then passed through a 250 µm test sieve (Fisher Scientific Company, Mentor, OH, USA) to obtain a free flowing powder, which was weighed and the yield was calculated as the percentage of the initial tuber weight.

3.2.2 Granular morphology and size estimation

3.2.2.1 Light microscopy

Granule morphology and size estimation was carried out according to the procedure outlined by Jayakody *et al.* (2007c, 2005). The size and shape of native starches were examined by a Leica Gallen III (Buffalo, NY, USA) light

microscope. Light micrographs were obtained using a light microscope (Nikon, Eclipse Model E 600, Japan) equipped with a Leica digital camera (Leica DFC-500, Heerbrugg, Switzerland) and Leica Application Suite (LAS) version 2.3.4 software (Heerbrugg, Switzerland). To minimize movement, starch granules were suspended in a 50% (v/v) glycerol-water mixture. The same field of view was photographed three times using a multi-focus module of the LAS. The range of granule size was determined by measuring the length and width of approximately 75 granules from a 1.0% (w/v) starch suspension (stained with 0.01M iodine) at 1000x magnification, measured with an ocular micrometer.

3.2.2.2 Birefringence

A Leica binocular microscope model DME (Leica Microsystems®, Wetzlar, Germany) equipped with a Micropublisher 3.3 RTV digital camera (QImaging, Surrey, BC, Canada) was used to observe the birefringence of starch granules. Samples were dispersed in water (~10% w/w) by stirring, and the dispersions then placed on a microscopic slide for examination. The images in polarized light were recorded at the same magnification (400x) for all starch samples.

3.2.2.3 Scanning Electron Microscopy (SEM)

The granule surface of the starches were examined by a FEI Quanta 400 environmental scanning electron microscope (Brno, Czech Republic). Starch samples were freed of granule clumps by sieving through a 250 µm mesh sieve. The samples were then mounted on a Cambridge type circular aluminum stubs with carbon electro-conductive adhesive tape (Electron Microscopy Science,

Hatfield, PA, USA). The starches on the stubs were spread evenly by viewing the granules through a stereoscopic microscope (Carl Zeiss, Stemi 2000-C, Wek Gottingen, Germany). Loosely bound granules were removed by a stream of low pressure dry air jet, and then the remaining granules were coated with gold (10 nm) for 60s at 50 mA using a EMS500 sputter coater (Electron Microscopy Science, Hatfield, PA, USA). The granules were then examined under the following conditions: accelerating voltage of 5.0 kV, emission current 100 µA, high vacuum mode (10^{-4} Pa), spot size 2 (range of 1-10), working distance 10.5~10.7 mm, Mode 300 V. Granules within a horizontal field width of 54.08 µm were photographed at a magnification of 5000x using an image integration mode of 128 frames (2 frames/sec or 50µsec/pixel/frame) and the Everhart-Thornley detector (ETD) (Jayakody *et al.* 2007c).

3.2.3 Starch damage

Damaged starch content was determined according to the method of Jayakody *et al.*, (2005). Starch samples (1.0 g, dry basis) in phosphate buffer (40 mL, 0.02M, pH 6.9) in a 125 mL Erlenmeyer flask was incubated with fungal α-amylase from *Aspergillus oryzae* (2500 Sigma units, 39.3 units/mg solid) in a water bath at 37°C for 15 min. The enzyme action was terminated by the addition of 10 mL of anhydrous trichloroacetic acid (10%, w/v). The digests were allowed to stand for 2 min and then centrifuged at 2000 rpm for 10 min. The supernatants were then neutralized to pH 7.0. The amount of reducing sugars in the supernatants (2.0 mL) were determined using the Somogyi-Nelson method (Nelson (1944),

Somogyi (1952). Controls without starch, but subjected to the above experimental conditions, were run concurrently.

The extent of starch damage was calculated using the equation shown below:

$$\text{Starch damage} = \frac{M}{W \times 1.05} \times 100$$

Where: M = mg maltose equivalents in the total digest (50 mL)
W= mg (dry basis) of native starch
1.05 is the molecular weight conversion of starch to maltose

3.2.3.1 Reducing sugar determination (Nelson (1944) & Somogyi, (1952))
Alkaline reagent:

Anhydrous sodium carbonate (Na_2CO_3, 25 g), Rochelle salt ($NaKC_4HO_6.4H_2O$, 25 g), sodium bicarbonate ($NaHCO_3$, 20 g), and anhydrous sodium sulfate (Na_2SO_4, 200 g) were dissolved in 800 mL of deionized water, and made up to one liter.

Copper reagent:

Copper sulfate penta hydrate ($CuSO_4.5H_2O$, 15 g) was dissolved in 75 mL of deionized water with two drops of concentrated H_2SO_4 and was made up to 100 mL.

Arsenomolybdate reagent:

Ammonium molybdate [$(NH_4)_6Mo_7O_{24}.4H_2O$, 25 g] was dissolved in deionized water (450 mL) and acidified with concentrated H_2SO_4 (21 mL). Sodium arsenate ($Na_2HAsO_4.7H_2O$, 3 g) was dissolved separately in deionized water (25 mL) and

then added slowly to the molybdate solution and vigorously mixed. The whole mixture was diluted to 500 mL, the volumetric flask was then covered with aluminum foil and incubated in a water bath (PolyScience, Model 2L-M PolyScience Niles, IL, USA) at 37°C for 24h.

Analytical procedure

A freshly prepared 1 mL aliquot of alkaline copper reagent (mixture of 25 parts of alkaline reagent and one part of copper reagent) was added to 2 mL of the starch supernatant and heated for 20 min in a boiling water bath. At the end of 20 min tubes were cooled rapidly in an ice-water bath to a temperature of 20°C. Arsenomolybdate reagent (1 mL) was rapidly pipetted directly to the solution mixture and vortexed. The resulting solution was kept for 5 min at room temperature for color development. The solution was then diluted with deionized water (6 mL). Absorbance was measured at 510 nm using a UV-visible spectrophotometer (Milton Roy, Spectronic-601, Rochester, NY, USA). The reagent blank was carried out following the same procedure with 2 mL of deionized water. A standard curve was established in order to calculate the glucose ($Y= 0.0044X$, $R^2= 0.9999$) and maltose ($Y=0.0023X$, $R^2=1$) equivalents in the sample.

3.2.4 Proximate analysis

3.2.4.1 Moisture content

Quantitative estimation of starch moisture content (native and annealed) was determined by the standard American Association of Cereal Chemists (AACC)

method (2000). The starch samples (5.00 ± 0.01 g, dry basis) were weighed into tight fitting lidded aluminum dishes and dried in an air forced oven (Fisher Scientific, model Fisher Isotemp® 615G, Pittsburgh, PA, USA) at 130 ± 1°C for 1h. The sample dishes were then removed and cooled in a desiccator. Four replicate were used in each determination.

Moisture content was calculated as the percentage weight loss of the sample.

$$\text{Moisture (\%)} = \frac{W_1 - W_2}{W_1 - W_O} \times 100$$

Where: W_1 = weight of sample, dish and lid before drying (g)
W_2 = weight of sample, dish and lid after drying (g)
W_O = weight of empty dish and lid (g)

3.2.4.2 Ash content

Ash content was determined by the standard AACC method (2000). Pre-weighed (5.00 ± 0.01 g) starch samples were transferred into clean, dry porcelain crucibles, and ignited over a Bunsen flame until thoroughly carbonized. The samples were then transferred to a pre-heated (550°C) muffle furnace (Lab Heat-Blue M model M30A-1C, Blue M Electric Co., Blue Island, IL, USA) and left until the samples were free from carbonaceous matter (~12h). The samples were cooled to room temperature in a desiccator and weighed. Four replicates were used for each determination.

The percentage ash was calculated from the following equation.

$$\text{Ash (\%)} = \frac{W_1 - W_2}{W_1 - W_O} \times 100$$

Where: W_1 = weight of sample, crucible and lid before ashing (g)
W_2 = weight of residue, crucible and lid after ashing (g)
W_O = weight of empty crucible and lid (g)

3.2.4.3 Phosphorous content

Total starch phosphorous was determined according to the method of Jayakody *et al*, (2005). Starch (5 mg db) was placed into screw-capped tubes (calibrated at the 5 mL level) and digested with concentrated H_2SO_4 (0.3 mL) for 12h at room temperature before charring. The partially-digested samples were heated using a micro-Bunsen burner until charring was completed, and the film of acid on the walls of the tubes was no longer viscous at the end of the digestion process. After the contents of the tubes had slightly cooled, hydrogen peroxide (30 µL, 30% [w/v]) was added (15 µL at a time) to hit the wall of the tube just above the digested mixture, and the tubes were well shaken. The tubes were then boiled for 1 min. Then allowed to cool to room temperature, and made up to a final volume of 3.6 mL with deionized water. For assay, anhydrous sodium sulfite [Na_2SO_3] (0.1 mL, 2.62M) was added with stirring, followed by addition of ammonium molybdate [$(NH_4)_6Mo_7O_{24}.4H_2O$] (1.0 mL, 0.0162M) and ascorbic acid (0.01 g). The contents of the tubes were vortex mixed and then heated for 10 min in a boiling water bath. After cooling to room temperature, the volume in the tubes were adjusted to 5.0 mL with deionized water, and the absorbance read at 822 nm using a UV-visible spectrophotometer (Milton Roy, Spectronic-601, Rochester, NY, USA). Four replicates and control samples were used for each determination. The phosphorous content was calculated from a standard

curve (Y=0.1784X, R^2 =0.9992) made using sodium dihydrogen phosphate (NaH_2PO_4. $2H_2O$) standards.

3.2.4.4 Nitrogen content

Nitrogen content was determined by the classical micro-Kjeldahl method (AACC 2000). Samples (0.3 g, dry basis) were weighed on nitrogen-free papers and placed in hard glass digestion tubes on a Buchi 430 digester (Buchi Laboratoriums- Technik AG, Flawill/Schweiz, Switzerland). Two Kjeltabs M pellets (Buchi Laboratoriums- Technik AG, Flawill/Schweiz, Switzerland) and 20 mL of concentrated H_2SO_4 acid were added to each digestion tube and the samples were digested until a clear solution was obtained. The digested samples were then cooled, diluted with 50 mL of nitrogen free water, 100 mL of 40% (w/v) NaOH was then added, and the released ammonia was steam distilled into 50 mL of 4% (w/v) boric acid (H_3BO_3) containing 12 drops of end point indicator (N-point indicator, EM Science, NJ, USA) using a Buchi 321 distillation unit until 150 mL of distillate was accumulated in the receiving flask. The amount of ammonia in the distillate was determined by titrating it against 0.05N H_2SO_4. Four replicates and control samples were used for each determination.

The percentage nitrogen was calculated using the equation shown below:

$$\text{Nitrogen (\%)} = \frac{(\text{Volume of } H_2SO_4 - \text{blank}) \times \text{Normality of } H_2SO_4 \times 14.0067}{\text{Sample weight [db] (g)}} \times 100$$

3.2.4.5 Starch lipids

3.2.4.5.1 Surface lipids

Surface lipids were determined according to the procedure outlined by Vasanthan & Hoover (1992b). The lipids were extracted at ambient temperature (25-27°C) by mixing native starch (5g, dry basis) with 100 mL of chloroform-methanol 2:1 (v/v) in a wrist action mechanical shaker (Burrell, Model 75, Burrell Corporation, Pittsburg, PA, USA) for 1h. The solution was then carefully filtered (Whatmann No. 4 filter paper) into a 250 mL round bottom flask and the residue was washed thoroughly with chloroform-methanol solution. The lipid-solvent mixture was then evaporated to dryness using a rotary evaporator (Rotavpor-R110, Buchi Laboratorimus-Technik AG, Flawill/Schweiz, Switzerland). The crude lipid extracts were purified by the method of Bligh and Dyer (1959) before quantification.

3.2.4.5.2 Bound lipids

Bound lipids were determined according to the procedure described by Vasanthan and Hoover (1992b). The residues from the chloroform methanol extractions were refluxed with n-propanol water 3:1 (v/v) in a Soxhlet apparatus ~85°C for 7h. The solvent was evaporated to dryness using a rotary evaporator (Rotavpor-R110, Buchi Laboratorimus-Technik AG, Flawill/Schweiz, Switzerland). The crude lipid residue was purified using the method of Bligh and Dyer (1959) before quantification.

The lipid content was calculated using the following equation:

$$\text{Lipid (\%)} = \frac{\text{(weight of flask and lipid after drying - weight of empty flask)}}{\text{Sample weight [db] (g)}} \times 100$$

3.2.4.5.3 Crude lipid purification (Bligh & Dyer 1959)

The crude lipid extracts (surface and bound) were purified by extraction in a seperatory funnel with chloroform/methanol/water (1:2:0.8, v/v/v) and forming a biphasic system at room temperature. Then the heavy chloroform layer was withdrawn into a pre-weighted 25 mL round bottom flask and evaporated to dryness in the rotary evaporator. The samples were then removed and dried at 60°C in an air forced oven (Fisher Scientific, model Fisher Isotemp® 615G, Pittsburgh, PA, USA). The dried lipids were cooled to room temperature in a desiccator.

3.2.4.6 Amylose

Apparent and total amylose contents of native starches were determined as described by Jayakody *et al.*, (2005).

3.2.4.6.1 Apparent amylose content

Starch (20 mg, dry basis) was weighed into a boiling tube and suspended in deionized water (6 mL). The contents of the tubes were vortex mixed for 30 s. Sodium hydroxide (2 mL, 1M) was then added and the mixture was vortex mixed for 60 s. The mouth of the tubes were covered with a inner layer of Parafilm® and an outer layer of aluminum foil and then heated in a water bath (with intermittent vortexing) at 85°C for 15 min. Tubes were then cooled to room

temperature and the contents transferred to 25 mL volumetric flask and diluted with deionized water. A 2.0 mL aliquot of diluted solution was mixed with phosphate buffer (45 mL, 0.06M, pH 8.0) and 1 mL I_2/KI solution (0.005M I_2 and 0.018M KI) in a 50 mL volumetric flask and then made up to the mark with the phosphate buffer. The contents were allowed to stand in the dark (30 min) at room temperature before taking absorbance measurements at 620 nm using a UV-visible spectrophotometer (Milton Roy, Spectronic-601, Rochester, NY, USA).

3.2.4.6.2 Total amylose

Total amylose content was also determined by the above procedure, but with prior defatting with hot n-propanol water (3:1 v/v) for 7 h. In order to correct for overestimation of apparent and total amylose content, amylose content was calculated from a standard curve ($Y=0.0059X + 0.0921$, $R^2 =0.9847$) using mixtures of pure potato amylose and amylopectin (over the range 0-100% amylose).

3.2.5 Starch structure analysis

3.2.5.1 Amylopectin branch chain length distribution

Isoamylase debranching of whole starch accompanied by high pressure anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was used to determine the branch chain length distribution of native and annealed starches (Jayakody *et al.*, 2005).

Starch was dispersed in 2 mL of 90% (v/v) DMSO at a concentration of 5 mg/mL by stirring in a boiling water bath for 20 min. After cooling, methanol (99.8%, 6 mL) was added with vortex mixing, and the tube placed in an ice bath for 30 min. The pellet, which was recovered by centrifugation (1,000 x g for 12 min), was dispersed in sodium acetate buffer (2 mL, 50 mM, pH 3.5) by stirring in a boiling water bath for 20 min. Following equilibration of the tube at 37°C, isoamylase (5 µL, 68,000 µ/mg protein) was added. The sample was incubated at 37°C with slow stirring for 22 h. The enzyme was inactivated by boiling for 10 min. An aliquot (200 µL) of the cooled debranched sample was diluted with NaOH (2 mL, 150 mM). The sample was filtered (0.45 µm nylon syringe filter) and injected into the HPAEC-PAD system (50 µL sample loop).

The HPAEC-PAD system consisted of a Dionex DX 600 equipped with an ED50 electrochemical detector with a gold working electrode, GP50 gradient pump, LC30 chromatography oven, and an AS40 automated sampler (Dionex Corporation, Sunnyvale, CA, USA). The standard triple potential waveform was employed, with the following periods and pulse potentials: T_1 = 0.40 s, with 0.20 s sampling time, E_1 = 0.05 V; T_2 = 0.20 s, E_2 = 0.75 V; T_3 = 0.40 s, E_3 = -0.15 V. Data were collected using Chromeleon software, version 6.50 (Dionex Corporation, Sunnyvale, CA, USA). Eluents were prepared in distilled deionized water with helium sparging; eluent A was 50 mM sodium acetate in 150 mM NaOH, and eluent B was 150 mM NaOH. Linear debranched were separated on a Dionex CarboPac™ PA1 analytical column with gradient elution (-5 min to 0 min, 40% A; 5 min, 60% A; 45 min, 80% A) at a column temperature of 26°C and

a flow rate of 1 mL/min. A CarboPac™ PA1 guard column was installed in front of the analytical column.

3.2.5.2 X-ray diffractometry and crystallinity

3.2.5.2.1 Powder X-ray diffraction

Powder X-ray diffraction was carried out by the method proposed by Jayakody *et al.*, (2007a). Starches for X-ray diffraction measurements were kept in a desiccator (at 25°C) over saturated K_2SO_4 (water activity (a_w)= 0.98) up to sorption equilibrium (3 weeks). X-ray diffractograms were obtained with a Rigaku RPT 300 PC X-ray diffractometer (Rigaku-Denki CO, Tokyo, Japan). The hydrated samples (0.5 g dry basis) were packed tightly into an elliptical aluminum holder (Appendix- **Figure 1**). The operating conditions were: target voltage 40 kV, target current 100 mA, aging time 5 min; scanning range 3 to 35°, step scan size 0.03°, scan speed 2.000°/min; step time 0.9 sec, divergence slit width 1.0°; scatter slit width 1.0° and receiving slit width 0.6mm. The moisture content of the samples was determined before and after scanning.

3.2.5.2.2 Starch crystallinity

Crystallinity of the starches was quantitatively estimated following the method of Nara and Komiya (1983). A line connecting the peak baselines was computer-plotted on the diffractogram. The area above the smooth curve was considered as the crystalline portion and the lower area between the smooth curve and a linear baseline was taken as the amorphous portion. The crystalline and amorphous areas were measured by integration using a software package

(Origin® version 6.0 Microcal Inc., Northampton MA, USA). The ratio of the upper area to the total diffraction area was calculated as the crystallinity [Appendix-**Figure 1**].

The following equation was used to determine the percent crystallinity:

$$\text{Crystallinity (\%)} = \frac{A_c}{A_c + A_a} \times 100$$

Where: A_c and A_a are the crystalline and amorphous area of the X-ray diffractogram.

3.2.6 Physicochemical properties

3.2.6.1 Gelatinization parameters

Gelatinization parameters of native starches were measured using a Seiko differential scanning calorimeter (DSC 210) (Seiko Instruments Inc, Chiba, Japan) equipped with a thermal analysis data station and data recording software. Heat flow and temperature calibrations were periodically performed using pure indium with a heat of fusion of 28.4 J/g and a melting temperature of 156.6°C.

Deionized water (11 µL) was added with a micro-syringe (MICROLITER®, #702, Hamilton Co. Reno, NV, USA) to starch (3.00 ± 0.01 mg, db) in the DSC pans and the contents were stirred (with micro-needle) the pans were then sealed reweighed and kept inside a glass vial at room temperature for 12h (for moisture equilibration). Sealed pans were then reweighed prior to scanning. The temperature was scanned from 25-130°C at rate of 10°C/min, then held at 130°C

for 5 min. For all measurements, a thermogram was recorded with an empty aluminum pan as a reference. During the scans, the space surrounding the sample chamber was flushed with dry nitrogen at a rate of 100 mL/min to avoid condensation. For each thermogram, gelatinization transition temperatures [onset (T_o), mid-point (T_p), and conclusion (T_c)] and enthalpy of gelatinization were measured using a DSC software (SSS 5300 Work Station, version 2.71U, 1996). The enthalpy of gelatinization (ΔH) was estimated by integrating the area between the thermogram and a base line under the peak and was expressed in terms of joules per gram (J/g) of dry starch (Jayakody 2001). Four replicates per sample were analyzed.

3.2.6.2 Granular swelling (SF)

The swelling factor was determined by a slight modification of the method of Tester and Morrison (1990a). Starch samples (50 mg, dry basis) were weighed into 25 mL screw-capped tubes, deionized water (5 mL) was added and the mixture heated in a constant temperature water bath between 60-90°C for 30 min (the tubes were vortexed every 5 min to resuspend the starch slurry). The tubes were then cooled rapidly to 20°C in an ice-water bath, blue dextran (0.5 mL) [Pharmacia, average MW 2 x 10^6, 5mg/mL] was added and the contents gently mixed. The tubes were then centrifuged at 1700xg/10min (IEC, Centra MP4 centrifuge, Madison, MA, USA). The absorbance of the supernatant was measured at 620 nm (Milton Roy, Spectronic-601, Rochester, NY, USA) against a reference without starch. The method measures only intra-granular water and hence the true swelling factor at the given temperature. The SF is reported as the

ratio of the volume of swollen starch granule to the volume of un-swollen starch granule (dry starch). At least four replicates and controls samples were used in this determination.

Free water (FW) [inter-granular water + supernatant water] is given by:

$$FW = 5.5\ (A_R/A_S) - 0.5$$

Where A_R and A_S are the absorbances of the reference and sample, respectively.

The initial volume (V_O) of weight of W (in mg) of starch (dry basis) is:

$$V_O = W/1{,}400 \text{ (assuming a density of the starch is 1,400 mg/mL)}$$

The intra-granular water content (V_1) in the swollen starch granule is thus:

$$V_1 = (\text{Total water} - \text{Free water})\ \text{mL}$$

$$V_1 = (5 - FW)\ \text{mL}$$

Hence the total volume of the swollen granule (V_2) is:

$$V_2 = (V_O + V_1)\ \text{mL}$$

Swelling factor (SF) by definition is:

$$SF = V_2/V_O$$

$$SF = 1 + V_1/V_O$$

This can also be simplified as follows:

$$SF = 1 + \{(7{,}700/W) \times [(A_S - A_R)/A_S]\}$$

3.2.6.3 Amylose leaching

Amylose content of the supernatant was determined according to the method (Jayakody *et al.* 2005) as follows. Starches (20mg db) in water (10 mL) were heated at 60-90°C in volume calibrated sealed tubes for 30 min (the tubes were vortex mixed every 5 min to resuspend the starch slurry). The tubes were then

cooled to room temperature and centrifuged at 2000 rpm for 10 min (IEC-Model HN-SII, Centrifuge, Needhamhts, MA, USA). Supernatant was diluted to 25 mL in a volumetric flask with deionized water. The diluted solution (2.0 mL) was mixed with phosphate buffer (45 mL, 0.06M, pH 8.0) and 1 mL I_2/KI solution (0.005M I_2 and 0.018M KI) and then adjusted to a final volume of 50 mL in a volumetric flask with the above buffer. The contents were allowed to stand in the dark (30 min) at room temperature before absorbance measurements at 620 nm using a UV-visible spectrophotometer (Milton Roy, Spectronic-601, Rochester, NY, USA). Extent of amylose leaching was expressed as mg of amylose leached per 100 g of dry starch. Three replicate starch and control samples were used in this determination.

3.2.6.4 Acid hydrolysis

Acid hydrolysis was carried by modification of the method of Jayakody and Hoover (2002). Starches were hydrolyzed in triplicate with HCl (1 g dry basis, starch/40 mL acid, 2.2M) at 35°C in a water bath (New Brunswick Scientific, G76D, Edison, NJ, USA) for periods ranging from 0 to 15 days. The starch slurries were vortex mixed daily to resuspend the deposited starch granules. Aliquots taken at specific time intervals were neutralized with 2.2M NaOH and centrifuged at 2,000 rpm/10 min (IEC-Model HN-SII, Centrifuge, Needhamhts, MA, USA). The amount of total reducing sugar in the supernatant was determined by the Somogyi-Nelson method (Nelson, 1994, Somogyi, 1952).

The extent of hydrolysis was calculated as shown below:

$$\text{Hydrolysis (\%)} = \frac{\text{Reducing sugar (as glucose)} \times 0.9 \times 100}{\text{Initial starch wt db (g)}}$$

3.2.6.5 Enzyme digestibility

Enzymatic digestibility studies on starches were conducted using a crystalline suspension of porcine pancreatic α-amylase in 2.9M sodium chloride containing 3mM calcium chloride (Sigma Chemical Co., St. Louis, MO, USA) in which the concentration of α-amylase was 32 mg protein/mL and the specific activity was 1,122 units/mg protein.

Starch granules (20 mg, db) were suspended in phosphate buffer (10mL, 0.02M, pH 6.9) containing 0.006M NaCl. A 5.5 µL of α-amylase suspension was added, the mixture gently mixed and digested at 37°C in a water bath (New Brunswick Scientific, G76D, Edison, NJ, USA) for periods ranging from 24 to 72h. The reaction mixtures were vortexed on a daily basis to resuspend the deposited granules. The digestion reaction was terminated by adding 5 mL of absolute ethanol to the digestion mixture. The hydrolysate was recovered by centrifugation (2000 rpm/5min, IEC HN-SII centrifuge, Madison, MA, USA) of the mixture. Aliquots of the supernatant were analyzed for reducing sugar content (Nelson, 1944; Somogyi, 1952). Controls without enzyme but subjected to the above experimental conditions were run concurrently (Jayakody *et al.*, 2007c). The reported values are the means of four replicates.

The extent of hydrolysis was calculated as shown below:

$$\text{Hydrolysis (\%)} = \frac{\text{Reducing sugar (maltose)}}{\text{Initial starch weight db (g)}} \times 0.95 \times 100$$

3.2.6.6 Pasting properties

A Rapid Visco™ Analyser RVA-4 (Newport Scientific Pty, Ltd, Warriewood, NSW, Australia) was employed to measure the pasting properties of starches (7% db, 27 g total weight). Native and annealed starches were equilibrated at 50°C for 1 min, heated at 6°C/min to 95°C, held at 95°C for 5 min, cooled at 6°C/min to 50°C, and held at 50°C for 2 min. The spindle speed was 960 rpm for the first 10 s (to disperse the sample) and then at 160 rpm for the remainder (~23min) of the experiment. The reported values are the means of duplicate measurements (AACC 2000).

3.2.6.7 Retrogradation

Retrogradation characteristics of the starches were determined by DSC according to the method of Jayakody *et al*, (2005). The samples were prepared with a starch to water ratio of 1:1. Starch was mixed with water using a micro-needle in order to facilitate even distribution of water in the mix. The pan was then hermetically sealed, reweighed and kept in a glass vial at room temperature for 12h for moisture equilibration. Sealed pans were reweighed prior to scanning. After the initial DSC run, sample pans containing the gelatinized starch were covered in a single thin layer with Teflon® film and then with a double layer of Saran film®. The covered pans were first immersed in a water bath at 4°C for 24 h and then immersed in a water bath at 40°C for periods ranging from 0 to

168 h (PolyScience, Model 2L-M PolyScience Niles, IL, USA). At the end of each time period, the covering films were carefully removed and the stored samples were equilibrated at 25°C for 1 h in a desiccator before reweighing and rescanning [Appendix - **Figure 2**]. The scanning temperature range and heating rate were identical to that used for the study of gelatinization parameters (section 3.2.6.1). Four replicates per sample was analyzed.

3.2.7 Annealing treatment

Starches were subjected to one step annealing. Native starch samples (30 g, dry basis) were weighed into 250 mL beakers. The samples were prepared with a starch to water ratio of 1:3. The slurry mixtures were covered and incubated at 55°C (approximately 10°C below the onset temperature of gelatinization) for 72h in water bath (New Brunswick Scientific, G76D, Edison, NJ, USA). At end of the incubation period, the water surrounding the annealed starch (removed by decantation) showed the absence of amylose. This indicated that amylose leaching does not occur during annealing. The treated starches were mixed with excess amount of deionized water and filtered (Whatmann No. 4 filter paper). The annealed starches were air dried at room temperature (25°C) and then passed through a 250 µm test sieve (Fisher Scientific Company, Mentor, OH, USA) to obtain a free flowing powder.

2.3.8 Statistical analysis

Analysis of variance (two way ANOVA) was performed by Tukey's HSD test ($P<0.05$) using Statistical Software SPSS version 14.0 for Microsoft Windows (SSPS Inc. Chicago, IL, USA).

CHAPTER 4

Results and Discussion

4.1 Granule morphology

Light microscopy (LM) and scanning electron microscopy (SEM) of the *Dioscorea* starches (native and annealed) are presented in **Figures 4-1** & **4-2**, respectively. Starch granules of *D. esculenta* and *D. alata* ranged in size from 3 to 10 μm and 30-50 μm, respectively [**Table 4-1**]. The prominent lamellation ('oyster-shell' striations) were clearly visible in both hingurala and raja-ala (*D. alata*) starches, but the rings were not distinct in the *D. esculenta* starches. This may have been due to their smaller granular size. Starch granules of *D. esculenta* were polygonal in shape [**Figure 4-2-A, B, C**], whereas those of *D. alata* were truncated spade shaped [**Figure 4-2-D, E**]. The granule surfaces of native and annealed starches appeared to be smooth and showed no evidence of fissures [**Figure 4-2**]. Starch granules of both, species exhibited a well defined birefringence pattern [**Figure 4-3**] under polarized light. This was indicative of a high degree of molecular orientation within the granule interior. Polarization crosses (hilum) were centric and eccentric for all *D. esculenta* and *D. alata* starches, respectively.

The granule morphology of the *Dioscorea* starches did not change on annealing [**Figures 4-1 (A-1, B-1, C-1) & 4-2 (D-1, E-1)**]. After annealing, the concentric growth rings [**Figure 4-1**] and birefringence [**Figure 4-3**] pattern remained unchanged. This is indicative that the granular and lamellar structure of the starches were not altered on annealing.

Figure 4-1 Light micrographs (500x) of *Dioscorea* starches

Native starches

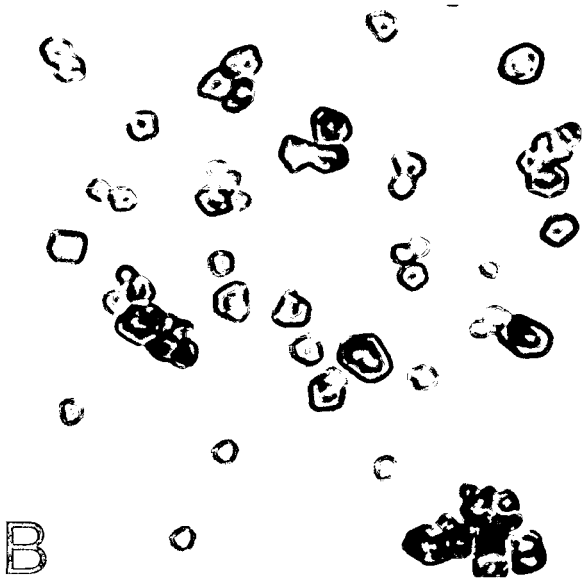
Dioscorea esculenta	*Dioscorea alata*
(A) kukulala	**(D)** hingurala
(B) java-ala	**(E)** raja-ala
(C) nattala	

Annealed starches

Dioscorea esculenta	*Dioscorea alata*
(A-1) kukulala	**(D-1)** hingurala
(B-1) java-ala	**(E-1)** raja-ala
(C-1) nattala	


A


A1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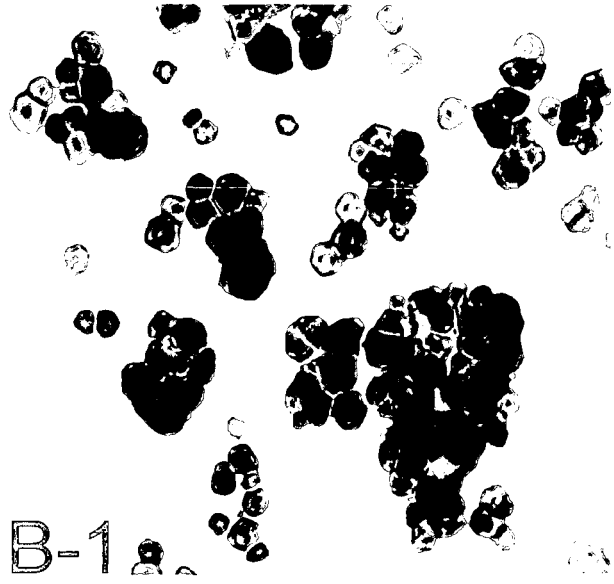
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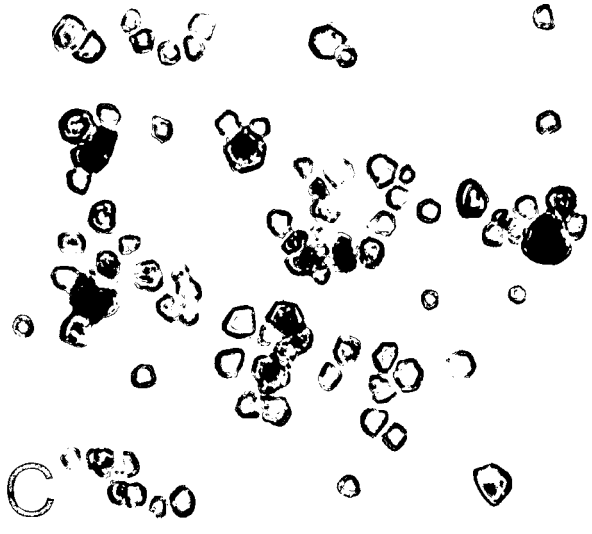
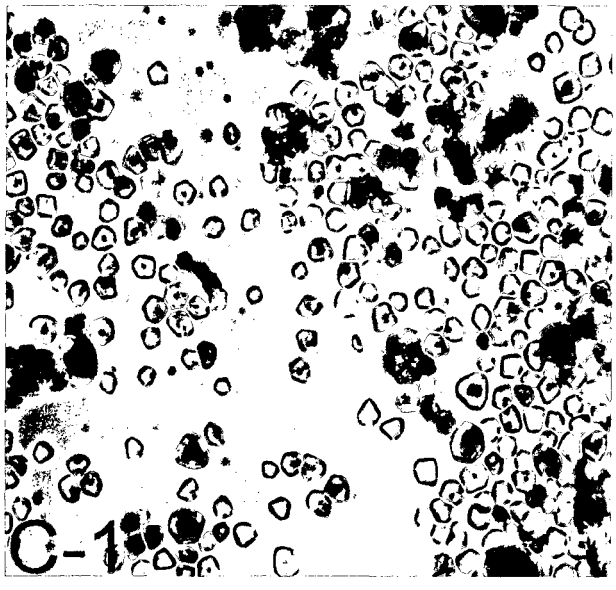

C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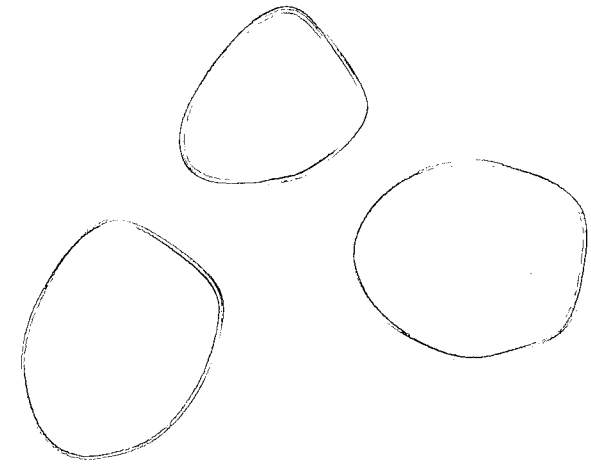
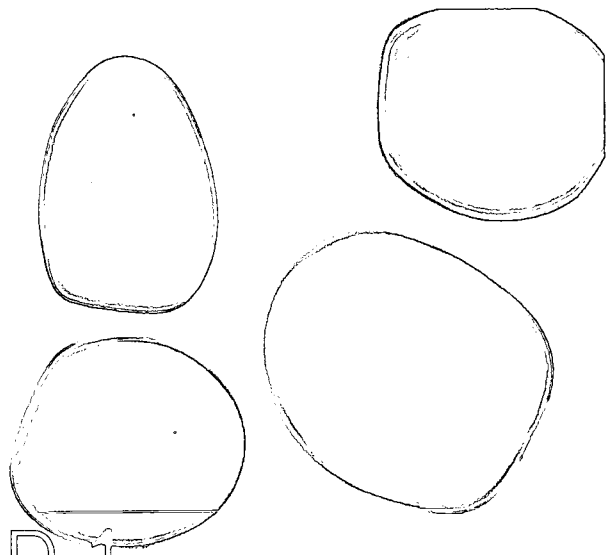
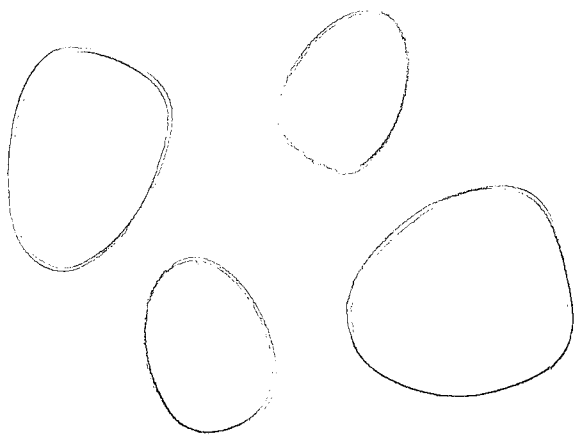


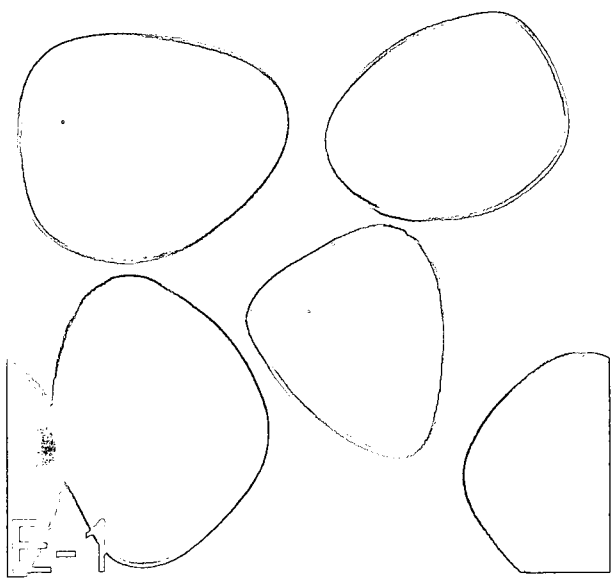
D



D-1



E



E-1

Figure 4-2 Scanning Electron Microscopy (5000x) of *Dioscorea* starches

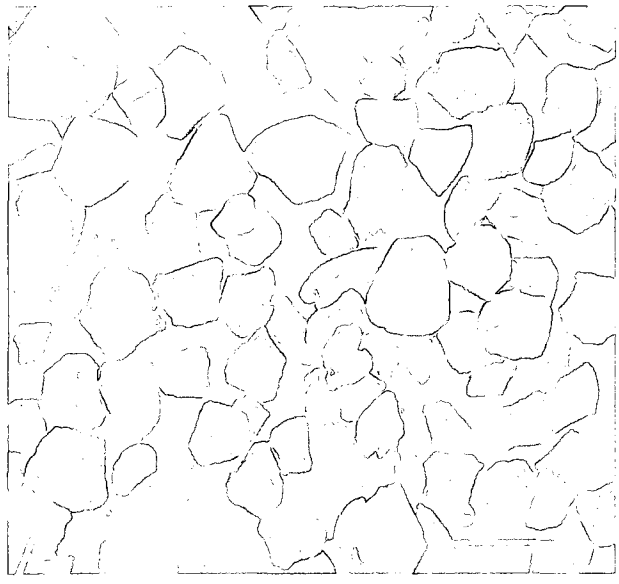
Native starches

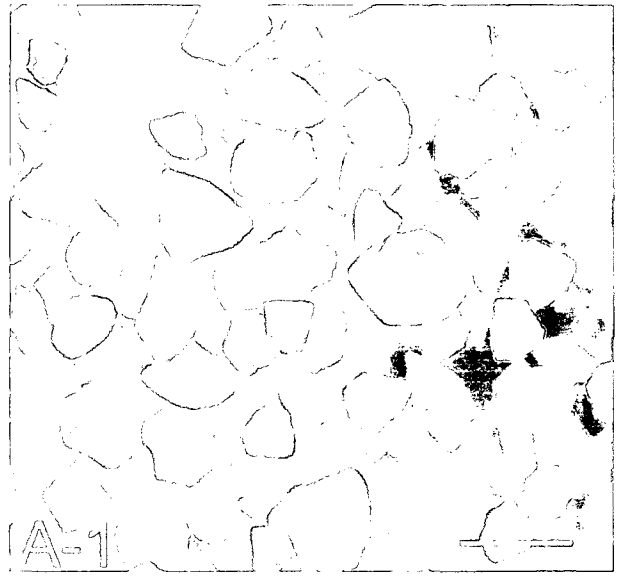
Dioscorea esculenta	*Dioscorea alata*
(A) kukulala	**(D)** hingurala
(B) java-ala	**(E)** raja-ala
(C) nattala	

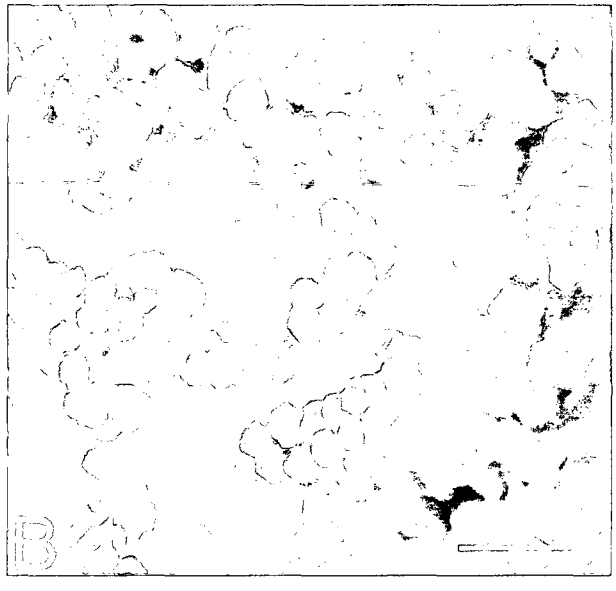
Annealed starches

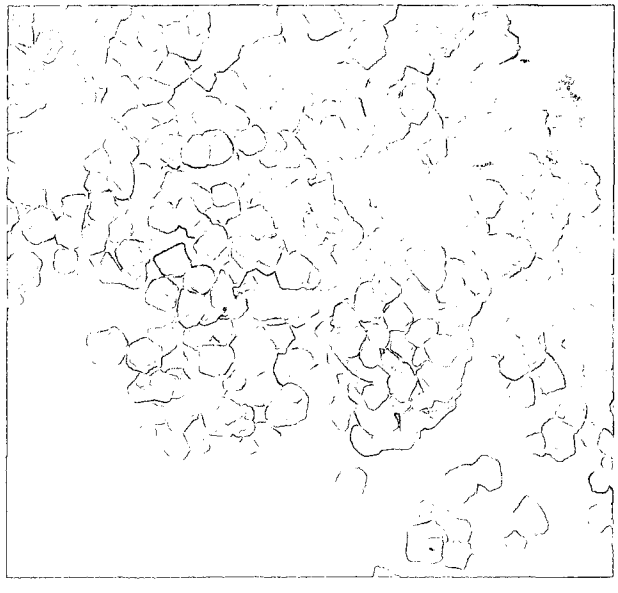
Dioscorea esculenta	*Dioscorea alata*
(A-1) kukulala	**(D-1)** hingurala
(B-1) java-ala	**(E-1)** raja-ala
(C-1) nattala	

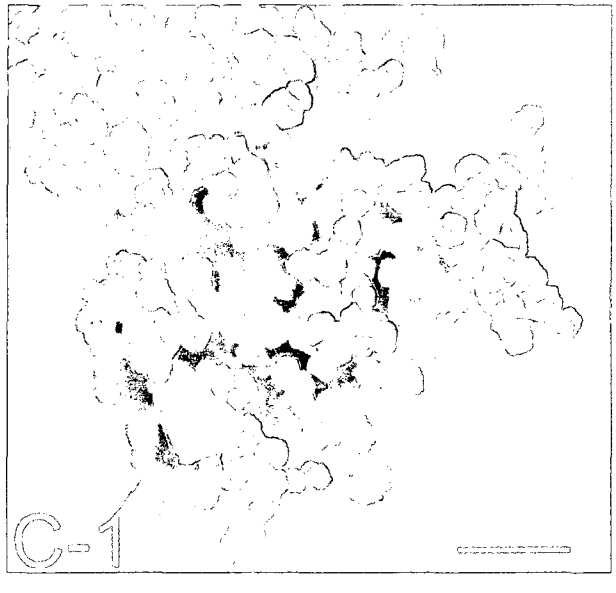
(Scale bar represents 12 μm)

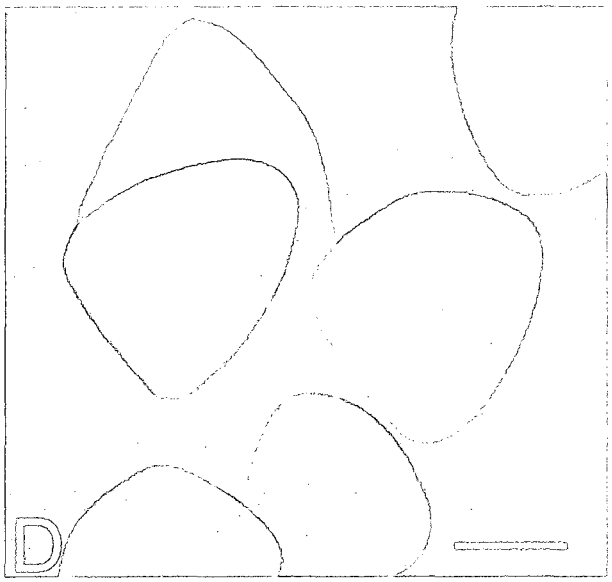


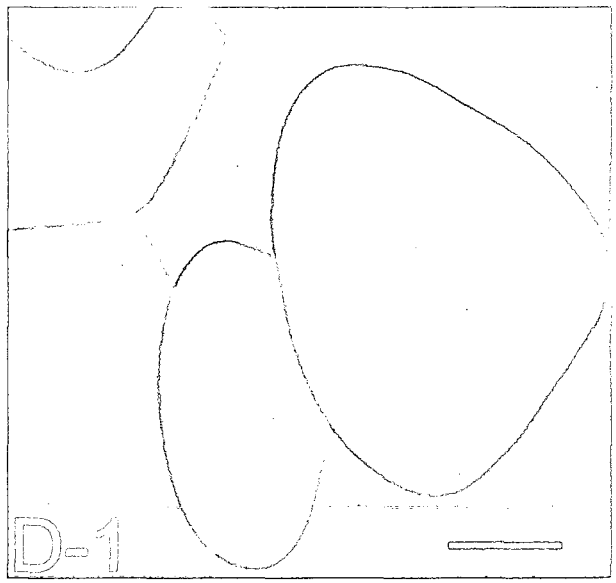

A-1

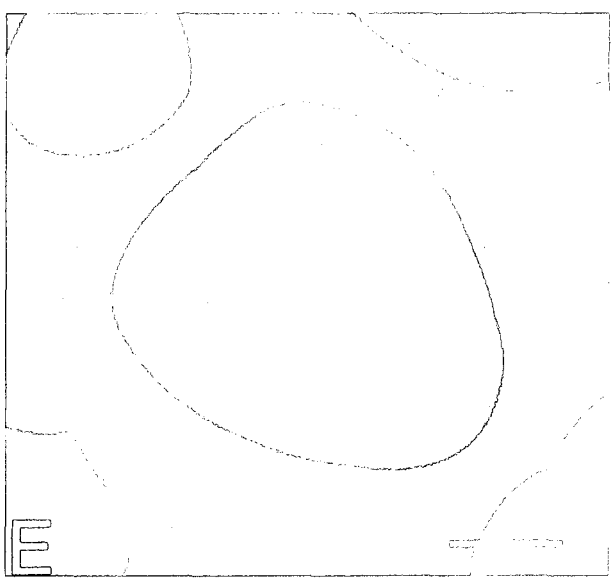

B




C-1


D


D-1


E

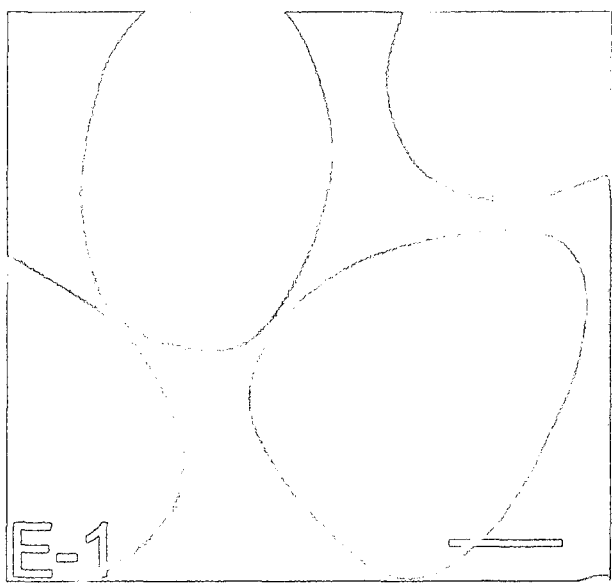

E-1

Figure 4-3 Birefringence (400x) of *Dioscorea* starches

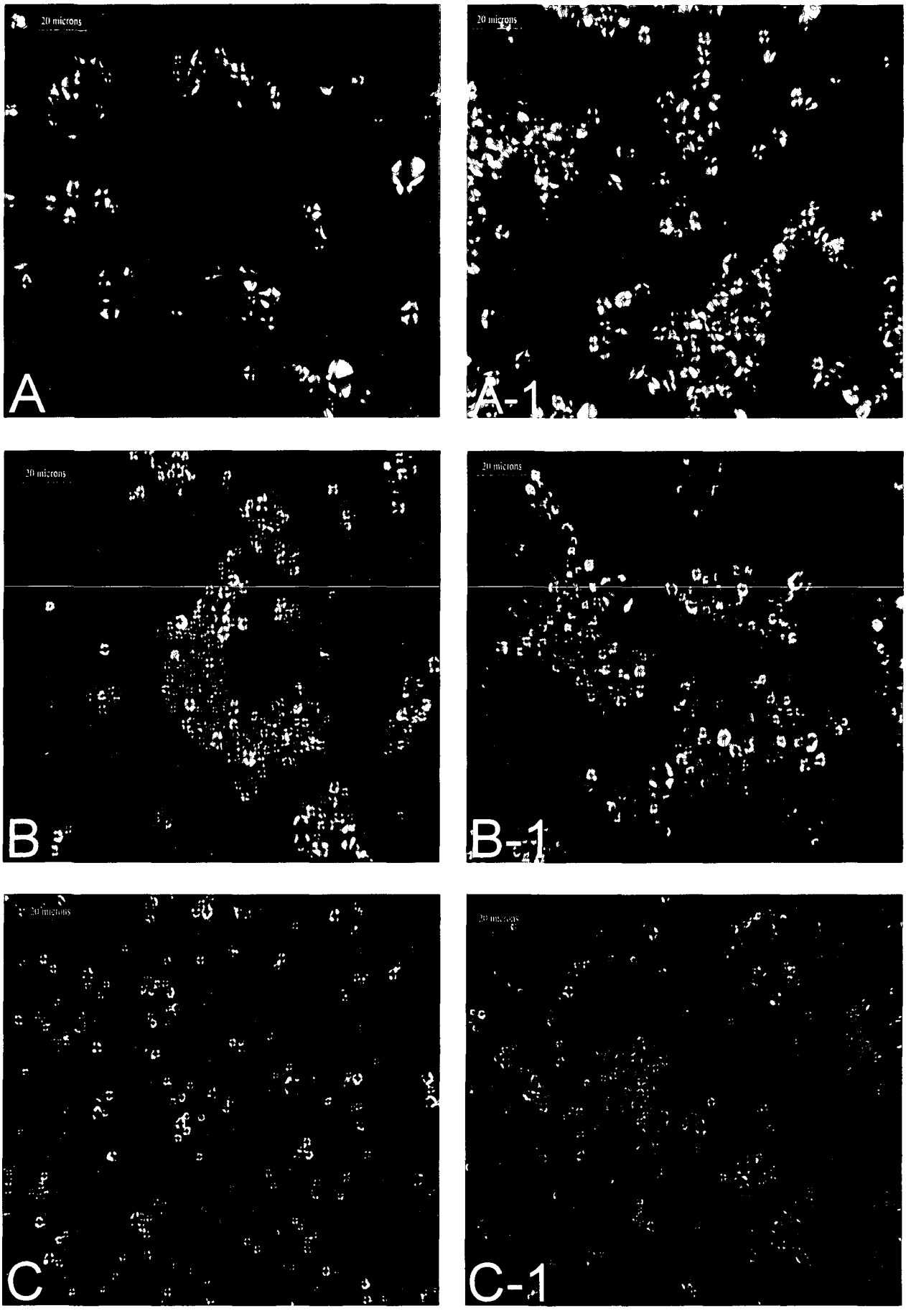
Native starches

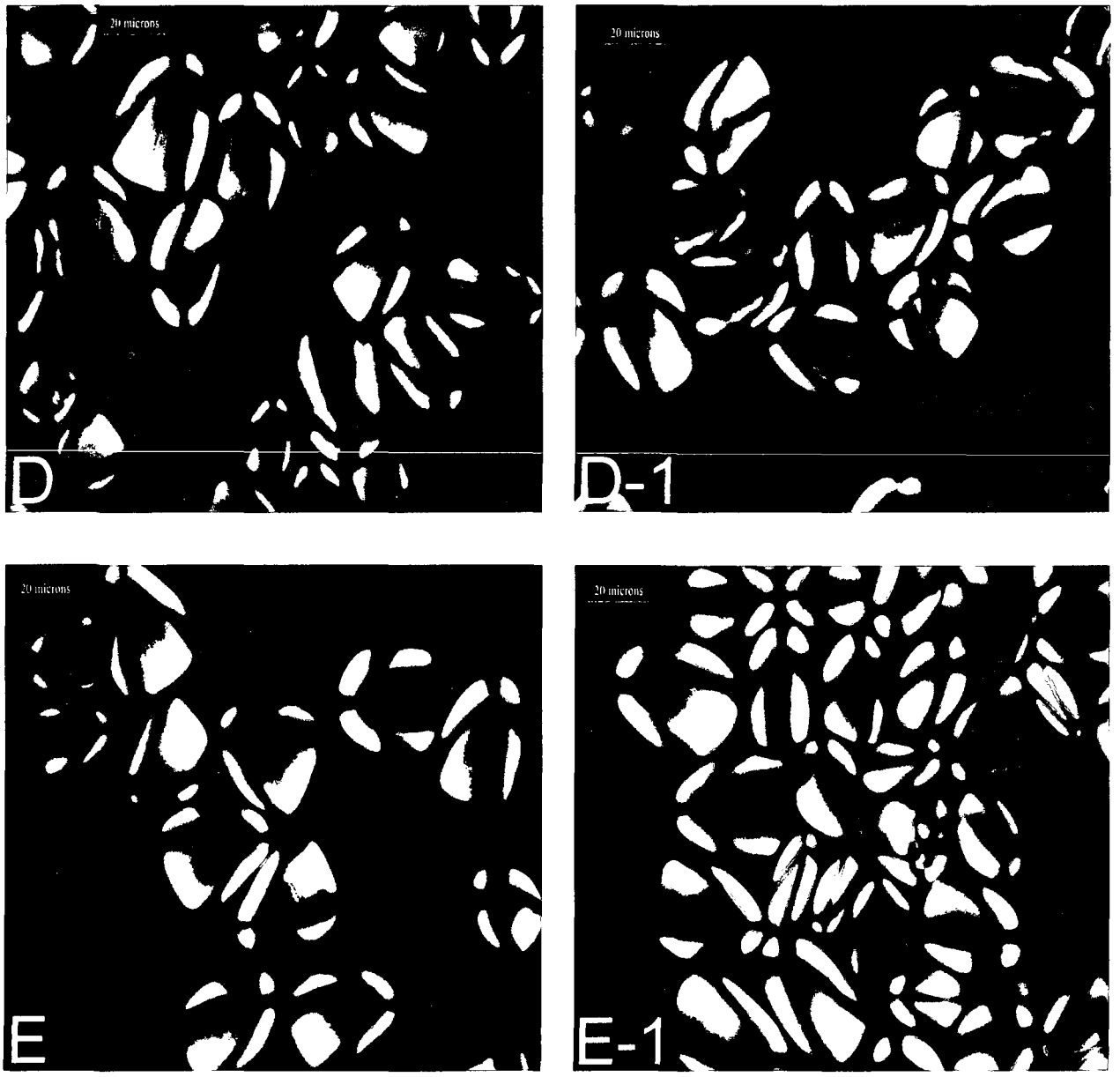
Dioscorea esculenta	*Dioscorea alata*
(A) kukulala	**(D)** hingurala
(B) java-ala	**(E)** raja-ala
(C) nattala	

Annealed starches

Dioscorea esculenta	*Dioscorea alata*
(A-1) kukulala	**(D-1)** hingurala
(B-1) java-ala	**(E-1)** raja-ala
(C-1) nattala	

(Scale bar represents 20 μm)


20 microns
A
20 microns
A-1
20 microns
B
20 microns
B-1
20 microns
C
20 microns
C-1


20 microns
D
20 microns
D-1
20 microns
E
20 microns
E-1

4.2 Chemical composition

The data on yield and composition are presented in **Table 4-1**. The purity of the starches was judged on the basis of composition [low ash 0.17-0.32%) and low nitrogen content (<0.03%)] and microscopic examination (absence of cell fragments and debris). The yield of starch from varieties of *D. esculenta* and *D. alata* ranged from 10.2 to 16.8% and 14.3 to 18.8%, respectively. This was within the range reported for most tuber starches (Moorthy 2002, Hoover 2001). The total phosphorus content of the starches from *D. esculenta* and *D. alata* ranged from 0.05 to 0.1% and 0.04 to 0.05%, respectively. The phosphorous content of all starches remained the same before and after defatting. This was indicative that phosphorus was present mainly in the form of monoester phosphate and/or inorganic phosphate. The total phosphorus content of *Dioscorea* starches were higher than those reported for sweet potato (0.023%), taro (0.021%), cassava (0.01%) kuzu (0.005%) and innala (0.02%) starches (Jayakody *et al.*, 2005, Srichuwong *et al.*, 2005c, Gunaratne & Hoover 2002, Lim *et al.*, 1994, Soni & Agarawal 1983). It was interesting to observe that the phosphorous content (0.10%) of one of the varieties (nattala) of *D. esculenta* was higher than that usually reported [**Table 2-7**] for tuber starches (0.006-0.091%). The total starch lipids (surface and bound) in varieties of native *D. esculenta* and *D. alata* starches ranged from 0.36 to 0.47% and 0.28 to 0.30%, respectively. These values were generally higher than those reported [**Table 2-6**] for most other *Dioscorea* (0.01-0.5%) and tuber and root (0.01-0.36%) starches, but were comparable to that reported for *D. alata* (0.5%), *D. ballophylla* (1.08%), kuzu

Table 4-1 Chemical composition (%) and granule morphology of native *Dioscorea* starches

Characteristics (%)	*D. esculenta*			*D. alata*	
	Kukulala	Java-ala	Nattala	Hingurala	Raja-ala
Starch yield (based on initial tuber weight)	16.81	10.21	12.22	14.25	18.80
Ash	0.17 ± 0.00[a]	0.22 ± 0.00[b]	0.32 ± 0.00[c]	0.13 ± 0.00[d]	0.17 ± 0.00[a]
Nitrogen	0.01 ± 0.00[a]	0.03 ± 0.00[b]	0.01 ± 0.00[b]	0.02 ± 0.00[c]	0.01 ± 0.00[d]
Phosphorous*	0.05 ± 0.00[a]	0.07 ± 0.00[b]	0.10 ± 0.00[c]	0.05 ± 0.00[a]	0.04 ± 0.00[d]
Lipid: Solvent extracted					
Chloroform-methanol[1]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.03 ± 0.00[b]	0.05 ± 0.00[c]	0.08 ± 0.00[d]
n-propanol-water[2]	0.39 ± 0.01[a]	0.35 ± 0.00[b]	0.44 ± 0.03[c]	0.25 ± 0.02[d]	0.20 ± 0.00[e]
Amylose content:					
Apparent[3]	20.38 ± 0.26[a]	16.19 ± 0.20[b]	15.58 ± 0.45[c]	24.73 ± 0.00[d]	29.29 ± 0.24[e]
Total[4]	23.97 ± 0.12[a]	20.07 ± 0.12[b]	19.98 ± 0.23[b]	26.98 ± 0.00[c]	31.02 ± 0.00[d]
Amylose complexed with lipids[5]	14.98[a]	19.33[b]	22.02[c]	8.34[d]	5.58[e]
Granule size range (μm)	8-10	4-5	3-4	30-40	35-45
Granule morphology	Polygonal	Polygonal	Polygonal	Truncated oval	Truncated spade

All data reported on dry basis and represent the mean of at least four replicates. Values followed by the same superscript in each row are not significantly different ($P<0.05$) by Tukey's HSD test.

[1] Lipids extracted by chloroform-methanol 2:1 (v/v) at 25°C (mainly unbound lipids)

[2] Lipids extracted by hot-n-propanol-water 3:1 (v/v) from the residue left after chloroform-methanol extraction (mainly bound lipids)

[3] Apparent amylose determined by iodine binding without removal of free and bound lipids

[4] Total amylose determined by iodine binding after removal of free and bound lipids

[5] $\frac{\text{Total amylose-apparent amylose}}{\text{Total amylose}} \times 100$

Phosphorus*- Phosphorus content remained the same before and after defatting

Starch damage was not detected for the native and annealed starches

(0.46%), arrowroot (0.31%), new cocoyam (0.30%), and canna (0.19%) starches [**Table 2-6**] (Karam *et al.*, 2006, Peroni *et al.*, 2006, Gunaratne & Hoover 2001, Soni *et al.*, 1990, Soni & Agarawal 1983). The total amylose content of native *D. esculenta* and *D. alata* varieties ranged from 19.98 to 23.97% and 26.98 to 31.02%, respectively. The above values were within the range (~10-36%) reported for the amylose content of native *Dioscorea* starches and for other tuber starches [**Table 2-2**]. Similar differences in amylose content between native starches from *D. esculenta* and *D. alata* have also been reported by other researchers (Karam *et al.*, 2006, Peroni *et al.*, 2006, Wang *et al.*, 2006, Amani *et al.*, 2004, Frietas *et al.*, 2004, Riley *et al.*, 2004, Farhat, *et al.*, 1999, Gallant *et al.*, 1982, Rašper & Coursey 1967). In tuber starches, the amount of lipid complexed amylose chains has been shown to range from 8.3 to 15.5% (Jayakody *et al.*, 2005, Gunaratne & Hoover 2002, Vasanthan & Hoover 1992). In this study, the lipid complexed amylose chains in varieties of native *D. esculenta* and *D. alata* ranged from 14.98 to 22.02% and 5.58 to 8.34%, respectively. Among the varieties of *D. esculenta*, kukulala showed the lowest content of lipid complexed amylose (14.98%) chains, in spite of its higher total amylose (23.97%) and bound lipid (0.39%) content. This suggests that most of the bound lipid in kukulala is probably trapped between amylose helices and/or between amylose (AM) and amylopectin (AMP) chains.

4.3 Amylopectin unit chain length distribution and average chain length

The chain length distribution and the average chain length ($\overline{CL}$) of amylopectins are presented in **Table 4-2**. Among the native *D. esculenta* starches, differences in DP 6-12, DP 13-24, DP 25-26 and $\overline{CL}$ 17.94-18.33% was marginal. However, the proportion of DP 37-50 was much higher in java-ala (4.16%) than in kukulala (3.17) and nattala [**Table 4-2**]. However, both *D. esculenta* and *D. alata* differed significantly from each other with respect to the proportion of short A chains (DP 6-12), medium chains (DP 25-36) and $\overline{CL}$ [**Table 4-2**]. Varities of both species did not exhibit the amylopectin chain length distribution characteristics of tuber (B-type) starches. Tuber starches have been shown to have a higher proportion of chains with DP > 37, a smaller proportion of chains with DP 6-12, and a larger $\overline{CL}$. For instance, in normal potato starch, DP > 37, DP 6-12 and $\overline{CL}$ have been reported to 28.6, 13.07 and 28.6%, respectively (McPherson & Jane 1999). However, in the native *D. esculenta* starches DP >37, DP 6-12 and $\overline{CL}$ ranged from 3.17 to 4.41, 24.57-25.85 and 17.93-18.33%, respectively. Whereas, the corresponding range for native *D. alata* starches were 4.46-4.87, 17.89-20.68 and 19.29-19.61%, respectively. There has been only one study (Srichuwong *et al.*, 2005a) on the amylopectin unit chain length distribution of *Dioscorea* starches. They showed that the proportion of unit chain length distribution of DP 6-8, 9-12, 13-24, 25-30 for *D. esculenta* were 11.6, 24.9, 56.2, and 7.3%, respectively. Whereas, the corresponding distributions for *D. alata* unit chain length were 3.9, 18.9, 67.5, 9.7%, respectively, but the variety of the starches were not specified. Hence, no comparison is possible.

Table 4-2 Branch chain length distribution & average chain length ($\overline{CL}$) of native and annealed *Dioscorea* starches

Starch source		% Distribution (DP_n)[1]				
		6-12	13-24	25-36	37-50	$\overline{CL}$[2]
	D. esculenta					
Kukulala						
	Native	25.85 ± 0.94	57.78 ± 0.02	13.19 ± 0.76	3.17 ± 0.16	17.94 ± 0.20
	Annealed	26.16 ± 0.96	57.63 ± 0.46	13.17 ± 0.60	3.04 ± 0.10	17.87 ± 0.11
Java-ala						
	Native	25.46 ± 2.33	56.55 ± 1.55	13.58 ± 1.23	4.41 ± 0.44	18.33 ± 0.20
	Annealed	25.90 ± 2.52	56.19 ± 1.55	13.53 ± 1.02	4.38 ± 0.06	18.27 ± 0.31
Nattala						
	Native	24.57 ± 1.95	59.64 ± 0.10	12.61 ± 1.64	3.17 ± 0.22	17.93 ± 0.41
	Annealed	25.33 ± 1.34	59.66 ± 0.73	12.05 ± 0.68	2.96 ± 0.08	17.73 ± 0.18
	D. alata					
Hingurala						
	Native	20.68 ± 2.45	57.40 ± 1.22	17.46 ± 1.01	4.46 ± 0.21	19.29 ± 0.39
	Annealed	20.97 ± 2.30	57.10 ± 1.11	17.30 ± 1.26	4.63 ± 0.08	19.31 ± 0.31
Raja-ala						
	Native	17.89 ± 3.41	59.76 ± 1.55	17.47 ± 1.72	4.87 ± 0.14	19.61 ± 0.52
	Annealed	18.32 ± 3.12	59.66 ± 1.57	17.03 ± 1.55	5.00 ± 0.01	19.58 ± 0.45

[1] DP_n : Indicates degree of polymerization

[2] Average chain length ($\overline{CL}$) calculated by $\Sigma(DP_n \times \text{peak area}_n)/\Sigma$ (peak area_n)

4.3.1 Effect of annealing on branch chain length distribution

The amylopectin branch chain length distribution remained unchanged in both *Dioscorea* species on annealing [**Table 4-2**]. This suggests that chain length elongation, hydrolysis or debranching of amylopectin chains did not occur on annealing. A similar finding was reported by Kohyama and Sasaki (2006) for potato, corn and wheat starches subjected to one step annealing at 20 and 50°C for 22h and by Tester *et al.*, (2005) for starches extracted from potato tubers stored at 15, 25 and 55°C (*in situ* annealing) for 7 days.

4.4 Powder X-ray diffraction and crystallinity

Tuber starches have been shown to exhibit a 'B' type X-ray diffraction pattern with reflections centered at 5.5-5.6°, 14.1°, 15.0°, 17.0°, 19.7°, 22.2° and 24° 2θ angles. Whereas, 'A' type starches (mainly cereals) exhibit reflections at 15.3°, 17.0°, 18.0°, 20.0° and 23.4° 2θ angles (Hizukuri *et al.*, 1983, Zobel 1988). In this study, granule crystallinity was determined at their maximum water absorption capacity, which was significantly different for each species [*D. esculenta* (30-35%) and *D. alata* (24-28%)] [**Figure 4-4**]. Starches of both species showed no significant change in moisture content during scanning. All three varieties of native *D. esculenta* starches, exhibited the 'B' type X-ray pattern [**Figure 4-4**].

Crystallinity of starches has been shown to increase with increase in hydration (Jayakody *et al.*, 2006, Buléon *et al.*, 1998, Cheetham & Tao 1998, Buléon *et al.*, 1987). The percentage crystallinity in the native *D. esculenta* starches followed

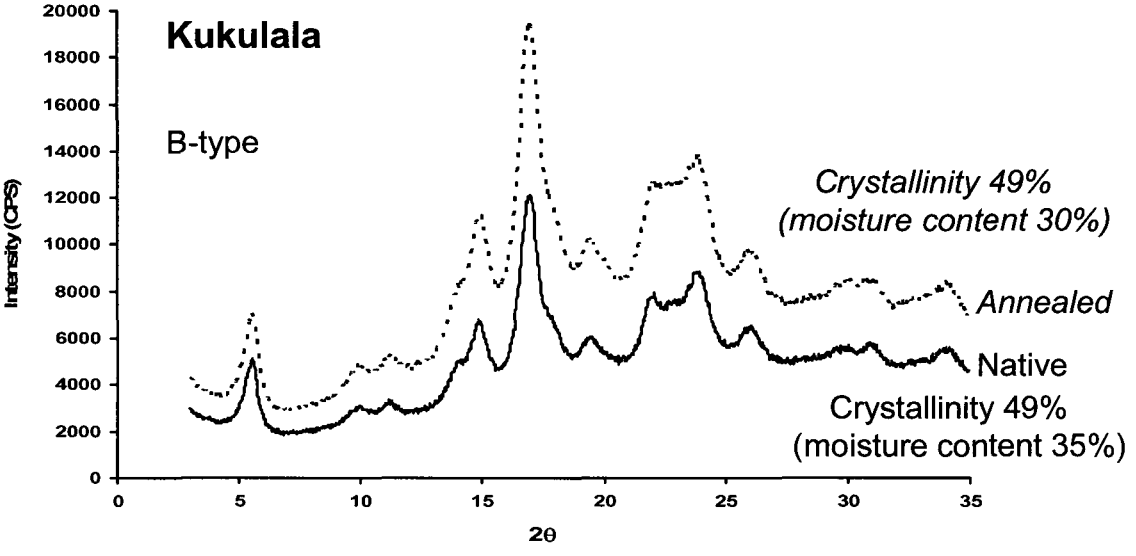
Figure 4-4 Polymorphic patterns and crystallinity (%) of *Dioscorea* starches

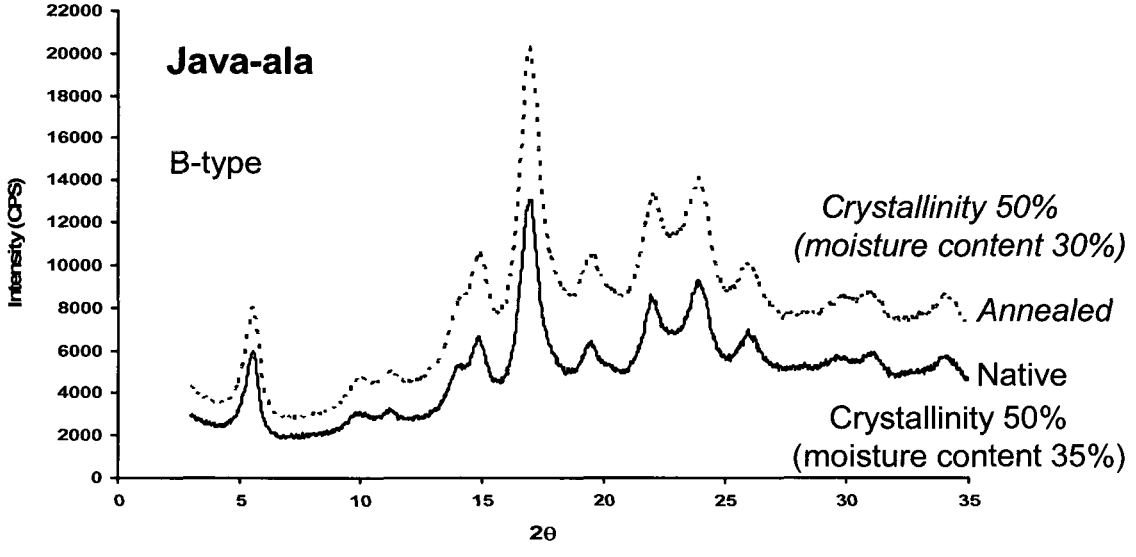
Native starches

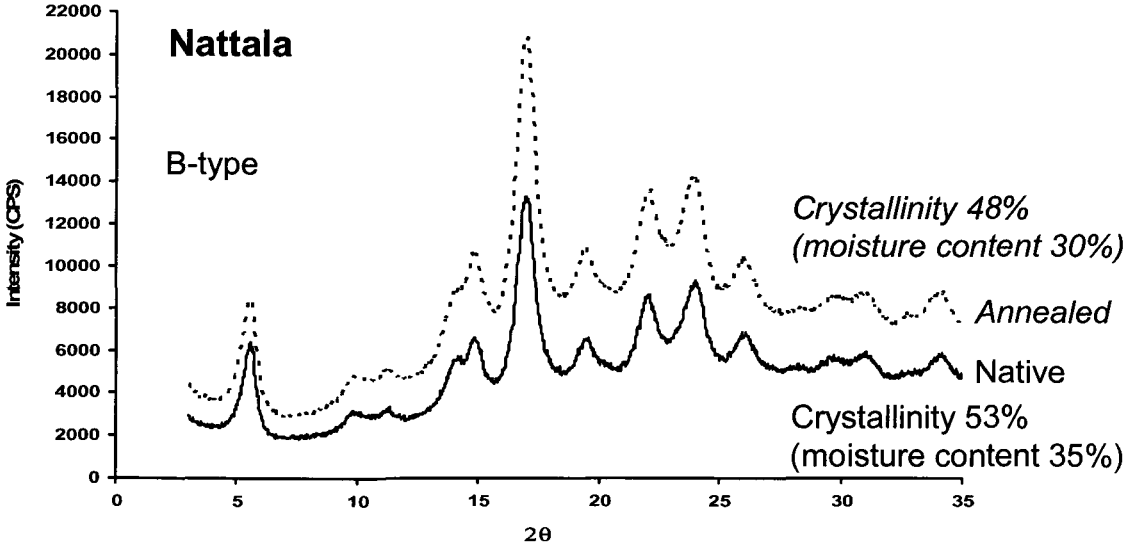
Dioscorea esculenta	*Dioscorea alata*
kukulala	hingurala
java-ala	raja-ala
nattala	

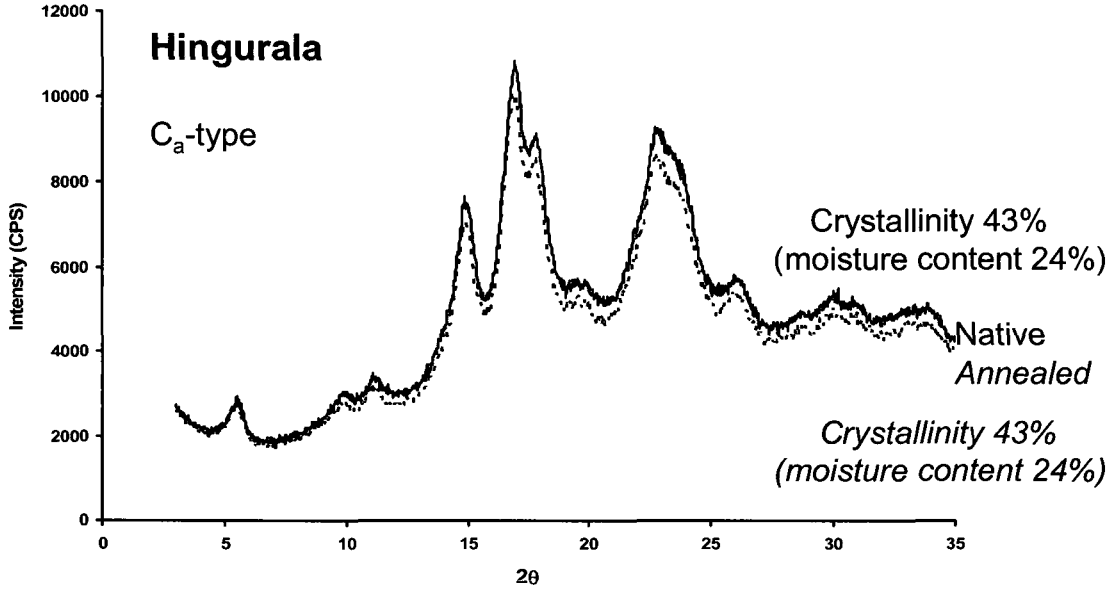
Annealed starches

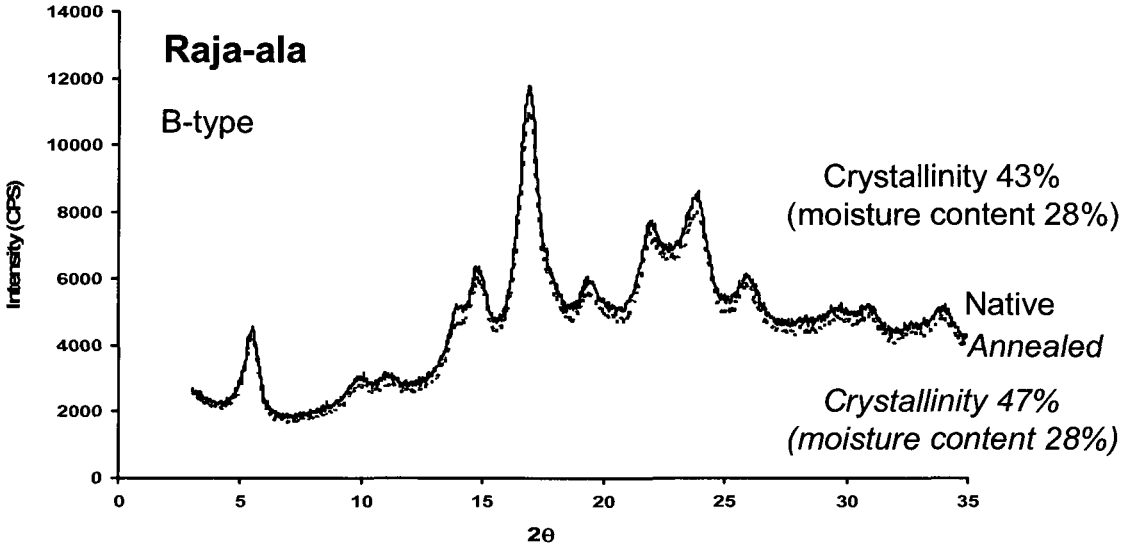
Dioscorea esculenta	*Dioscorea alata*
kukulala	hingurala
java-ala	raja-ala
nattala	


Kukulala
B-type
Crystallinity 49%
(moisture content 30%)
Annealed
Native
Crystallinity 49%
(moisture content 35%)
Intensity (CPS)
2θ


Java-ala
B-type
Crystallinity 50%
(moisture content 30%)
Annealed
Native
Crystallinity 50%
(moisture content 35%)
Intensity (CPS)
2θ


Nattala
B-type
Crystallinity 48%
(moisture content 30%)
Annealed
Native
Crystallinity 53%
(moisture content 35%)
Intensity (CPS)
2θ


Hingurala
C_a-type
Crystallinity 43%
(moisture content 24%)
Native
Annealed
Crystallinity 43%
(moisture content 24%)
Intensity (CPS)
2θ
0
2000
4000
6000
8000
10000
12000
0
5
10
15
20
25
30
35


Raja-ala
B-type
Crystallinity 43%
(moisture content 28%)
Native
Annealed
Crystallinity 47%
(moisture content 28%)
Intensity (CPS)
2θ
0
2000
4000
6000
8000
10000
12000
14000
0
5
10
15
20
25
30
35

the order: nattala > java-ala > kukulala. Starch crystallinity has been shown to be influenced by: (1) crystallite size, (2) orientation of the double helices (within the crystallites) to the X-ray beam, (3) the number of crystallites that are properly aligned to diffract the X-ray beam, (4) shape of the crystallites, (5) interaction between crystallites, (6) direction of crystal growth, (7) extent of packing of the double helices within the crystalline lamella, (8) average chain length of amylopectin, (9) ratio of short chain to long chain fraction of amylopectin, (10) amylose content, (11) extent of disruption of amylopectin crystallites by amylose, (12) starch moisture content, (13) botanical source, (14) pretreatments and (15) diffractometer settings (Jayakody *et al.*, 2006, Jayakody *et al.*, 2005, Gunaratne & Hoover 2002, Cheetham & Tao 1998, Hoover & Vasanthan 1994a, Stute 1992). The higher degree of crystallinity exhibited by native nattala starch suggests interplay of the following factors: (1) presence of larger crystallites, (2) larger number of crystallites (3) a more highly ordered crystalline structure, and (4) better orientation of the crystallites to the X-ray beam. This seems plausible, since differences in equilibrated moisture content [**Figure 4-4**], amylose content [**Table 4-1**] and amylopectin average chain length [**Table 4-2**] between nattala, kukulala and java-ala (*D. esculenta*) are too small to make any significant impact on starch crystallinity.

Mukrbeck *et al.*, (1991) have shown by studies on native potato starches containing different levels of total phosphate, that the degree of crystallinity is reduced by high phosphate levels. They postulated that this could be due to dislocations in the amylopectin clusters induced by the bulky phosphate groups

interfering with the build up of the structure during biosynthesis. On this basis, native nattala starch having the highest phosphate level among the native *D. esculenta* starches [**Table 4-1**] should have exhibited the lowest level of crystallinity. The results [**Table 4-1**] suggests that in nattala, the effect of phosphate on crystallinity may have been negated by the interplay of the factors such as number and size of the crystallites, a highly ordered crystalline structure and their orientation to the X-ray beam.

In the native *D. alata* starches, only raja-ala exhibited a 'B' type X-ray diffraction pattern. Whereas, hingurala exhibited a 'C'-type pattern. Hizukuri *et al.* (1960) classified the 'C'-type spectrum into C_a, C_b and C_c based on the extent of their resemblance to 'A' and 'B' type or between the two types, respectively (section 2.3.6.1.2.2.1.1). On this basis, the X-ray spectra of hingurala could be classified as a 'C_a' type. Both native hingurala and raja-ala exhibited the same level (43%) of crystallinity [**Figure 4-4**].

All starches exhibited a peak at ~2θ=19.4° [**Figure 4-4**] which has been shown to be indicative of the presence of crystalline V-amylose-lipid complexes (Biliaderis & Galloway 1989, Zobel 1988, Hoover & Hadizyev 1981). It was interesting to observe, that the intensity of this peak in both *D. esculenta* and *D. alata* starches increased with increase in the amount of lipid complexed amylose chains [**Table 4-1**]. However, it must be borne in mind, that differences in the intensity of the V-amylose-lipid complex peak among the starches may also reflect the extent to which the V-amylose-lipid complexes are organized into three dimensional

structures (long range order). It is difficult to compare the X-ray crystallinities of the *Dioscorea* starches [**Figure 4-4**] with those published in the literature for tuber starches (Shujun *et al.*, 2006a, Yusuph *et al.*, 2003, Vasanthan *et al.*, 1999, Buléon *et al.*, 1987, Nara & Komiya 1983, Nara *et al.*, 1978) for the following reasons: (1) crystallinities have not been determined at the maximum water absorption capacities of the starches, (2) crystallinity data have been published without any mention of the moisture content at which measurements were performed, (3) crystallinity of many tuber starches have been determined by calculating the proportion of crystallinity within the starch granules using as reference, materials with zero and 100% crystallinity. '0%' reference representing fully amorphous material (example-freeze-dried gelatinized starch). Whereas, 100% reference being generated by using quartz (Vasanthan 1994) or extensive acid hydrolysis of starch in which all the amorphous material has been eroded (Tester *et al.*, 2004). The crystallinity of some varieties of *D. esculenta* (kukulala, java-ala) and *D. alata* (hingurala) starches remained unchanged on annealing [**Figure 4-4**]. However, crystallinity decreased in nattala but increased in raja-ala starches on annealing [**Figure 4-4**].

In potato starch, crystallinity has been shown to remain unchanged on annealing (Tester *et al.*, 2005, Hoover & Vasanthan 1994a). McPherson and Jane (1999) have shown by ^{31}P NMR, that phosphorous in tuber starches (potato, waxy potato, yam, sweet potato) are mainly in the form of phosphate monoesters with minor amounts (<0.001%) as inorganic phosphate [**Table 2-7**]. Hizukuri (1996), Gracza (1965) and Schoch (1942) and have shown that phosphate monoesters

are found exclusively on amylopectin. Lim *et al.*, (1994) showed by ^{31}P NMR the absence of lipid phosphorous in several tuber and root starches [**Table 2-7**]. In the present study, the total phosphorus content remained unchanged on defatting [**Table 4-1**], implying that phosphorus in the *Dioscorea* starches are mainly in the form of phosphate monoesters esterified at the C-6 and C-3 positions in amylopectin. Since crystallinity is due mainly to amylopectin, the decrease in crystallinity observed in nattala starch on annealing [**Figure 4-4**] could be attributed to its phosphate monoester content (0.10%) [**Table 4-1**] being higher than those of the other *Dioscorea* starches (0.04 to 0.07%) [**Table 4-1**]. Perfection of the double helices (forming the crystalline structure) during annealing of nattala starch may have caused reorientation of the bulky phosphate groups (located within the crystalline region), resulting in crystallites being oriented in a crystalline array that may have been different to that present in native starch [**Figure 4-4**]. This would then explain the decreased crystallinity observed in annealed nattala starch [**Figure 4-4**].

Amylose leaching studies [Table 4-4] showed that interactions involving AM-AM and/or AM-AMP chains on annealing were more extensive in raja-ala than in the other starches. This suggests that the increase in crystallinity in raja-ala on annealing is due to the formation of additional crystallites (resulting from interactions between AM-AM and/or AM-AMP chains). The unchanged crystallinity exhibited by java-ala, kukulala and hingurala starches on annealing [**Figure 4-4**] suggests that new crystallites may have formed on annealing, but

are either smaller in number and/or in size or are improperly oriented to make a significant impact on crystallinity.

4.5 Gelatinization parameters

The gelatinization temperatures, [onset (T_o), mid point (T_p) and conclusion (T_c)], gelatinization temperature range (T_c-T_o) and gelatinization enthalpy (ΔH) are presented in **Table 4-3**. T_o, T_p, T_c, T_c-T_o and ΔH of native *D. esculenta* starches were lower than those of native *D. alata* starches [**Table 4-3**]. Among native *D. esculenta* starches, java-ala and nattala exhibited only marginal differences in T_o, T_p, T_c, T_c-T_o and ΔH. In kukulala, T_o and T_p were similar to those of nattala and java-ala, however, T_c, T_c-T_o and ΔH were slightly higher [**Table 4-3**]. In the *D. alata* starches, all of the above gelatinization parameters were higher in hingurala [**Table 4-3**]. It is not possible to compare the above results with published data, due to differences in starch: water ratio, heating rate and other differences in methodology [**Table 2-8**]. Complicating matters is the fact that in many studies the varieties have not been specified. Tester (1997) postulated that gelatinization parameters are controlled, in part, by the molecular structure of amylopectin, starch composition and granular architecture. Noda *et al.* (1996) showed, by studies on wheat and sweet potato starches, that DSC parameters are influenced by the molecular architecture of the crystalline region, which corresponds to the distribution of amylopectin short chains (DP 6-11) and not by the proportion of crystalline region which corresponds to the amylose to amylopectin ratio. The

Table 4-3 Gelatinization parameters of native and annealed *Dioscorea* starches

Starch source	Gelatinization parameters[1]				
	T_o (°C)[2]	T_p (°C)[2]	T_c (°C)[2]	T_c-T_o (°C)[3]	ΔH (J/g)[4]
D. esculenta					
Kukulala					
Native	72.30 ± 0.20[a]	75.73 ± 0.15[a]	85.40 ± 0.50[a]	13.10	18.07 ± 0.10[a]
Annealed	75.15 ± 0.06[b]	78.18 ± 0.17[b]	86.33 ± 0.10[b]	11.18	19.09 ± 0.05[b]
Java-ala					
Native	72.55 ± 0.07[a]	75.00 ± 0.00[c]	82.00 ± 0.00[c]	9.45	17.32 ± 0.00[c]
Annealed	74.50 ± 0.25[c]	77.11 ± 0.15[d]	83.48 ± 0.38[d]	8.98	17.57 ± 0.04[d]
Nattala					
Native	72.45 ± 0.07[a]	75.60 ± 0.15[a]	82.25 ± 0.35[c]	9.80	17.90 ± 0.06[e]
Annealed	74.51 ± 0.25[c]	77.79 ± 0.47[e]	83.07 ± 0.32[d]	8.56	18.05 ± 0.05[a]
D. alata					
Hingurala					
Native	78.17 ± 0.06[d]	85.13 ± 0.06[f]	92.70 ± 0.06[e]	14.53	18.98 ± 0.09[f]
Annealed	80.50 ± 0.16[e]	85.58 ± 0.05[g]	93.00 ± 0.08[e]	12.50	19.45 ± 0.19[g]
Raja-ala					
Native	75.45 ± 0.07[b]	78.40 ± 0.14[b]	85.70 ± 0.28[a]	10.25	18.60 ± 0.00[h]
Annealed	78.55 ± 0.13[f]	80.68 ± 0.15[h]	87.25 ± 0.55[f]	8.70	18.74 ± 0.05[i]

All data reported on dry basis & represent the mean of at least four replicates. Values followed by the same superscript in each column is not significantly different (P<0.05) by Tukey's HSD test.

[1]Starch: water ratio = 1:3 (w/w dry basis).
[2]T_o, T_p, T_c, indicate the temperature of the onset, midpoint and end of gelatinization, respectively.
[3]T_c-T_o indicates the gelatinization temperature range.
[4]Enthalpy of gelatinization ΔH (J/g)

above authors showed that a low T_o, T_p, T_c and ΔH values reflect the presence of abundant short amylopectin chains, this suggests that the higher proportion of DP 6-12 chains [**Table 4-2**] in the *D. esculenta* starches may have been mainly responsible for their gelatinization parameters being lower than those of the *D. alata* starches [**Table 4-3**]. This seems plausible, since the difference in gelatinization parameters (*D. alata* > *D. esculenta*) cannot be explained in terms of differences in the amount of: (1) lipid complexed amylose chains [**Table 4-1**], (2) amylose content [**Table 4-1**], (3) total phosphorus content [**Table 4-1**] or (4) granule crystallinity [**Figure 4-4**]. The influence of the proportion of DP 6-12 chains on gelatinization parameters was also evident among varieties of native *D. esculenta* starches [**Table 4-2**]. For instance, the small difference in the gelatinization parameters [**Table 4-3**] could be attributed to the absence of significant differences in the proportion of DP 6-12 chains among the native *D. esculenta* starches [**Table 4-2**]. Whereas, in the native *D. alata* starches, the differences in T_o, T_p, T_c and ΔH between native hingurala and raja-ala [**Table 4-3**] indicate that interactions between amylopectin chains are stronger in hingurala. This seems plausible, since differences in composition [**Table 4-1**], crystallinity [**Figure 4-4**] and proportion of DP 6-12 chains [**Table 4-2**] between these starches are significant.

Annealing increased T_o, T_p, T_c and decreased T_c-T_o in all starches [**Table 4-3**]. Similar changes on annealing has also been observed in potato [**Table 2-14**] (Tester *et al.* 2005, Genkina *et al.* 2004a, Jacobs *et al.* 1998a,b,c, Hoover & Vasanthan 1994a), sweet potato (Genkina *et al.*, 2004b) and cassava (Gomes *et*

al., 2004) starches. Increase in T_o, T_p and T_c has been attributed to perfection of pre-existing crystallites. The increase in crystal perfection on annealing has been attributed to lengthening of amylopectin pre-existing double helices caused by coiling of previously uncoiled ends and (Tester *et al.*, 2005, Genkina *et al.*, 2004a) to the improvement of their registration (Hoover & Vasanthan 1994a). In general, the extent of increase in T_o, T_p and T_c on annealing was nearly the same for all *Dioscorea* starches [**Table 4-3**]. T_c-T_o reflects variations in crystalline stability; annealing has been shown to minimize these variations (Hoover & Vasanthan 1994a). Annealing decreased T_c-T_o in all starches [**Table 4-3**]. The extent of this decrease followed the order: hingurala > kukulala > raja-ala > nattala > java-ala [**Table 4-3**]. This was not surprising, since the largest and smallest variations in crystalline stability were present in native hingurala and java-ala starches, respectively [**Table 4-3**]. The ΔH of all *Dioscorea* starches increased slightly on annealing (kukulala > hingurala > java-ala > nattala > raja-ala) [**Table 4-3**]. The extent of this increase although marginal was significant ($P < 0.05$). Marginal increases in ΔH have also been observed in annealed potato (Tester *et al.*, 2005, Genkina *et al.*, 2004a, Hoover & Vasanthan 1994a), sweet potato (Genkina *et al.*, 2004b) and cassava (Gomes *et al.*, 2004) starches. The increase in ΔH could be attributed to crystal perfection and/or to melting of crystallites formed between AM-AM and/or AM-AMP chains during annealing.

4.6 Amylose leaching (AML) and swelling factor (SF)

The extent of AML and SF in the temperature range 60-90°C are presented in **Tables 4-4** & **4-5**, respectively. AML has been shown to be influenced by total amylose content, extent of interaction between amylose-amylose (AM-AM) and/or amylose-amylopectin (AM-AMP) chains within the native granule and on the amount of lipid complexed amylose chains (Jayakody *et al.*, 2005, Nakazawa & Wang 2003, Gunaratne & Hoover 2002, Hoover & Vasanthan 1994a). In all native starches, AML increased with temperature. *D. alata* starches exhibited AML only at temperatures exceeding 80°C [**Table 4-4**]. Whereas, AML occurred at lower temperatures (<80°C) in the *D. esculenta* starches [**Table 4-4**]. This suggests that interactions between AM-AM and/or AM-AMP chains are stronger in the native *D. alata* starches. At temperatures beyond 85°C [**Table 4-4**], the extent of AML in native *D. alata* starches (raja-ala> hingurala) was much higher than in the native *D. esculenta* (java-ala > kukulala > nattala) starches [**Table 4-4**]. This is indicative of the lower amylose content [**Table 4-1**] in the latter. The results suggest that the extent of AML among the native *D. esculenta* and between native *D. alata* starches is influenced by the interplay of differences in: (1) percentage of lipid complexed amylose chains [**Table 4-1**], (2) total amylose content [**Table 4-1**] and (3) the extent of interaction between AM-AM and/or AM-AMP chains. The extent of AML in the *Dioscorea* starches was much lower than those reported for potato, true yam, taro and new cocoyam starches (Gunaratne & Hoover 2002). For instance, at 80°C, the extent of AML in the *Dioscorea* starches was in the range 0.55 to 2.16% [**Table 4-4**]. Whereas, at the same

Table 4-4 Amylose leaching (%) of native and annealed *Dioscorea* starches in the temperature range 60-90°C

Variety	60°C	70°C	80°C	85°C	90°C
D. esculenta					
Kukulala					
Native	0.00 ± 0.00[a]	0.69 ± 0.00[a]	1.94 ± 0.03[a]	3.88 ± 0.02[a]	5.75 ± 0.07[a]
Annealed	0.00 ± 0.00[a]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	1.69 ± 0.05[b]	2.35 ± 0.09[b]
Java-ala					
Native	0.00 ± 0.00[a]	0.92 ± 0.02[c]	2.16 ± 0.06[c]	4.97 ± 0.01[c]	6.19 ± 0.05[c]
Annealed	0.00 ± 0.00[a]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	2.29 ± 0.00[d]	3.29 ± 0.02[d]
Nattala					
Native	0.00 ± 0.00[a]	0.46 ± 0.04[d]	1.55 ± 0.08[d]	2.85 ± 0.02[e]	5.58 ± 0.01[e]
Annealed	0.00 ± 0.00[a]	0.00 ± 0.00[b]	0.00± 0.00[b]	1.16 ± 0.08[f]	2.05 ± 0.06[f]
D. alata					
Hingurala					
Native	0.00 ± 0.00[a]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	6.76 ± 0.04[g]	13.20 ± 0.07[g]
Annealed	0.00 ± 0.00[a]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	1.94 ± 0.03[h]	5.92 ± 0.00[h]
Raja-ala					
Native	0.00 ± 0.00[a]	0.00 ± 0.00[b]	0.55 ± 0.01[e]	8.63 ± 0.05[i]	13.60 ± 0.09[i]
Annealed	0.00 ± 0.00[a]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[j]	1.17 ± 0.00[j]

All data reported on dry basis and represent the mean of three replicates. Values followed by the same superscript in each column are not significantly different ($P<0.05$) by Tukey's HSD test.

temperature, the values reported for native potato, true yam, taro and new cocoyam starches were 22.0, 13.0, 22.1, and 2.9%, respectively (Gunaratne & Hoover 2002). The large difference in AML between *Dioscorea* and other tuber starches cannot be attributed total amylose content (TAC) or to the amount of lipid complexed (LCA) chains. For instance, raja-ala starch with a higher TAC (31.0%) [**Table 4-1**] and a lower LCA (5.6%) [**Table 4-1**] than potato, true yam, taro and new coco yam starches (TAC 26.4-28.1%, LCA, 10.4-15.5%) (Gunaratne & Hoover 2002) exhibited no AML at 80°C. This suggests that interaction between AM-AM and /or AM-AMP chains within native granules are much stronger in *Dioscorea* than in other tuber starches.

In all starches, AML decreased on annealing [**Table 4-4**]. The decrease in AML on annealing has been attributed to: (1) interaction between amylose chains, (2) decrease in granular swelling, and (3) increase in V-amylose-lipid content (Tester *et al.*, 2000, Hoover & Vasanthan 1994a,b). The extent of decrease in AML at 90°C on annealing was more pronounced in the *D. alata* (raja-ala > hingurala) than in the *D. esculenta* (nattala ~ kukulala > java-ala) starches. This indicates stronger interactions between AM-AM and/or AM/AMP chains during annealing of *D. alata* starches. It is likely, that due to their higher amylose content [**Table 4-1**], the amylose chains in native *D. alata* starches are more compactly packed than in the native *D. esculenta* starches. Consequently, interactions between AM-AM and/or AM-AMP chains on annealing will be more pronounced in the former. The extent of reduction in AML on annealing in the *Dioscorea* starches was much higher than that reported for potato starch (Hoover & Vasanthan 1994a). For

instance, at 80°C, AML was zero (decreased by 100%) in all annealed *Dioscorea* starches. Whereas, at the same temperature, the difference between native and annealed potato starch (Hoover & Vasanthan 1994a) was 2.1%. This large difference in AML reduction between the *Dioscorea* starches and potato starch was rather surprising, since the extent of interaction between AM-AM and/or AM-AMP chains during annealing should have been more pronounced in potato starch since its amylopectin average chain length ($\overline{CL}$ =28.1, Gunaratne & Hoover 2002) and degree of polymerization (DP) of amylose (DP = 4850 Gunaratne & Hoover 2002) is longer than those of the native *Dioscorea* starches ($\overline{CL}$ = 17.6 -19.5) **[Table 4-2]**, DP 1800-2000 (Gunaratne & Hoover 2002, Suzuki *et al.*, 1986). This suggests, that it is the arrangement of amylose chains (loose or compactly packed) rather than the $\overline{CL}$ and DP of amylopectin and amylose, respectively, that has the greatest influence on the extent of interaction between AM-AM and /or AM/AMP chains during annealing. The greater relative reduction in AML in the annealed *Dioscorea* starches is indicative of amylose chains in the native *Dioscorea* starches being more compactly packed than in native potato starch.

Swelling factor (SF) also increased with increase in temperature [**Table 4-5**]. At all temperatures, SF was lower in native *D. alata* starches. SF has been shown to be influenced by: (1) phosphate monoester content (Jayakody *et al.*, 2005, Srichuwong *et al.*, 2005c, Noda *et al.*, 2004, Suzuki *et al.*, 1994, Galliard & Bowler 1987), (2) amount of lipid complexed amylose chains (Jayakody *et al.*, 2005, Hoover & Manuel, 1995, Tester & Morrison 1990, Swinkels 1985a, Hoover

Table 4-5 Swelling factor of native and annealed *Dioscorea* starches in the temperature range 60-90°C

Variety	60°C	70°C	80°C	85°C	90°C
D. esculenta					
Kukulala					
Native	3.27 ± 0.20[a]	7.86 ± 0.00[a]	32.52 ± 0.42[a]	43.31 ± 0.00[a]	53.61 ± 0.11[a]
Annealed	0.00 ± 0.00[b]	1.99 ± 0.34[b]	19.77 ± 0.00[b]	24.88 ± 0.37[b]	31.97 ± 0.19[b]
Java-ala					
Native	3.50 ± 0.00[c]	11.85 ± 0.22[c]	35.45 ± 0.00[c]	45.73 ± 0.00[c]	54.29 ± 0.33[c]
Annealed	0.00 ± 0.00[b]	3.73 ± 0.54[d]	26.67 ± 0.75[d]	32.43 ± 0.47[d]	41.31 ± 0.30[d]
Nattala					
Native	4.55 ± 0.00[d]	18.22 ± 0.00[e]	39.00 ± 0.00[e]	51.69 ± 0.12[e]	64.97 ± 0.42[e]
Annealed	0.00± 0.00[b]	8.59± 0.54[f]	29.62 ± 0.63[f]	32.20 ± 0.65[f]	44.87 ± 0.70[f]
D. alata					
Hingurala					
Native	2.45 ± 0.35[e]	3.13 ± 0.00[g]	6.26 ± 0.19[g]	16.27 ± 0.13[g]	38.64 ± 0.00[g]
Annealed	0.00 ± 0.00[b]	0.00 ± 0.00[h]	3.99 ± 0.36[h]	7.57 ± 0.35[h]	21.68 ± 0.44[h]
Raja-ala					
Native	2.81 ± 0.50[e]	3.83 ± 0.00[i]	14.28 ± 0.00[i]	24.27 ± 0.13[i]	36.60 ± 0.00[i]
Annealed	0.00 ± 0.00[b]	0.00 ± 0.00[h]	6.16 ± 0.00[j]	11.65 ± 0.32[j]	20.55 ± 0.50[j]

All data reported on dry basis and represent the mean of at least four replicates. Values followed by the same superscript in each column are not significantly different ($P<0.05$) by Tukey's HSD test.

& Hadziyev 1981) and (3) granule crystallinity (Jayakody *et al.*, 2005). Differences in SF between native *D. alata* (raja-ala>hingurala) [**Table 4-5**] starches in the range 60-85°C can be attributed to the higher content of lipid complexed amylose chains [**Table 4-1**] and to a greater interaction between amylopectin chains [**Table 4-3**] in hingurala. This seems plausible, since differences in crystallinity [**Figure 4-4**] and total phosphorus content [**Table 4-1**] between hingurala and raja-ala were marginal. The reversal in SF at 90°C (hingurala>raja-ala) indicates that the number of hydrogen bonds (between amylopectin chains) that are destroyed in the temperature range 85-90°C is higher in hingurala (due to stronger interactions between amylopectin chains) than in raja-ala. This would the explain the steeper SF increase exhibited by hingurala in the temperature range 85-90°C thus stands explained.

The higher SF shown by native *D. esculenta* starches [**Table 4-5**] reflects the interplay of differences in: (1) crystallinity (*D. esculenta* > *D. alata* [**Figure 4-4**]), (2) total phosphorus content (*D. esculenta* > *D. alata* [**Table 4-1**]) and (3) extent of interaction between AM-AM and/or AM-AMP chains (*D. alata* > *D. esculenta* [**Table 4-4**]). SF differences among the native *D. esculenta* starches (kukulala, java-ala, nattala) reflects the interplay of differences in: (1) total phosphorus content (nattala > java-ala > kukulala [**Table 4-1**] (2) crystallinity (nattala > java-ala > kukulala [**Figure 4-4**] and (3) extent of interaction between AM-AMP and/or AM-AMP chains (nattala > kukulala > java-ala [**Table 4-4**]. The presence of lipid complexed amylose chains has been shown to be correlated to resistance to granular swelling (Tester & Morrison 1990a, Hoover & Hadziyev 1981), however,

it is unlikely that the observed differences in SF among the native *D. esculenta* starches is due to solely differences in the amount of lipid complexed amylose chains [**Table 4-1**], since nattala, having the highest content of lipid complexed amylose chains [**Table 4-1**] exhibited the highest SF at all temperatures [**Table 4-5**]. This suggests that the influence of amylose-lipid complexes on SF differences among the native *D. esculenta* starches is negated by crystallinity, total phosphorous content and glucan chain interactions.

At all temperatures, the SFs of the native *Dioscorea* starches were lower [**Table 4-5**] than that reported for potato starch (Hoover & Vasanthan 1994a) but were generally higher (with the exception of hingurala) than those reported for true yam, taro and new cocoyam starches (Gunaratne & Hoover 2002). For instance, at 80°C, the SFs of the all native *Dioscorea* starches ranged from 6.26 to 39.0 [**Table 4-5**]. Whereas, at the same temperature, potato, true yam, taro and new cocoyam exhibited a SF of 60, 26, 36, 18, respectively (Gunaratne & Hoover 2002). The difference in SF between potato and the *Dioscorea* starches could be attributed to the crystallinity of the native *Dioscorea* starches (43-53%) [**Figure 4-4**] being higher than that of potato starch 24-30% (Gunaratne & Hoover 2002, Cooke & Gidley 1992, Zobel 1988), and to amylose chains being more compactly packed in the *Dioscorea* starches.

In all starches, SF decreased on annealing [**Table 4-5**]. The extent of this decrease was more pronounced in the *D. alata* than in the *D. esculenta* starches. The higher extent of SF reduction in the *D. alata* starches, suggests that the

higher extent of AML reduction observed in the *D. alata* starches on annealing [**Table 4-4**] is also influenced by their lower SF that results from annealing. The results showed that the decrease in SF on annealing is influenced to a large extent by the interplay between the extent of crystalline perfection (a decreased T_c-T_o on annealing reflects an increase in crystalline perfection) [**Table 4-3**] and on the extent of interaction involving AM-AM and/or AM-AMP chains [**Table 4-4**]. Both crystalline perfection and AM-AM and/or AMP interactions would decrease hydration of the amorphous regions, thereby decreasing granular swelling. Decreased granular swelling on annealing has also been observed in potato starch (Hoover & Vasanthan 1994a, Kuge & Kitamura 1985). However, the extent of this decrease in annealed potato starch (Hoover & Vasanthan 1994a) is much lower than that observed in the annealed *Dioscorea* starches. For instance, at 80°C, annealing decreased SF in potato starch by 24.7%. Whereas, the corresponding decrease for the *Dioscorea* starches was in the range 29-54% [**Table 4-5**]. This indicates that the degree of interaction between AM-AM and/or AM-AMP chains during annealing is less pronounced in potato starch.

4.7 Pasting characteristics

The pasting properties of the *Dioscorea* starches measured using a rapid viscoanalyzer (RVA) are presented in **Figure 4-5**. Native *D. esculenta* starches exhibited higher peak viscosities, lower pasting temperatures, a greater degree of viscosity breakdown and lower set-back values than *D. alata* starches [**Figure 4-5**]. The native *D. alata* starches differed significantly from each other with respect to peak viscosity (raja-ala > hingurala), viscosity breakdown (hingurala

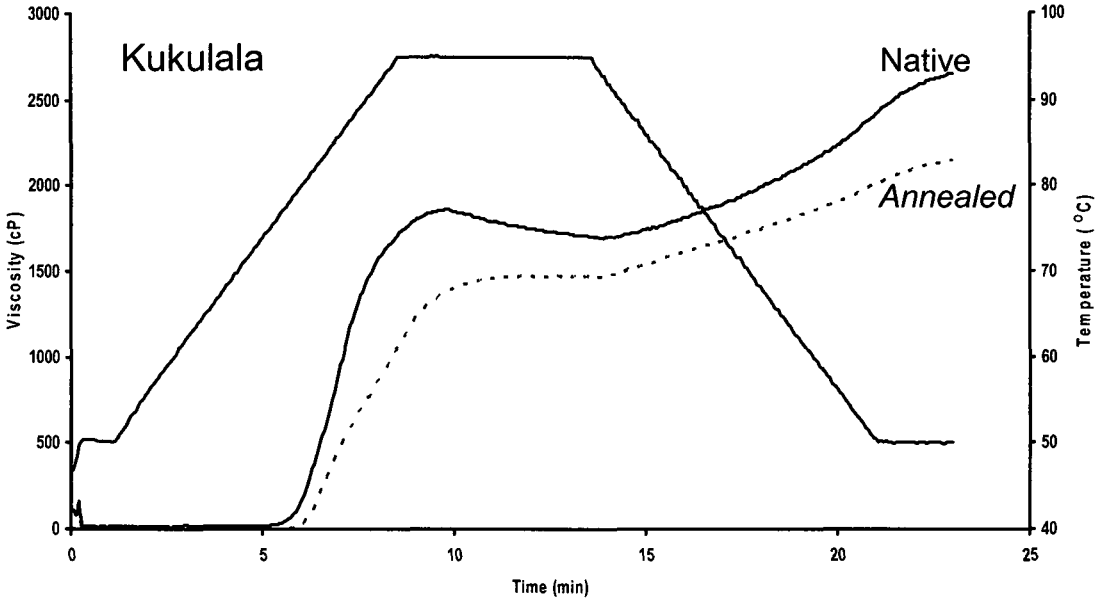
Figure 4-5 Pasting characteristics of *Dioscorea* starches

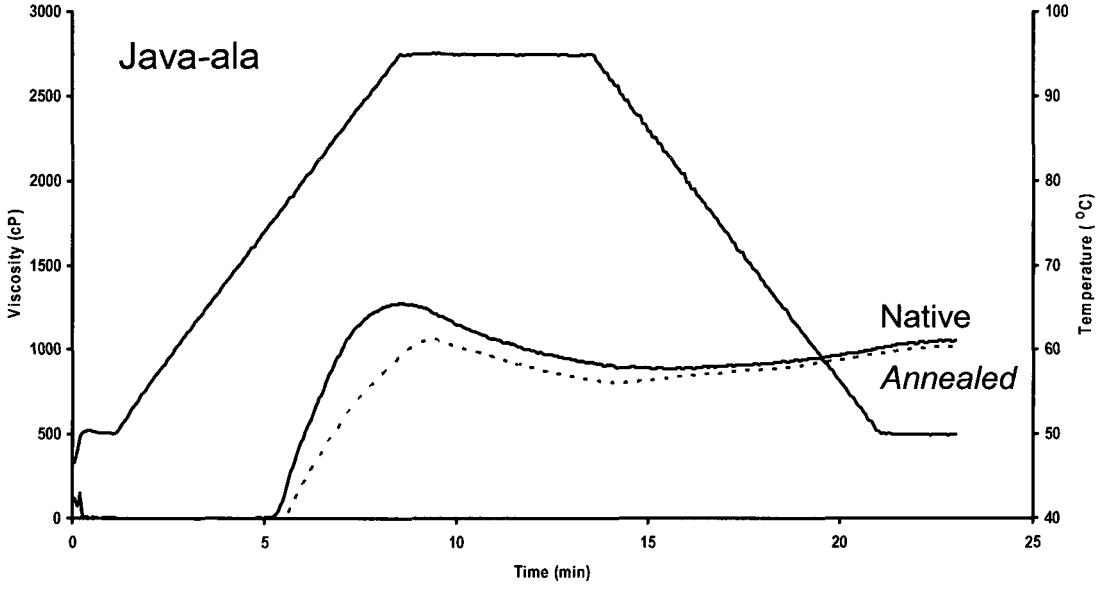
Native starches

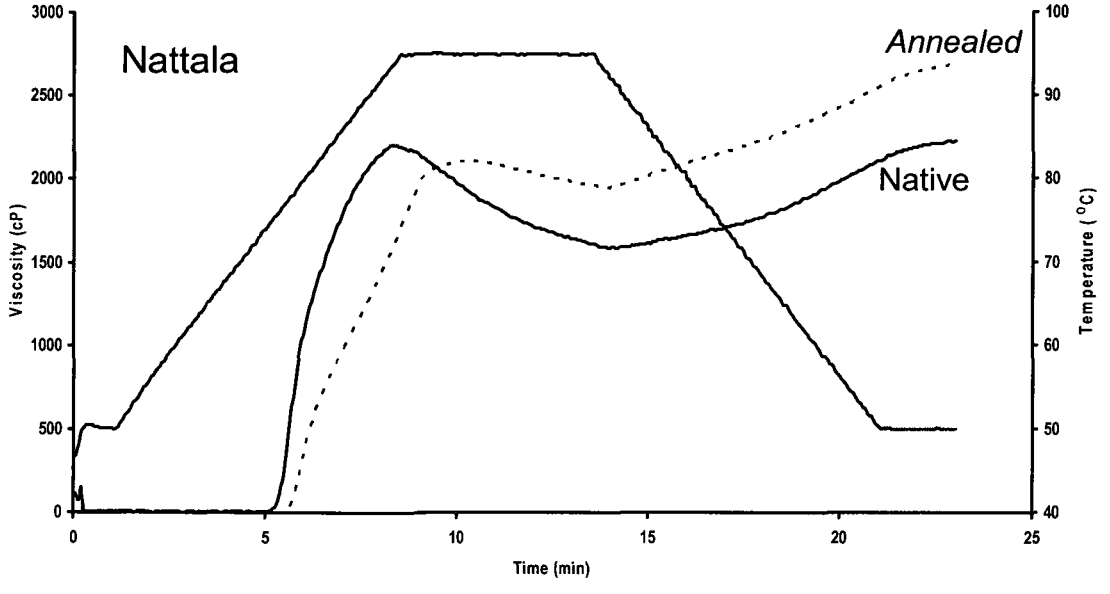
Dioscorea esculenta	*Dioscorea alata*
kukulala	hingurala
java-ala	raja-ala
nattala	

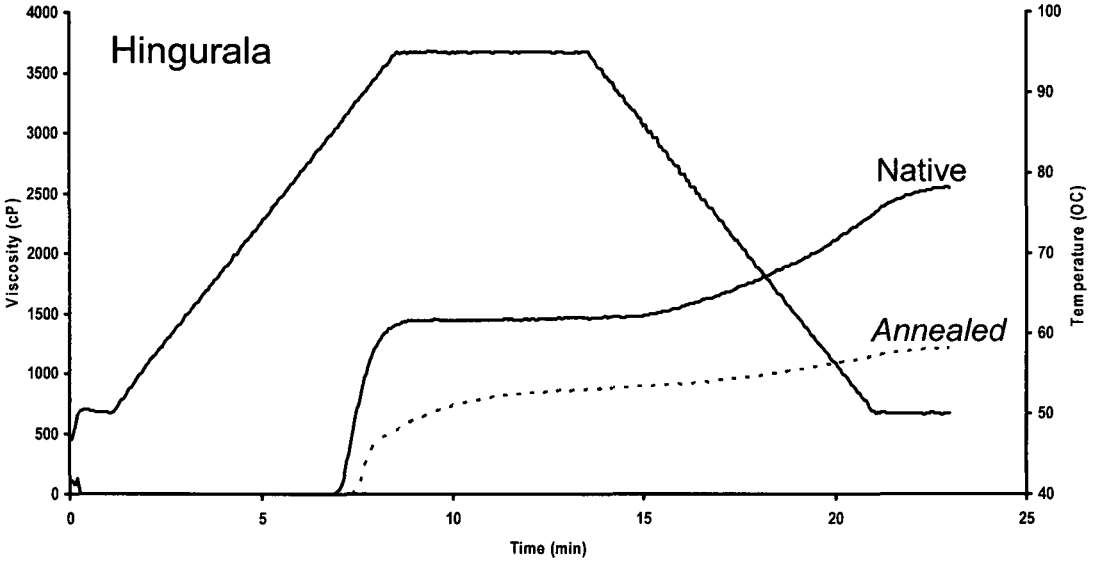
Annealed starches

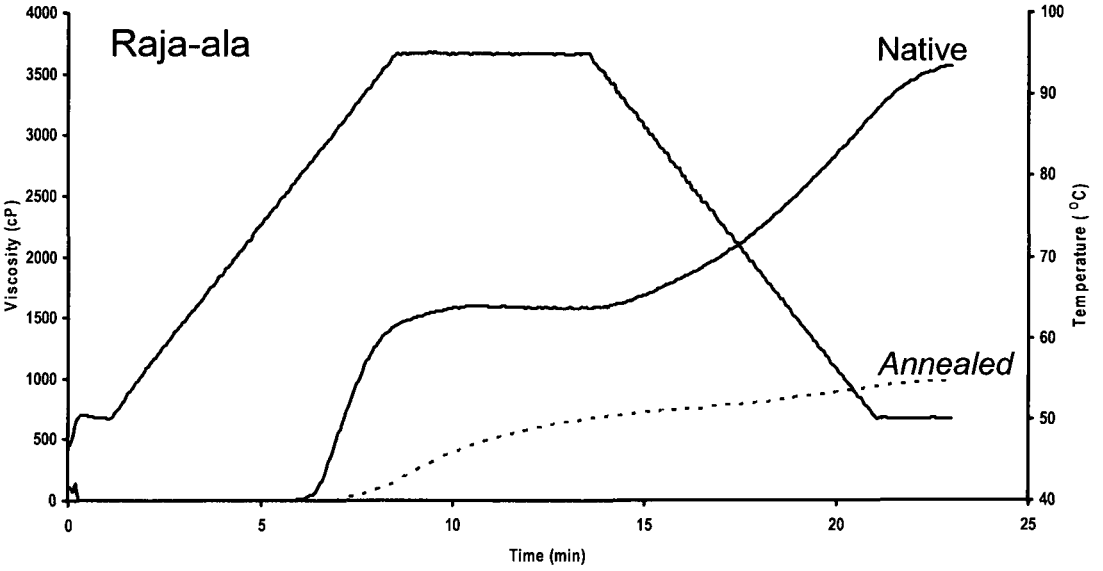
Dioscorea esculenta	*Dioscorea alata*
kukulala	hingurala
java-ala	raja-ala
nattala	


Kukulala
Native
Annealed
Viscosity (cP)
Temperature (°C)
Time (min)
3000
2500
2000
1500
1000
500
0
100
90
80
70
60
50
40
0
5
10
15
20
25


Java-ala
Native
Annealed
Viscosity (cP)
Temperature (°C)
Time (min)
3000
2500
2000
1500
1000
500
0
100
90
80
70
60
50
40
0
5
10
15
20
25


Nattala
Annealed
Native
Viscosity (cP)
Temperature (°C)
Time (min)
3000
2500
2000
1500
1000
500
0
100
90
80
70
60
50
40
0
5
10
15
20
25


Hingurala
Native
Annealed
Viscosity (cP)
Temperature (OC)
Time (min)
4000
3500
3000
2500
2000
1500
1000
500
0
100
90
80
70
60
50
40
0
5
10
15
20
25


Raja-ala
Native
Annealed
Viscosity (cP)
Temperature (°C)
Time (min)
4000
3500
3000
2500
2000
1500
1000
500
0
100
90
80
70
60
50
40
0
5
10
15
20
25

> raja-ala) set-back (raja-ala > hingurala) and pasting temperature (hingurala > raja-ala). Viscosity development during heating of starch granules in water has been attributed to the formation of tightly packed array of swollen deformable granules, to friction between swollen granules, and the amount of leached amylose (Singh *et al.*, 2006, Amani *et al.*, 2004, Gebre-Mariam & Schmidt 1998, Jacobs *et al.* 1995, Hoover & Vasanthan 1994b, Zeigler *et al.*, 1993, Lineback & Rašper 1988, Doublier *et al.*, 1987, Evans & Haisman 1979, Miller 1973, Schoch & Maywald 1968). The viscosity rise during cooling of a heated starch suspension has been attributed to interactions between leached amylose chains which leads to the formation of a gel (Miles *et al.*, 1985, Bowler *et al.*, 1980). The increase in viscosity on cooling is a measure of the retrogradation tendency of the starch. In the *D. alata* starches, difference in pasting temperatures between native hingurala and raja-ala suggests that bonding forces between glucan chains are stronger in the former. The higher peak viscosity exhibited by raja-ala could be attributed to its higher degree of amylose leaching [**Table 4-4**], greater swelling factor [**Table 4-5**] and larger granule size [**Table 4-1**]. Singh *et al.*, (2006) and Srichuwong *et al.*, (2005c) have postulated that starches with larger granules might occupy more volume and thus enhance viscosity. The higher resistance of granules of native raja-ala starch to viscosity breakdown (due to granule fragmentation) during the holding cycle at 95°C, can be attributed to its larger granule size [**Table 4-1**] and more extensive amylose leaching [**Table 4-4**]. It is likely, that the highly swollen raja-ala granules may have become resistant to shear due to granules becoming embedded within the amylose network during

the holding cycle. The higher set-back (mainly due to amylose gelation) shown by raja-ala could be attributed to its greater degree of amylose leaching and/or to more rigid unfragmented granules embedded within the amylose network.

The native *D. esculenta* starches differed significantly with respect to peak viscosity (nattala > kukulala > java-ala), viscosity breakdown during the holding cycle (nattala > java-ala > kukulala) and degree of set-back (kukulala > nattala > java-ala) [**Figure 4-5**]. Amylose leaching [**Table 4-4**] and swelling factor [**Table 4-5**] measurements showed that among the *D. esculenta* starches, interaction between glucan chains was stronger in nattala. This would then explain the ability of nattala starch to swell to a higher degree and attain a higher degree of viscosity during the heating cycle [**Figure 4-5**]. The extent of viscosity breakdown during the holding cycle (at 95°C) is more pronounced in nattala [**Figure 4-5**] due to susceptibility of the highly swollen granules [**Table 4-5**] to shear. Between native kukulala and java-ala starches, the extent of amylose leaching [**Table 4-4**] and granular swelling [**Table 4-5**] was more pronounced in the latter. Therefore, theoretically, the viscosity rise during the heating cycle [**Figure 4-5**] should have been higher in java-ala starch. It is likely, that the causative factor influencing the viscosity differences between java-ala and kukulala (kukulala > java-ala) starches may have been due to the large difference in granular size between the starches (kukulala > java-ala) [**Table 4-1**]. The viscosity rise during the heating cycle is higher in kukulala starch due to greater friction between swollen granules. The lower extent of viscosity breakdown and the high degree of set-back shown by kukulala starch can be attributed to its

larger granules becoming embedded in the leached amylose network. It is difficult to compare the RVA results obtained in this study with those reported for other tuber starches, due to differences in starch concentration and to the methodology (Brabender viscoamylogram, micro viscoanalyzer) used for determination of pasting characteristics. There are only two reports in the literature (Srichuwong *et al.*, 2005c, Amani *et al.*, 2004) where the RVA pasting characteristics of native *D. alata* and *D. esculenta* starches have been compared. However, the starch concentration used in the above studies were different 8% (Srichuwong *et al.*, 2005c), and 4% (Amani *et al.*, 2004) w/w [**Table 2-10**] and only a few RVA parameters were reported by Amani *et al.*, (2004). These studies showed that pasting temperatures, peak viscosity and final viscosity (at 50°C) of *D. alata* starches were higher than those of *D. esculenta* starches, and that *Dioscorea* starches were more resistant to viscosity breakdown than potato or cassava starches.

In the *D. esculenta* starches [**Figure 4-5**], peak viscosity decreased (kukulala > java-ala > nattala) on annealing. However, annealing increased peak time (kukulala > nattala > java-ala), pasting temperature (kukulala > nattala ~ java-ala) and thermal stability (kukulala-ala > nattala > java-ala). The set-back decreased on annealing in kukulala, but increased in both nattala and java-ala (nattala > java-ala) starches [**Figure 4-5**]. A similar trend was also exhibited by the *D. alata* starches [**Figure 4-5**], where annealing decreased peak viscosity (raja-ala > hingurala), but increased peak time (raja-ala > hingurala), pasting temperature (raja-ala > hingurala), and thermal stability (hingurala > raja-ala). Whereas, set-

back decreased in raja-ala, but increased in hingurala on annealing [**Figure 4-5**]. The decrease in peak viscosity and the increase in thermal stability and pasting temperature on annealing was generally higher in the *D. alata* than in the *D. esculenta* starches [**Figure 4-5**]. However, a similar comparison cannot be made between the starch species with respect to changes in set-back on annealing, since set-back decreased in raja-ala (*D. alata*) and kukulala (*D. esculenta*), but increased in java-ala (*D. esculenta*), nattala (*D. esculenta*) and hingurala (*D. alata*) starches. Similar changes in pasting properties on annealing has also been observed (Jacobs *et al.*, 1995, Hoover & Vasanthan 1994a, Stute 1992) in potato starch. The magnitude of the changes in pasting properties on annealing between potato and the *Dioscorea* starches cannot be compared due to differences in concentrations and on the type of instrument used for viscosity measurements.

In all the *Dioscorea* starches, the decrease in peak viscosity on annealing can be attributed to decreased swelling factor (SF) [**Table 4-5**] and amylose leaching (AML) [**Table 4-4**]. The effect of annealing on peak viscosity is more pronounced in the *D. alata* than in the *D. esculenta* starches [**Figure 4-5**] due to a greater reduction in SF [**Table 4-5**] and AML [**Table 4-4**] in the former. The increase in thermal stability exhibited by all the annealed *Dioscorea* starches [**Figure 4-5**] is mainly due to the decrease in SF [**Table 4-5**]. For instance, among the *D. esculenta* starches [**Figure 4-5**], the increase in thermal stability on annealing is more pronounced in kukulala starch, due to its greater decrease in SF [**Table 4-5**]. The thermal stability increase on annealing in java-ala is lower than in nattala

starch [**Figure 4-5**] due to a less pronounced decrease in SF for java-ala [**Table 4-5**]. The thermal stability increase on annealing is of nearly the same order of magnitude in raja-ala and hingurala, [**Figure 4-5**] due to a similarity in the extent of decrease in SF [**Table 4-5**] in both starches. The increased pasting temperatures exhibited by all *Dioscorea* starches on annealing is influenced by the decrease in SF [**Table 4-5**] and increased interaction between glucan chains (AM-AM, and/or AM-AMP) [**Table 4-4**]. This seems plausible, since the increase in pasting temperatures on annealing was more pronounced in the *D. alata* than in the *D. esculenta* starches.

An increase in the amount of leached amylose, large granule size and rigid swollen granules have been shown to increase the amount of set-back (viscosity at the end of the cooling cycle- trough viscosity [**Figure 4-5**]) (Jacobs *et al.*, 1995, Hoover & Vasanthan 1994a, Loh 1992). As shown earlier, the extent of decrease in SF on annealing varied widely among the *D. esculenta* starches, with a greater decrease in kukulala starch [**Table 4-5**]. On the other hand, variations in the extent of decrease in AML among these starches was less pronounced [**Table 4-4**]. In kukulala, the large decrease in SF [**Table 4-5**] combined with a minor decrease in AML [**Table 4-4**] may have mediated the effect of increased granule rigidity (increases set-back), thereby reducing net set-back in annealed kukulala [**Figure 4-5**]. The slight increase in set-back exhibited by annealed java-ala and nattala (nattala > java-ala) [**Figure 4-5**] starches, suggest that the extent of decrease in SF on annealing in the above starches was probably not large enough to negate the effect of increased granule rigidity on set-back. The extent

of increase in set-back is higher in nattala starch showed a higher decrease in SF than java-ala on annealing [**Table 4-5**]. In the *D. alata* starches, the extent of decrease in SF on annealing was nearly the same in both raja-ala and hingurala. However, the extent of decrease in AML was much higher in annealed raja-ala starch [**Table 4-4**]. This would then explain the decreased set-back in annealed raja-ala [**Figure 4-5**] starch.

4.8 Acid hydrolysis

The extent of hydrolysis (2.2M HCl, 35°C) of native and annealed *D. esculenta* and *D. alata* starches are presented in **Figure 4-6**. Starches from both of the above species did not exhibit the typical biphasic pattern (a faster rate of hydrolysis followed by a slower rate) during the time course (15 days) of hydrolysis [**Figure 4-6**]. The faster rate has been attributed to the hydrolysis of the amorphous domains (amorphous background and the thin amorphous lamella within the crystalline region) of the starch granule, whereas during the second stage, the crystalline regions are slowly degraded (Jayakody *et al.,* 2005, Jayakody & Hoover 2002, Hoover 2000, Billaderis *et al.*, 1981). Several researchers have shown that the time taken for the degradation of the crystalline region by H_3O^+ can vary widely (9-25 days) depending on the starch source (Srichuwong *et al.*, 2005a, Jayakody 2001, Hoover 2000, McPherson & Jane 1999). The data suggests that during the time course of hydrolysis, only the amorphous regions were degraded by H_3O^+. This could be attributed to the high level of crystallinity (43-53%) in the *Dioscorea* starches [**Table 4-5**]. Unlike the *Dioscorea* starches, potato starch has been shown (Jacobs *et al.*, 1998a, Hoover

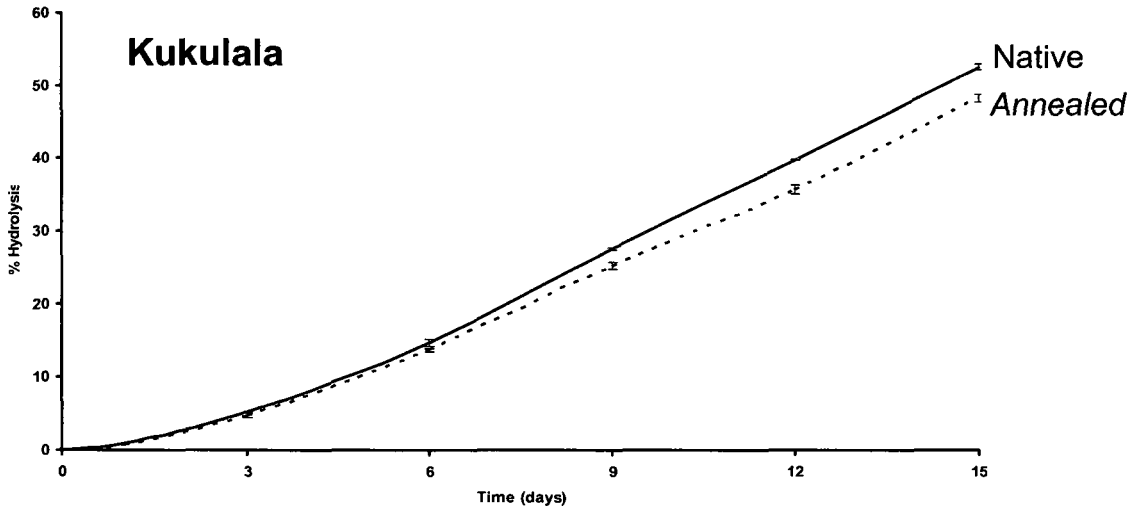
Figure 4-6 Acid hydrolysis (2.2M HCl, 35°C) of *Dioscorea* starches

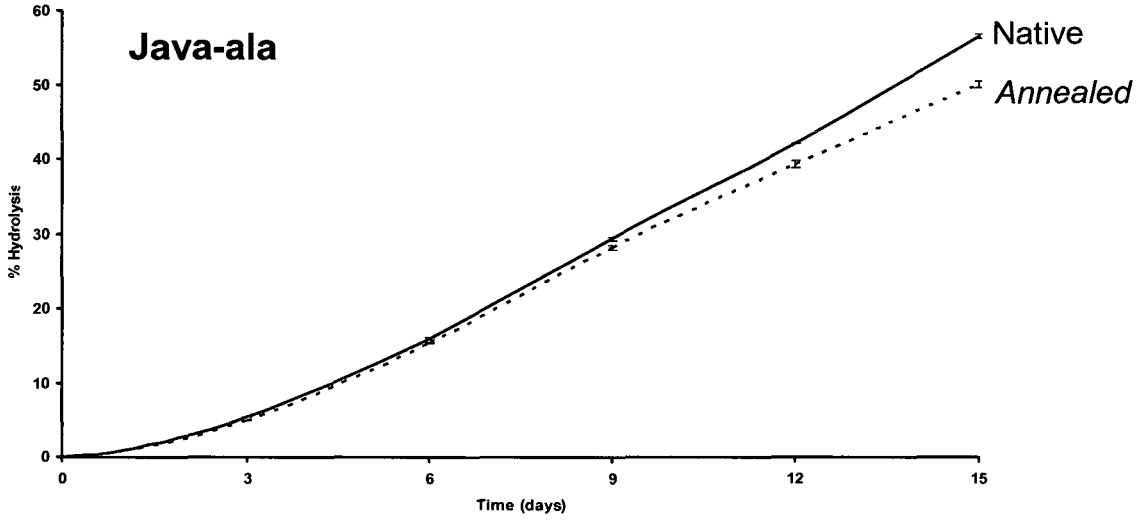
Native starches

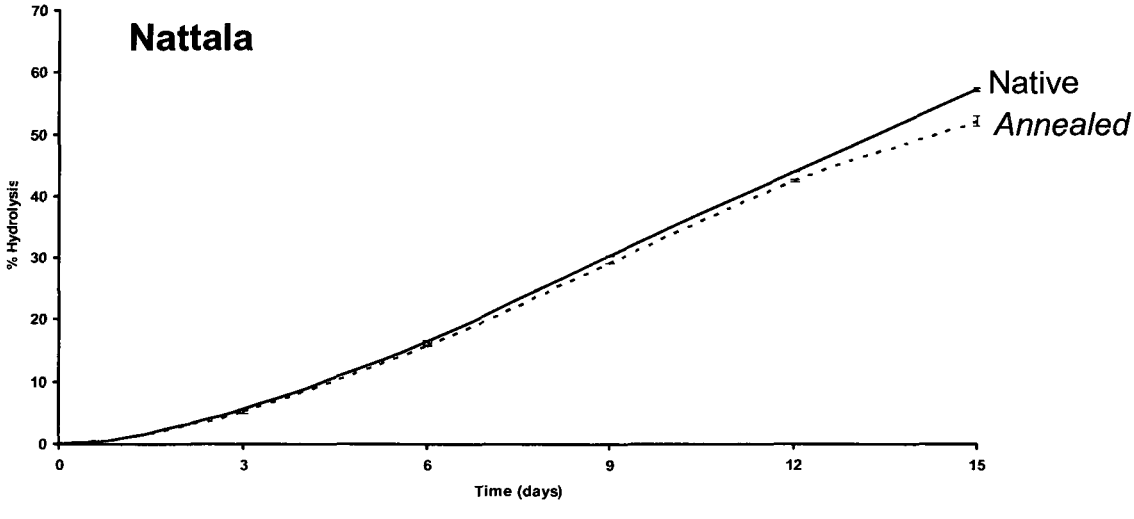
Dioscorea esculenta	*Dioscorea alata*
kukulala	hingurala
java-ala	raja-ala
nattala	

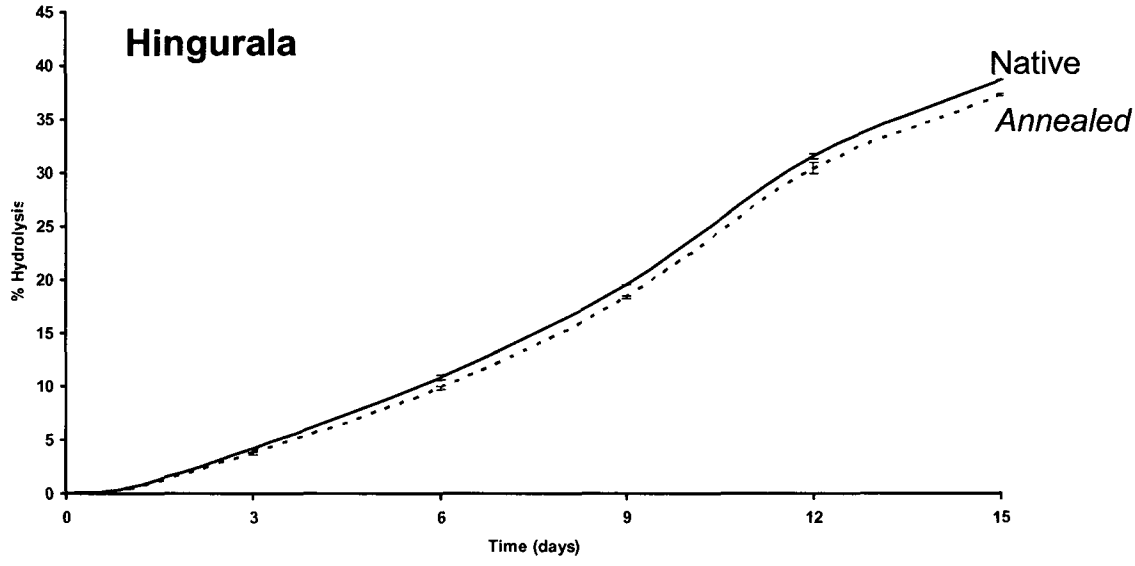
Annealed starches

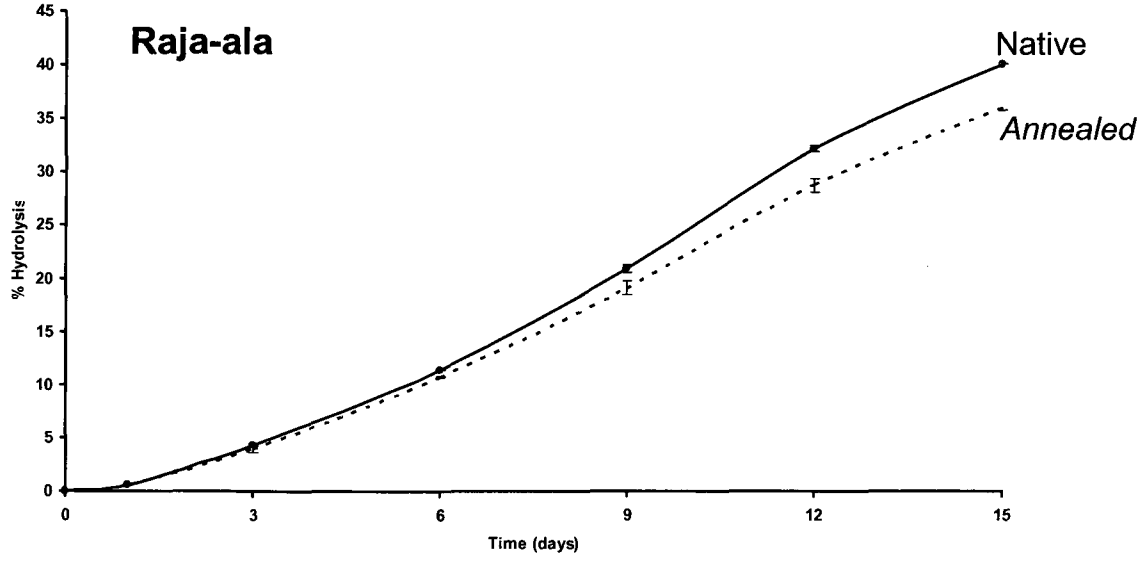
Dioscorea esculenta	*Dioscorea alata*
kukulala	hingurala
java-ala	raja-ala
nattala	


Kukulala
Native
Annealed
% Hydrolysis
Time (days)
0
10
20
30
40
50
60
0
3
6
9
12
15


Java-ala
Native
Annealed
% Hydrolysis
Time (days)
0
10
20
30
40
50
60
0
3
6
9
12
15


Nattala
Native
Annealed
% Hydrolysis
Time (days)
0
10
20
30
40
50
60
70
0
3
6
9
12
15


Hingurala
Native
Annealed
% Hydrolysis
Time (days)
0
5
10
15
20
25
30
35
40
45
0
3
6
9
12
15


Raja-ala
Native
Annealed
% Hydrolysis
Time (days)
0
5
10
15
20
25
30
35
40
45
0
3
6
9
12
15

& Vasanthan 1994a) to exhibit a biphasic pattern. This is not surprising since the crystallinity of native potato starch (24-28%) (Yusuph *et al.*, 2003, Zobel & Senti 1960) is much lower than that of the *Dioscorea* starches. The rate and extent of hydrolysis of native *D. alata* starches were lower than those of *D. esculenta* starches [**Figure 4-6**]. The extent of hydrolysis of native *D .alata* starches followed the order: raja-ala > hingurala. Whereas, the corresponding order for the native *D. esculenta* starches was: nattala > java-ala > kukulala [**Figure 4-6**].

Differences in the extent of acid hydrolysis among starches has been attributed to differences in: (1) granule size (Jayakody *et al.*, 2005, Jayakody & Hoover, 2002, Jayakody 2001, Vasanthan & Bhatty 1996, Billiaderis *et al.*, 1981), (2) amount of lipid complexed amylose chains (Waduge *et al.,* 2006, Morrison *et al.*, 1993a), (3) extent of interaction between glucan chains (Hoover & Manuel 1996), (4) amylopectin chain length distribution (Srichuwong *et al.*, 2005c) and (5) phosphorus content (Jayakody *et al.*, 2005, Hoover 2000). The slower extent of hydrolysis of native *D. alata* starches [**Figure 4-6**] could be attributed to the interplay of the following factors: (1) stronger interaction between AM-AM and/or AM-AMP chains within the granule interior, (2) larger granule size [**Table 4-1**], (3) lower phosphorus content [**Table 4-1**] and (4) a lower proportion of DP 6-12 chains [**Table 4-2**]. Amylose-lipid complexes have been shown to be resistant to acid hydrolysis (Waduge *et al.*, 2006, Morrison *et al.*, 1993a,b). However, in this study, the resistance of amylose-lipid complexes to acid hydrolysis was not evident [**Figure 4-6**], since *D. esculenta* starches having a much higher content

of amylose-lipid complexes (14.9-22.0%) were hydrolyzed to a greater extent than the *D. alata* starches (5.6-8.3%) [**Table 4-1**]. It is likely, that the smaller granule size [**Table 4-1**] and higher proportion of DP 6-12 chains [**Table 4-2**] in the native *D. esculenta* starches may have eclipsed the effect of amylose-lipid complexes on acid hydrolysis. The difference in the extent of hydrolysis between the native *D. alata* starches (raja-ala > hingurala) [**Figure 4-6**] can be attributed to stronger interaction between AM-AM and/or AM-AMP chains [**Table 4-4**] and to a higher content of lipid complexed amylose chains in hingurala [**Table 4-1**]. This seems plausible, since the starches did not differ significantly with respect to granule size [**Table 4-1**] amylopectin chain length distribution [**Table 4-2**]. Difference in hydrolysis among the native *D. esculenta* starches (nattala > java-ala > kukulala) [**Figure 4-6**] could be attributed to differences in: (1) granule size (kukulala > java-ala > nattala) [**Table 4-1**], (2) phosphorus content (nattala > java-ala > kukulala) [**Table 4-1**], (3) interaction between AM-AM and/or AM-AMP chains (java-ala > kukulala > nattala) and (4) amount of lipid complexes amylose chains (nattala > java-ala > kukulala) [**Table 4-1**]. The observed difference in the extent of acid hydrolysis among the native *D. esculenta* starches [**Figure 4-6**] suggest that the combined effect of factors 1 & 2 negates the effect of factors 3 & 4. The extent of hydrolysis in the native *Dioscorea* starches [**Figure 4-6**] was much lower than that reported for native potato, cassava, yam (species not specified) and sweet potato (Gunaratne & Hoover 2002, McPherson & Jane 1999). In these starches, hydrolysis exceeded 70% after 12 days. However, in the same time period, hydrolysis ranged from 25-40% in the *Dioscorea* starches

[**Figure 4-6**]. This could be attributed to the crystallinity of the native *Dioscorea* starches being much higher (43-53%) [**Figure 4-4**] than that reported for potato (24-28%), true yam (32%), cassava (38%) and sweet potato (38%) (Yusuph *et al.*, 2003, Gunaratne & Hoover 2002, Zobel & Senti 1960) starches.

Annealed starches exhibited a similar hydrolysis pattern [**Figure 4-6**] as their native counterparts. There was no significant difference in the rate of hydrolysis between the native and annealed starches. However, annealing decreased the extent of hydrolysis in all starches. The extent of this decrease in the *D. esculenta* starches followed the order: java-ala (11.0%) > nattala (9.0%) > kukulala (8.0%). Whereas in the *D. alata* starches, the corresponding values were 9 and 10%, respectively in hingurala and raja-ala starches. A smaller decrease (~5%) in acid hydrolysis on annealing as also been reported for potato starch (Jacobs *et al.*, 1998a, Hoover & Vasanthan 1994a). The decrease in acid hydrolysis on annealing has been shown to be influenced by the following factors: (1) perfection of starch crystallites, (2) formation of V-amylose lipid complexes and (3) formation of amylose double helices (Waduge *et al.*, 2006, Jacobs *et al.*, 1998a Hoover & Vasanthan 1994a). However, X-ray diffraction studies [**Figure 4-4**] showed the absence of any increase in V-amylose-lipid complex formation on annealing. Furthermore, crystalline perfection (due to alignment of double helices of amylopectin) cannot be considered as a factor influencing acid hydrolysis of annealed starches, since crystallites were not attacked during the time course of hydrolysis. Amylose leaching studies [**Table 4-4**] showed the interactions occur between amylose-amylose and/or amylose-

amylopectin chains on annealing. These interactions may have led to the formation of double helices within the amorphous domains of the granule, thus decreasing the susceptibility of annealed starches towards H_3O^+.

4.9 Enzyme hydrolysis

The susceptibilities of native *Dioscorea* starches towards hydrolysis by porcine pancreatic α-amylase are presented in **Table 4-6**. After 72h of hydrolysis, native *D. esculenta* and *D. alata* starches were hydrolyzed to the extent of 54.1-77.6% and 7.1-33.7%, respectively [**Table 4-6**]. Among native *D. esculenta* starches, the extent of hydrolysis followed the order: java-ala > kukulala > nattala. Whereas, in the *D. alata* starches, hingurala was hydrolyzed to a greater extent than raja-ala [**Table 4-6**]. Differences in the in-*vitro* digestibility of starches among and within species have been attributed to the interplay of many factors such as (1) botanical source (Srichuwong *et al.*, 2005a, Jacobs *et al.*, 1998c, Ring *et al.*, 1988, Gallant & Bouchet 1986, Snow & O'Dea 1981, Fuwa *et al.*, 1979, Rašper *et al.*, 1974, Gallant *et al.*, 1972), (2) granular morphology (Valetudie *et al.*, 1993, Fujita *et al.*, 1989, Colonna *et al.*, 1988), (3) granular size (Noda *et al.*, 2005, Snow & Glover 1997, Valetudie *et al.*, 1993, Ring *et al.*, 1988, Snow & O'Dea 1981, Leach & Schoch 1961), (4) surface area (Kong *et al.*, 2003, Guraya *et al.*, 2001, Leloup *et al.*, 1992, Knutson *et al.*, 1982), (5) amylose/amylopectin ratio (Hoover & Sosulski 1985), (6) extent of molecular association between glucan chains (Dreher *et al.,* 1984), (7) degree of crystallinity (Hoover & Sosulski 1985), (8) amylose-lipid complexes (Holm *et al.,* 1983; Hoover & Manuel 1995), and (9) unit cell structure (Jane 2006, Jane *et al.,*

Table 4-6 Hydrolysis (%)[1] of native and annealed *Dioscorea* starches by porcine pancreatic α-amylase

Species	Variety		% Hydrolysis Day-1	Day-2	Day-3
D. esculenta	Kukulala	Native	43.20 ± 0.00[a]	59.63 ± 0.00[a]	67.66 ± 0.00[a]
		Annealed	35.78 ± 0.33[b]	51.62 ± 0.34[b]	59.67± 0.53[b]
	Java-ala	Native	41.75 ± 0.28[c]	64.64 ± 0.22[c]	77.63 ± 0.00[c]
		Annealed	34.27± 0.17[d]	52.53± 0.30[d]	64.59 ± 0.13[d]
	Nattala	Native	32.41 ± 0.33[e]	46.47± 0.43[e]	54.11 ± 0.46[e]
		Annealed	35.01 ± 0.54[f]	50.03 ± 0.21[f]	57.93 ± 0.78[f]
D. alata	Hingurala	Native	19.28 ± 0.44[g]	28.62 ± 0.28[g]	33.69 ± 0.33[g]
		Annealed	14.76 ± 0.05[h]	22.10 ± 0.00[h]	25.75 ± 0.13[h]
	Raja-ala	Native	3.87 ± 0.00[i]	5.92 ± 0.46[i]	7.11 ± 0.28[i]
		Annealed	2.71 ± 0.33[j]	4.14 ± 0.06[j]	4.82 ± 0.12[j]

[1]All data reported on dry basis and represent the mean of four replicates. Values followed by the same superscript in each column are not significantly different (P<0.05) by Tukey's HSD test.

1992). Several researchers (Lauro *et al.,* 1999, Colonna *et al.,* 1988, Leach & Schoch 1961) have shown that α-amylase can simultaneously solubilize both amorphous and crystalline regions of starch granules. The difference in hydrolysis between *D. esculenta* and *D. alata* starches is mainly influenced by the smaller granular size [**Table 4-1**], lower amylose content [**Table 4-1**] and weaker interaction between AM-AM and/or AM-AMP chains [**Table 4-4**] in the former. The combined effect of these three factors may have negated the effect of crystallinity (*D. esculenta* > *D. alata*) and content of lipid complexed amylose chains (*D. esculenta* > *D. alata*) on hydrolysis. In the *D. alata* starches, the higher susceptibility of hingurala starch towards α-amylase reflects its smaller granular size [**Table 4-1**] and lower amylose content [**Table 4-1**]. It is likely, that the combined effect of those two factors culminate the effect of lipid complexed amylose chains (hingurala > raja-ala) on the extent of hydrolysis. Among the native *D. esculenta* starches, the lower susceptibility of nattala towards α-amylase can be attributed to its higher crystallinity [**Figure 4-4**] and more extensive interaction between AM-AM and/or AM-AMP chains [**Table 4-4**]. Whereas, the difference in susceptibility between kukulala and java-ala starches can be attributed to the smaller granule size [**Table 4-1**] and to a lower degree of interaction between glucan chains in the latter. A meaningful comparison of variations in the extent of hydrolysis between the *Dioscorea* and other tuber starches cannot be made due to differences in α-amylase source, reaction times and quantity of enzyme used.

Annealing decreased the susceptibility of kukulala, java-ala, hingurala and raja-ala towards α-amylolysis [**Table 4-6**]. The extent of this decrease was more pronounced in the *D. alata* (raja-ala > hingurala) than in the *D. esculenta* (java-ala > kukulala) starches. However, annealing increased the susceptibility of nattala starch towards α-amylolysis [**Table 4-6**]. Decreased α-amylase susceptibility on annealing has also been reported for potato starch (Jacobs *et al.*, 1998c, Hoover & Vasanthan 1994a). The decrease in α-amylase susceptibility seen in some of the *Dioscorea* starches on annealing [**Table 4-6**] can be attributed to interaction between AM-AM and/or AM-AMP chains on annealing. These interactions would decrease the accessibility of the α-D-(1→4) glycosidic bonds to α-amylase. Amylose leaching [**Table 4-4**] studies showed that on annealing, the extent of interaction between AM-AM and/or AM-AMP chains of the *D. esculenta* starches followed the order: nattala ~ kukulala > java-ala [**Table 4-4**]. On this basis, nattala should also have shown a decreased susceptibility towards α-amylolysis on annealing. Zhang *et al.*, (2006) and Planchot *et al.*, (1997a) have postulated that the crystalline region of the starch granule has a major influence in defining the rate and extent of α-amylolysis. The latter authors have shown by linternerization studies on potato, wheat and maize starches that susceptibility towards α-amylase increases with the extent of crystalline perfection. X-ray diffraction data [**Figure 4-4**] showed that among the *Dioscorea* starches, crystallinity decreased in nattala on annealing. This was attributed to a change in crystallite orientation rather than to crystallite disruption. This suggests that the increased hydrolysis exhibited by nattala starch [**Table**

4.4] on annealing, may have been due to crystallite reorientation. Crystallite reorientation may have rendered the α-D-(1→4) glycosidic bonds that are buried with the crystalline domains more accessible to α-amylase. The results indicate that the hydrolysis of annealed nattala starch is influenced to a greater extent by crystallite reorientation rather than by glucan chain interactions (AM-AM, and/or AM-AMP). As shown earlier, AM-AM and/or AM-AMP interactions [**Table 4.4**] and the extent of crystallite perfection [**Table 4.3**] was of a higher order of magnitude in kukulala than in java-ala starch. Therefore, it was surprising to note that the decrease in α-amylolysis on annealing was less pronounced in kukulala starch. It must be borne in mind, that crystalline perfection on annealing can lead to the creation of void areas within the crystalline lamella that can lead to susceptibility to attack (Nakazawa & Wang 2004). DSC data [**Table 4-3**] showed that the amount of crystallites being perfected on annealing was more pronounced in kukulala than in java-ala starch, consequently, more void areas may have been created within the crystalline lamella of kukulala, resulting in starch crystallites being more accessible to α-amylase in annealed kukulala than in annealed java-ala starch.

The extent of decrease in α-amylolysis on annealing is more pronounced in the *D. alata* (raja-ala > hingurala) than in the *D. esculenta* starches (java-ala > kukulala) [**Table 4-6**] due to stronger interactions between AM-AM and/or AM-AMP chains in the *D. alata* starches [**Table 4-4**]. In the *D. alata* starches, the extent of decrease in α-amylolysis on annealing is more pronounced in raja-ala [**Table 4-6**] due to increased crystallinity [**Table 4-5**] and stronger interactions

between AM-AM and/or AM-AMP chains on annealing [**Table 4-4**]. Scanning electron microscopy [**Figure 4-3**] showed that the granule surface and granule size of all *Dioscorea* starches remained unchanged on annealing. Thus, the observed changes to α-amylolysis on annealing can be attributed solely to structural changes within the granule interior.

4.10 Retrogradation

DSC of retrograded native starches have been shown to exhibit: (1) a broad peak in the range 55-85°C, which has been assigned to crystallization arising from associations between the outer A chains of amylopectin (Srichuwong *et al.*, 2005a, Fredriksson *et al.*, 1998, Zobel & Stephen 1995, Kalichevsky *et al.*, 1990, Ring *et al.*, 1987, Russel 1987), (2) a sharp peak in the range 98-104°C due to melting of the amylose-lipid complex (Russell 1987, Kugimiya & Donovan 1981), and (3) a broad peak due to melting of crystallized amylose chains formed by extensive interactions between amylose chains with DP ~ 50. This peak has normally been observed only at temperatures exceeding 140°C (Roulet *et al.*, 1988). Since bound lipids were present only in trace quantities, the endotherm due to melting of the amylose-lipid complex was not also, and the one associated with the melting of crystallized amylose chains was not detected due [**Table 4-1**] to pan failure at temperatures exceeding 130°C. Thus, the enthalpy of retrogradation ΔH_R mainly represents the unravelling and melting of the double helices formed by associations between the outer A chains of amylopectin during storage (40°C for 7 days) of the gelatinized starch gels.

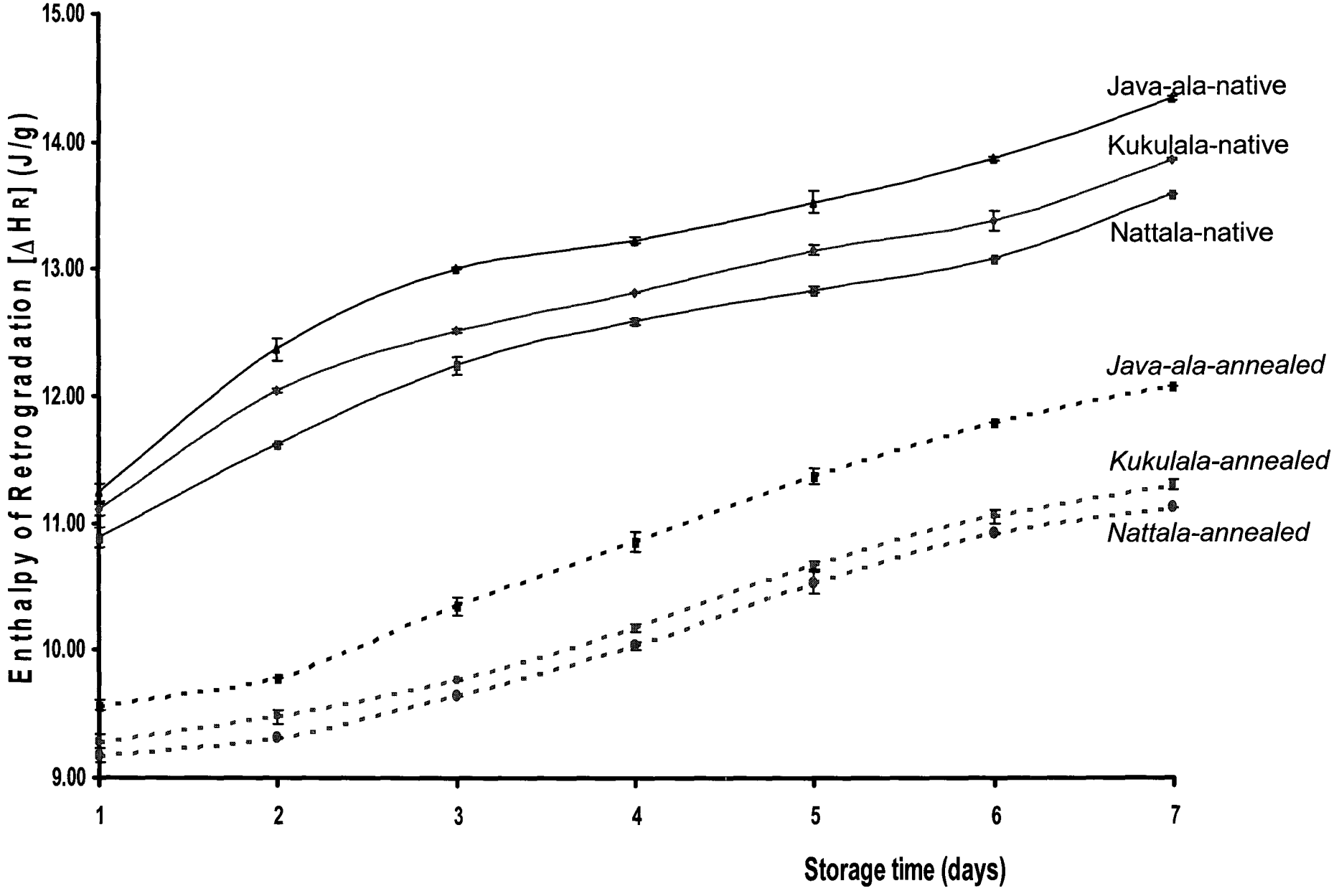
Figure 4-7 Enthalpy of retrogradation (ΔH_R) of *Dioscorea* starches

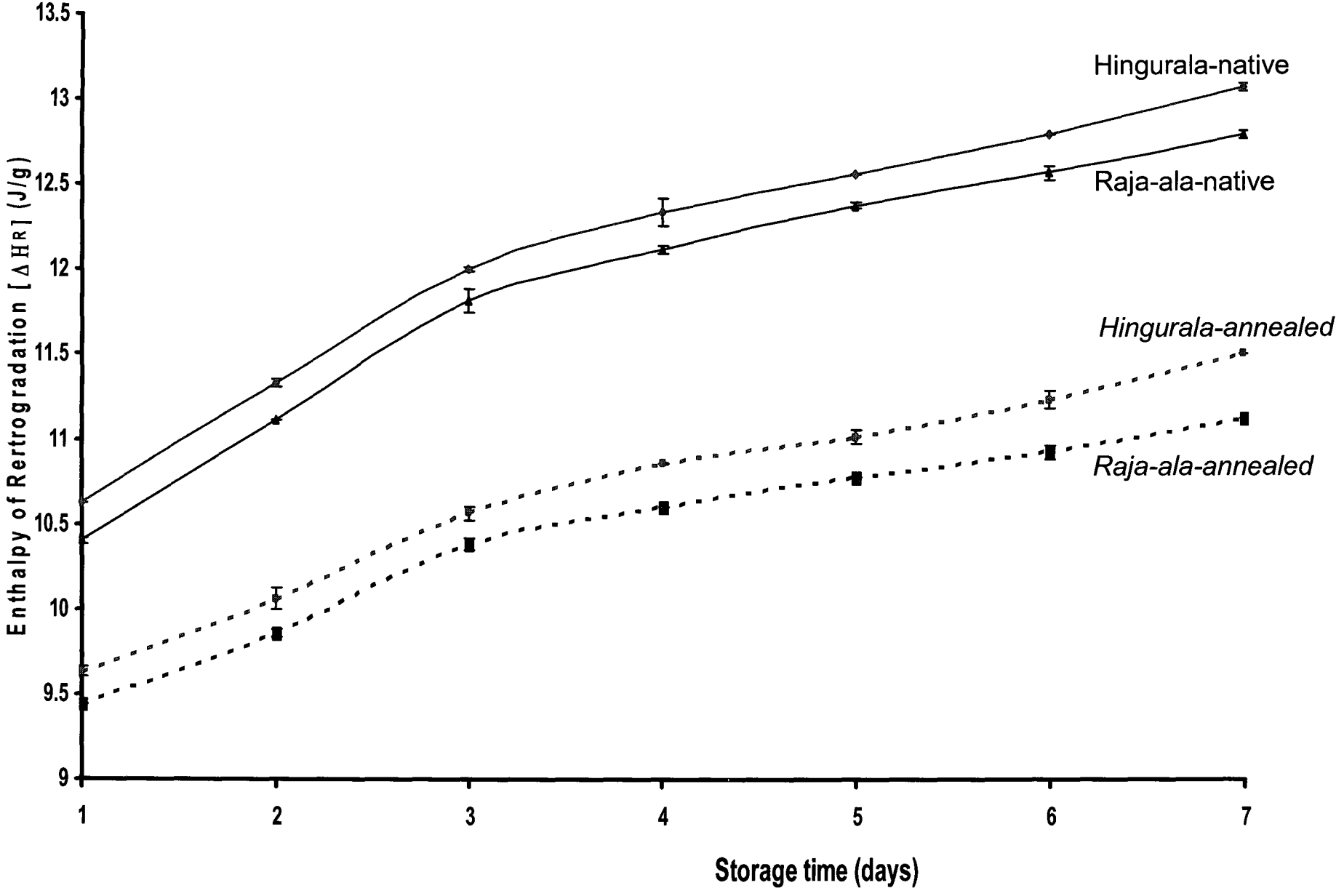
Native starches

Dioscorea esculenta	*Dioscorea alata*
kukulala	hingurala
java-ala	raja-ala
nattala	

Annealed starches

Dioscorea esculenta	*Dioscorea alata*
kukulala	hingurala
java-ala	raja-ala
nattala	


15.00
14.00
13.00
12.00
11.00
10.00
9.00
Enthalpy of Retrogradation [ΔHR] (J/g)
1
2
3
4
5
6
7
Storage time (days)
Java-ala-native
Kukulala-native
Nattala-native
Java-ala-annealed
Kukulala-annealed
Nattala-annealed


13.5
13
12.5
12
11.5
11
10.5
10
9.5
9
Enthalpy of Rertrogradation [ΔHR] (J/g)
1
2
3
4
5
6
7
Storage time (days)
Hingurala-native
Raja-ala-native
Hingurala-annealed
Raja-ala-annealed

The ΔH_R of native *D. esculenta* (java-ala > kukulala > nattala) starches were higher than those of the *D. alata* (hingurala > raja-ala) starches. In all starches, ΔH_R increased rapidly during the first 3 days of storage [**Figure 4-7**]. Thereafter, the increase was gradual. Differences in ΔH_R among starches have been explained on the basis of amylopectin unit chain length distribution (Lai *et al.*, 2000, Ward *et al.*, 1994, Shi & Seib, 1992, Kalichevsky *et al.*, 1990, Fredriksson *et al.*, 1988), and phosphate monoester content (Jane *et al.*, 1996). The main difference in amylopectin unit chain length distribution between native *D. esculenta* and *D. alata* starches was in the proportion of short (DP 6-12) chains (*D. esculenta* > *D. alata*) [**Table 4-2**]. Ward *et al.,* (1994) and Würsch and Gumy (1994) postulated that an increase in molar proportion of short chains with DP 6-9 inhibits retrogradation. Whereas, an increased molar proportion of unit chains with DP 14-24 increases the extent of retrogradation. On this basis, the *D. alata* starches should have shown a higher ΔH_R than the *D. esculenta* starches. Thus the observed differences in the extent of retrogradation (ΔH_R) between the two *Dioscorea* species cannot be attributed to amylopectin unit chain length distribution.

Studies have shown that in tuber and root starches, phosphorus is primarily in the form of starch phosphate monoester derivatives (Lim *et al.*, 1994, Lim & Seib 1993, Hizukuri *et al.*, 1983), mainly found on amylopectin. Jane *et al.*, (1996) have shown that starch phosphate monoesters slow retrogradation due to repulsion between negative charges. On this basis, the ΔH_R (enthalpy of retrogradation) of native *D. esculenta* starches should have been lower than that

of the *D. alata* starches, since the phosphate monoester content of the *D. esculenta* starches (0.05-0.10%) are higher than those of the *D. alata* (0.04-0.05%) [**Table 4-1**] starches. The results [ΔH_R *D. esculenta* > ΔH_R *D. alata*)] indicate that the higher amylopectin content of the *D. esculenta* starches [**Table 4-1**] negates the effect of phosphorous content [**Table 4-1**] on ΔH_R . Among the *D. esculenta* starches differences in ΔH_R between nattala and java-ala [**Figure 4-7**] is mainly due to differences in phosphorous content (nattala > java-ala [**Table 4-1**]), since there was no significant difference in amylopectin content between nattala and java-ala starches [**Table 4-1**]. In kukulala, the amount of phosphorus and amylopectin are lower than in nattala and java-ala [**Table 4-1**]. Therefore, theoretically, kukulala should have either exhibited a higher ΔH_R (if phosphorus content had been the main causative factor influencing ΔH_R) or a lower ΔH_R (if amylopectin content had been the main causative factor influencing ΔH_R) than nattala and java-ala. The observed order of retrogradation in the *D. esculenta* starches (ΔH_R java-ala > ΔH_R nattala) suggests that differences in ΔH_R between nattala and kukulala is due to the lower phosphate monoester content of kukulala [**Table 4-1**] negating the influence of amylopectin content (nattala>kukulala) [**Table 4-1**] on ΔH_R. Whereas the difference in ΔH_R between java-ala and kukulala is due to the higher amylopectin content of java-ala negating the influence of differences in phosphate monoester content [**Table 4-1**] between the two starches (java-ala>nattala). The difference in ΔH_R between the native *D. alata* starches (hingurala > raja-ala) can be attributed to the variation in

amylopectin content (hingurala > raja-ala) and phosphorus content (hingurala > raja-ala).

There are no reports in the literature comparative studies on the rate and extent of retrogradation of native and annealed cereal, legumes, tuber and root starches. Annealing decreased the extent of retrogradation in all starches [**Figure 4-7**]. For instance, during the storage period (40°C for 7 days) the ΔH_R of native kukulala, java-ala, nattala, hingurala and raja-ala starches. Increased by 2.76, 3.10, 2.69, 2.45 and 2.39 J/g, respectively. However, in annealed kukulala, java-ala, nattala, hingurala and raja-ala starches the increase in ΔH_R was 2.10, 2.51, 1.94, 1.88 and 1.68 J/g respectively. The difference in the magnitude of ΔH_R between native and annealed starches (native > annealed) indicate that annealing decreases the number of double helices formed between the outer chains of amylopectin during retrogradation. Amylose leaching studies [**Table 4-4**] showed that interaction between amylose chains (AM) and the outer chains of amylopectin (AMP) are possible during annealing. It is likely that these interactions are of a higher order of magnitude than those between AMP-AMP chains, and are, hence, not destroyed during gelatinization. Consequently, during gel storage, AM-AMP interactions (formed during annealing) could restrict the ability of adjacent AMP chains to form double helices by lowering the extent of interaction during retrogradation by steric hindrance and/or by reducing AMP chain mobility. The extent of reduction in ΔH_R on annealing is higher in the *D. esculenta* than in the *D. alata* starches due to their higher AMP content in the former [**Table 4-1**].

Advancement of knowledge & significance of the present study

1. In this study, many of the techniques that have been used to study starch structure and functional properties were extensively modified.

2. This is the first time that a detailed study has been made on the structure-property relationships between different varieties of *Dioscorea* species, and on the effect of annealing on their morphology, structure and functional properties.

3. Research on annealing has been mainly carried out on a single species. This study showed how differences in composition and structure among different varieties of a particular species can influence changes to physicochemical properties on annealing.

4. The study has significantly advanced the state of knowledge on the impact of annealing on: (i) crystallinity, (ii) starch polymorphism (iii) pasting properties and (iv) enzyme and acid susceptibility.

5. The study showed for the first time how variations in phosphate monoester content influence changes within the crystalline domain on annealing.

6. The results from this investigation could be used by food processors to tailor the properties of annealed *Dioscorea* starches (by different moisture/ temperature/ time combinations) to a level that is presently met by chemical modification.

7. The thesis reports the first study on the impact of annealing on starch retrogradation. Many researchers had assumed that native and annealed starches may exhibit nearly the same extent of retrogradation, since all interactions formed on annealing are disrupted during gelatinization. However, this study showed that this assumption was erroneous, as some interactions (amylose-amylopectin) do survive gelatinization and have a major impact in modifying the retrogradation properties of annealed starches.

8. This study showed that comparison of X-ray diffraction patterns and crystallinities of native annealed starches is more meaningful, only at their maximum water absorption capacities.

Summary and Conclusion

The main thrust of this thesis was to gain a deep insight into the impact of one step annealing on the granule morphology, composition, physicochemical properties and molecular structure of *Dioscorea* starches with varying composition and molecular structure. Suggestions for new avenues of research are also given.

The first part of thesis involved a detailed study of the surface characteristics, granule size distribution, proximate composition, molecular structure and properties of starches from varieties of *Dioscorea esculenta* (kukulala, java-ala, nattala) and *Dioscorea alata* (hingurala, raja-ala) tubers grown under the same environmental conditions in Sri Lanka. The study showed that the granule surface of all starches appeared to be smooth and showed no evidence of fissures. The granule size ranged from 3 to 10 μm and 30-50 μm, respectively, in the *D. esculenta* and *D. alata* starches. Starch granules of both species exhibited well-defined birefringence patterns under polarized light. Polarization crosses (hilum) were centric for *D. esculenta* and eccentric for *D. alata* starches. There was considerable variation in total amylose, phosphorous, bound lipid, lipid complexed amylose chains, amylose leaching, granule swelling, gelatinization parameters, pasting characteristics, extent of retrogradation, susceptibility towards acid, enzyme hydrolysis, amylopectin unit chain length distribution, X-ray diffraction pattern and crystallinity among the starches belonging to the two species. The unit chain length distribution of debranched amylopectin of the starches showed that the proportion of short branched chain (DP 6-12), medium

chains (DP 25-36) and average chain length of amylopectin were higher in the *D. esculenta* starches. However, variations in amylopectin structure among varieties of both species were marginal. *D. esculenta* starches exhibited a B-type X-ray diffraction pattern, though, both B- and C-type patterns were observed in the *D. alata* starches. The results indicated that the variations in physicochemical properties between the two species and among varieties were influenced by the interplay of factors such as granule size, crystallinity, magnitude of interaction between glucan chains (within the native granule), phosphorous content, amylopectin unit chain length distribution, amylose/amylopectin ratio and the number of branch points in close proximity to amylopectin clusters. Some of the *Dioscorea* starches exhibited high thermal stability (e.g. raja-ala) and low retrogradation rates (e.g. nattala). Thus, these starches can be used in foods with minimal modification.

The second part of thesis was to determine the impact of one step annealing on the composition, morphology, structure and physicochemical properties of starches from different varieties of *D. esculenta* and *D. alata* starches. The granule composition, morphology, birefringence, concentric granule growth rings, amylopectin chain length distribution and X-ray patterns remained unchanged on annealing in all starches. However, though crystallinity remained the same in kukulala, java-ala (*D. esculenta*), and hingurala (*D. alata*) starches, in nattala (*D. esculenta*) crystallinity decreased and in raja-ala (*D. alata*) starches crystallinity increased on annealing. In all starches, annealing increased the gelatinization transition (T_o, T_p, T_c) temperatures, decreased the gelatinization temperatures

range (T_c-T_o) and increased gelatinization enthalpy (ΔH). The magnitude of increase in T_o, T_p, and T_c was nearly the same in all starches, but the extent of decrease in T_c-T_o (which reflects the extent of crystallites perfection) varied among the starches. The increase in ΔH was a reflection of increased crystalline perfection and/or glucan chain interactions during annealing. Annealing decreased swelling factor (SF) and amylose leaching (AML) in all starches (*D. alata*>*D. esculenta*). The decrease in SF was influenced by crystalline perfection and extent of interaction between glucan chains on annealing, which in turn lead to a decrease in AML on annealing.

In both *D. alata* and *D. esculenta* starches, annealing decreased peak viscosity and increased pasting temperature, peak time and thermal stability, however, set-back decreased in some varieties (e.g. raja-ala), but increased (e.g. nattala) in others. The changes in viscosity parameters was more pronounced in the *D. alata* starches. The decrease in peak viscosity was influenced by the decrease in SF and AML. Whereas, the increase in pasting temperatures and thermal stability was influenced by the decrease in SF and increased interaction between glucan chains on annealing. The variations in the extent of set-back on annealing was influenced by SF and AML, and by granule rigidity. Annealing decreased the extent of acid hydrolysis in both *D. alata* and *D. esculenta* starches. The extent of this decrease was nearly similar in the varieties of both species. This decrease was the result of formation of double helices (resistant to acid hydrolysis) resulting from glucan chain interactions during annealing. With the exception of one variety (nattala), all varieties of *D. alata* and *D. esculenta*

starches exhibited decreased susceptibility (*D. alata>D. esculenta)* towards α-amylase hydrolysis on annealing. This was attributed to glucan chain interactions and increased crystalline perfection. The increased α-amylolysis exhibited by nattala starch on annealing, was attributed to changes in crystalline orientation (resulting from its higher phosphate monoester content in that variety). The extent of retrogradation of all starches decreased on annealing, differing for each of the varieties. The decreased retrogradation on annealing was attributed to amylose-amylopectin interactions (formed during annealing) restricting (by steric hindrance and/or by reducing chain mobility) the ability of the outer chains of amylopectin to form double helical associations. The results showed that changes to physicochemical properties in the *Dioscorea* starches on annealing is influenced by: (1) native starch structure (amylopectin chain length distribution, arrangement of amylose chains in the granule interior), (2) native starch composition (phosphate monoester content, amylose/amylopectin ratio) and (3) structural changes on annealing (crystallite perfection, crystallite reorientation interaction between amylose-amylose and/or amylose-amylopectin chains).

Directions for future research

1. This study showed that structural changes occur within the amorphous and crystalline domains of the granule on annealing. These changes could alter the reactivity of the granule (either increase or decrease) towards reagents that are used in the food industry to improve starch functionality. For instance, acetic anhydride in an alkaline medium is used to decrease retrogradation, whereas phosphorous oxychloride in an alkaline medium is used to impart thermal and shear stability. Furthermore, the reordering and self association of the starch components on annealing could also alter the location of the reaction site for acetic anhydride and phosphorous oxychloride. Changes in reactivity and location site could alter starch functionality. Thus, annealing before chemical modification may result in novel starch properties. Research geared to physical modification is important, since it is unlikely that any new chemical modification or genetic modifications of existing commercially based starches will be allowed.

2. The rate of starch digestion in food is altered by factors that are extrinsic and intrinsic to food. The extrinsic factors include the food particle size, viscosity of the digest, α-amylase inhibitors, and the level of α-amylase in an individual starch. Whereas, intrinsic factors include swelling and solubilization of starch granules, extent of amylopectin branching and the physical association of glucan chains. Starch has been classified into rapidly digestible (RDS), slowly digestible (SDS), and resistant starch (RS). RDS is rapidly and completely digested in the small intestine, while SDS is slowly but completely digested in the small intestine.

Presently, RS is produced mainly by cross-linking using mixtures of sodium trimetaphosphate/sodium tripolyphosphate. It is likely that annealing may increase RS levels in high amylose starches by (i) growth of perfection of existing crystallites (ii) interaction between amylose-amylose and/or amylose-amylopectin. It is hypothesized, that SDS levels could also be increased somewhat by annealing, whereas, RS levels could be increased substantially, if starches are annealed prior to cross-linking. This approach may decrease the amount of cross-linking reagent needed for modification (a desirable feature). A study geared to understanding the effect of annealing and/or chemical modification on SDS & RS formation is needed to improve the nutritional profiles of grain-based foods.

3. A comparison of the structure and physical properties of starches extracted from *Dioscorea* tubers (used in this study) stored at different storage temperatures (could trigger *in vivo* annealing) with those of starches extracted from freshly harvested tubers, and then subjected to *in vitro* annealing, may provide an excellent opportunity to further understand temperature induced structural transformations. This study is important, since thermal energy is the major variable cost of annealing. Thus, more research should be focused towards *in situ* annealing under natural conditions.

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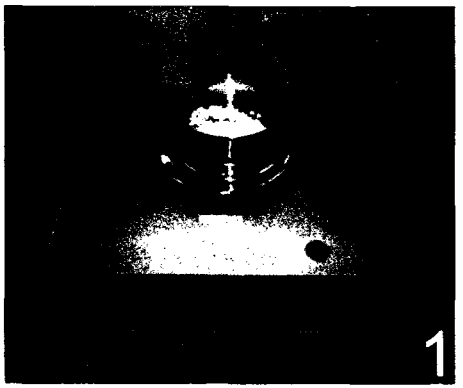
Appendix

Figure 1- Protocol for X-ray analysis

1. Weigh (0.5 g, db) starch into a moisture pan
2. Moisture equilibrate the pans over saturated K_2SO_4/3 weeks at room temperature
3. Reweigh the moisture equilibrated pan
4. Note sample appearances before and after moisture equilibration:
    (A) Before moisture equilibration (free flowing powder form)
    (B) Moisture absorbed sample (lumpy appearance)
5. Pack the starch into an elliptical aluminium holder (exposure area 289 mm^2)
6. Quickly press the starch into the holder
7. Well pack the sample into the holder (starch thickness 3 mm)
8. Scotch tape the back of the holder
9. Front side of the holder (note sample is pressed against glass pad)
10. Smoothen surface of the pressed sample (exposure side)
11. Scan the starch under the given X-ray diffraction settings
12. Calculate crystallinity by drawing a base line and connecting the base of the major peaks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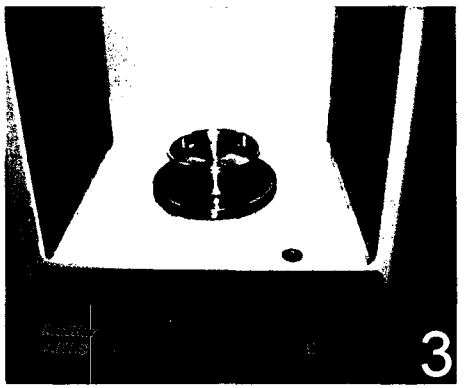
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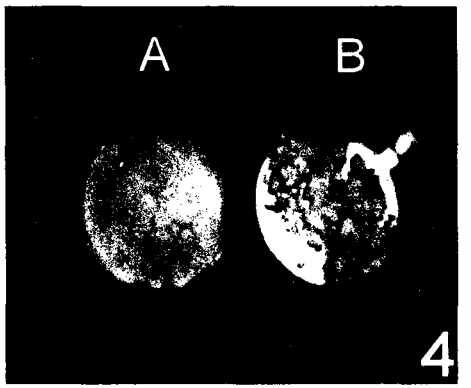
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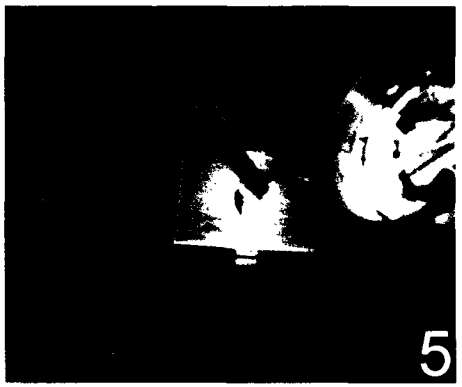
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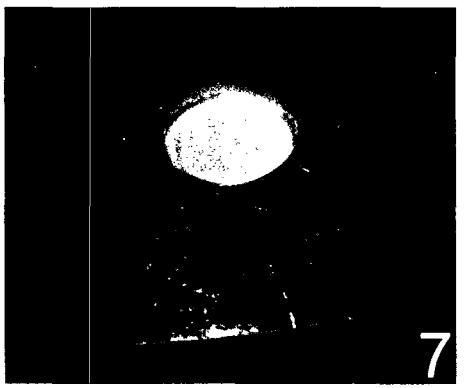
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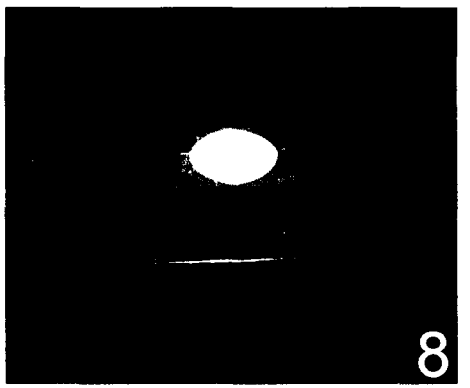
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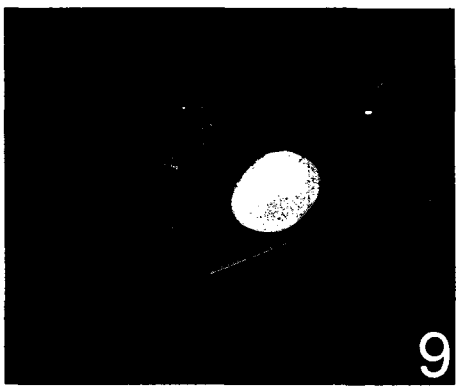
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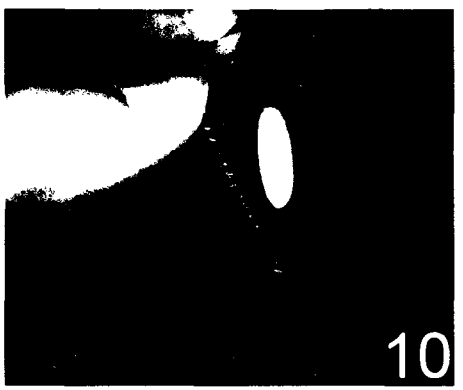
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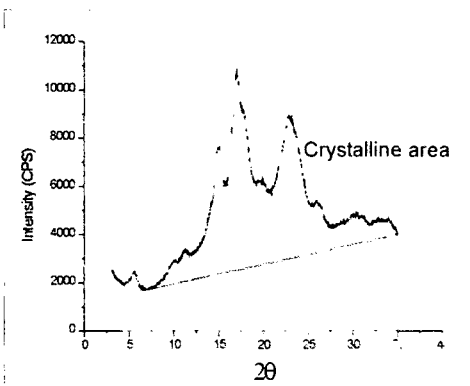
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Intensity (CPS)
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15
20
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Figure 2- Protocol for determination of retrogradation

1. Mix starch with distilled water in side a DSC pan using a micro-needle

2. Stretch a piece of Teflon®

3. Wrap the pan containing gelatinized starch with the stretched Teflon®

4. Label the wrapped gelatinized pans

5. Wrap of the individual samples with a Saran film®

6-8. Remove the excess wrapper (note all sequential steps)

9. Wrap sample replicates in a large piece of Saran film®

10. Attach wrapped samples to a glass rod (each set for daily analysis)

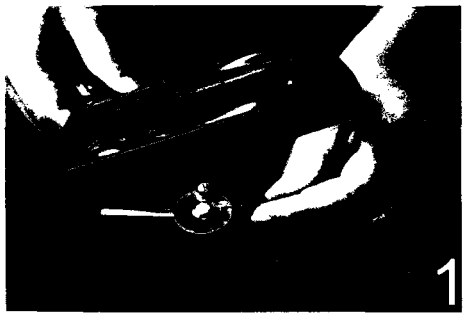
11. Nucleation step: immerse samples in water at 4°C/24h

12. Propagation step: store nucleated samples at 40°C for various time periods

13. Remove Saran film®

14-15. Carefully remove the Teflon® cover

16. DSC pan is ready for rescanning (note non corroded DSC pan)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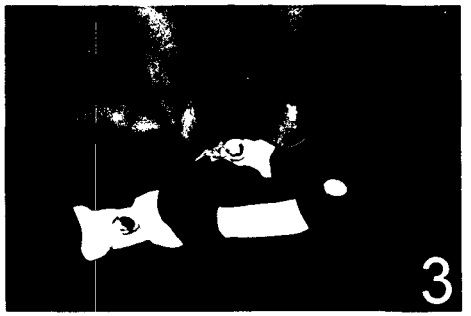
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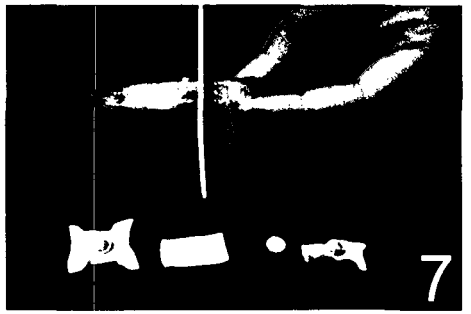
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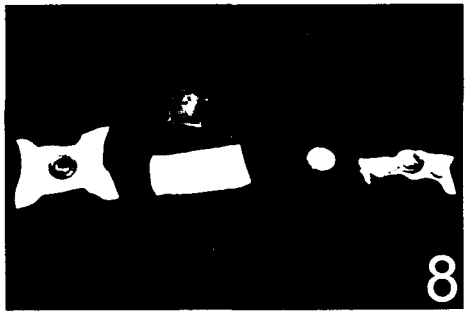
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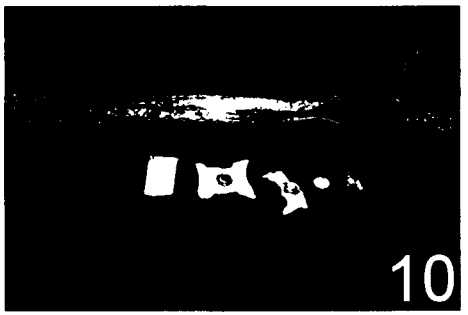
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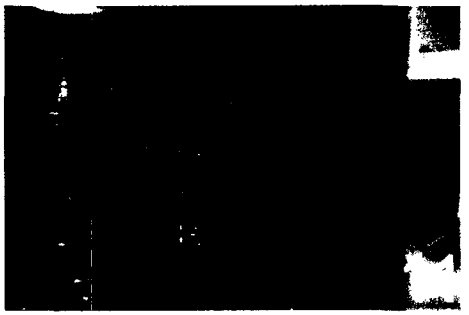

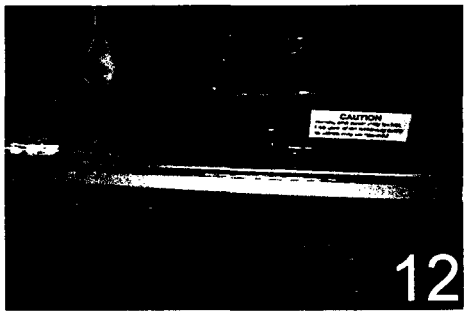

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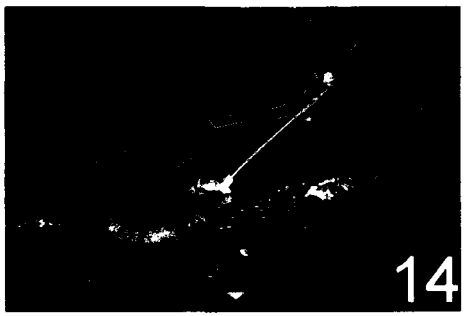
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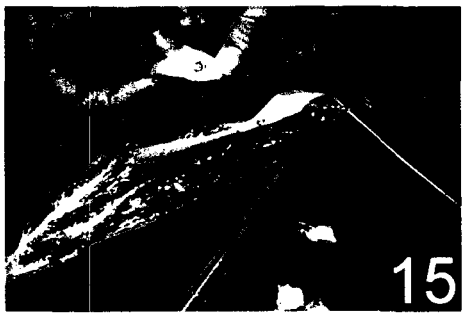
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16

ACADEMIC MERITS

(1) Publications:

(A) Research papers & articles (selected):

1. **Jayakody, L.**, Lan, H., Hoover, R., Chang., P., Liu. Q., & Weber, E., (2007). Composition, molecular structure and physicochemical properties of starches from grass pea (*Lathyrus sativus* L.) cultivars grown in Canada. *Food Chemistry*, **105**, 116-125.

2. **Jayakody, L.**, Hoover, R., Liu. Q., Weber, E., (2007). Studies on tuber starches. II. Molecular structure, composition and physicochemical properties of yam (*Dioscorea* sp.) starches grown in Sri Lanka. *Carbohydrate polymers*, **69**, 148-163.

3. Jayakody, J.A.L.P (2006). Traditional food grinding and pounding equipments in Sri Lanka. *Spolia Ceylanica*, **40**, 66-72.

4. **Jayakody, L.**, Hoover, R., Liu. Q., Weber, E., (2005). Studies on tuber and root starches. I. Structure and physicochemical properties of innala (*Solenostemon rotundifolius*) starches grown in Sri Lanka. *Food Research International*, **38** (6), 615-629.

5. Jayakody, L., (2005). Health benefits of omega fatty acids in fish and fish oil. *Vidusara Science Journal*, **18** (12), 16-17.

6. **Jayakody L.** & Hoover, R., (2002). The effect of lintnerization on cereal starch granules. *Food Research International*, **35**, 665-680.

(B) Abstracts in conference proceedings/presentations (selected):

1. **L. Jayakody**, R. Hoover, Q. Liu, and E. Donner (2007). Impact of annealing on the structure and properties of *Dioscorea* Starches. American Association of Cereal Chemists (AACC), Texas, USA, p. A 46.

2. **L. Jayakody**, R. Hoover, and D. Harnett (2007). Characterization of Starches from Kithul, Palmyra, Jackfruit flesh & Seeds Grown in Sri Lanka. Aldrich Interdisciplinary research conference, Memorial University, Canada, pp.11-13.

3. **L. Jayakody**, R. Hoover, P. Chang, H. Lan, Q. Liu, and E. Donner (2006). Composition, molecular structure, and physicochemical properties of starches from two grass pea (*Lathyrus sativus* L.) cultivars grown in Canada, 6th Canadian pulse research workshop (Pulse innovation project symposium), Hilton Garden Inn, Saskatchewan, Canada. p. 26.

4. **L. Jayakody**, R. Hoover, Q. Liu, and E. Donner (2006). Molecular structure, composition and physicochemical properties of yam (*Dioscorea* sp.) starches cultivated in Sri Lanka. Institute of Food Science & Technology (IFT), Orlando, USA.

5. **L. Jayakody**, R. Hoover, P. Chang, H. Lan, Q. Liu, and E. Donner (2006). Molecular structure and physicochemical properties of grass pea (*Lathyrus sativus* L.) starches. Canadian Institute of Food Science & Technology (CIFST), Montreal, Canada, p. 211.

6. **L. Jayakody**, R. Hoover, C. Fraser, and R. Coleman (2006). Effect of hydration on crystallinity and polymorphic composition of legume starches. Canadian Institute of Food Science & Technology (CIFST), Montreal, Canada, p.212.

7. **L. Jayakody** and R. Hoover (2006). Studies on tropical tuber starches. Aldrich Interdisciplinary research conference, Memorial University of Newfoundland, Canada, pp, 14-15.

8. **L. Jayakody**, & Hoover, R,. (2004). Structure and physicochemical properties of Innala (*Solenostemon rotundifolius*) starches grown in Sri Lanka. Canadian Institute of Food Science & Technology (CIFST), Guelph, Canada, pp. 76-77.

9. Heenatigalage, P., and **Jayakody, L.** (1998). Development of protein rich soft drink, Sri Lanka Association for the Advancement of Science (SLAAS) proceeding of fifty fourth annual session, B-118, University of Sri Jayawardanepura, Colombo, Sri Lanka p. 119.

(C) Other Scholarly Contributions:

2006 Reviewer: Manuscript number: FOODCHEM-D-06-01359 for *Food Chemistry*.

(D) Papers in Preparation:

1. **Jayakody, L.**, Hoover, R., Liu. Q., & Weber, E. Studies on tuber starches. III. The impact of annealing on the molecular structure and properties of *Dioscorea* starches grown in Sri Lanka.

2. **Jayakody, L.**, & Hoover, R. The impact of annealing on the molecular structure and physicochemical properties of cereal, tuber and legume starches- A Review

(2) Scholarships, Fellowships, Assistantships & Awards (selected):

2007 : American Association of Cereal Chemists International Student Travel Award

2007 : NSERC Visiting Fellowship in Canadian Government Laboratories

2006 : Merit award for oral presentation, Institute of Food Technologists (IFT), USA.

2006 : Award for Excellence in Research, Graduate Student Union, Memorial University, Canada

2006 : Fellow of the School of Graduate Studies, Memorial University, Canada.

2006 Session Monitor, IFT Annual Conference, Orlando, USA

2005/2006 : Barrowman Biochemistry Graduate Travel Award, Memorial University, Canada.

2006 : Graduate Fellowship, School of Graduate Studies, Memorial University, Canada.

2003/2004 : Barrowman Biochemistry Graduate Travel Award, Department of Biochemistry, Memorial University, Canada.

(3) Patents:

An improved Kjeldahl digester (Patent was filled in Canada Patent Office, 5th October, 2005).