ลักษณะสมบัติและนิกซ์ทามาไลเซชันของสตาร์ชถั่วมะแฮะ
Cajanus cajan L. Millsp

นาง ปิยะนุช รสเครือ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาเทคโนโลยีทางอาหาร ภาควิชาเทคโนโลยีทางอาหาร
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2553

CHARACTERIZATION AND NIXTAMALIZATION OF PIGEON PEA *Cajanus cajan* L. Millsp. STARCH

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A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Food Technology
Department of Food Technology
Faculty of Science
Chulalongkorn University
Academic Year 2010

Thesis Title	Characterization and nixtamalization of pigeon pea *Cajanus cajan* L. Millsp. starch
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ปิยะนุช รสเครือ : ลักษณะสมบัติและนิกซ์ทามาไลเซชันของสตาร์ชถั่วมะแฮะ *Cajanus cajan* L. Millsp. (CHARACTERIZATION AND NIXTAMALIZATION OF PIGEON PEA *Cajanus cajan* L. Millsp. STARCH) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : ผศ.ดร. พาสวดี ประทีปะเสน, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : รศ.ดร. สายวรุฬ ชัยวานิชศิริ, อ.ดร.ศศิกานต์ กู้พงษ์ศักดิ์, Dr. Thierry Tran. 163 หน้า.

งานวิจัยนี้ศึกษาองค์ประกอบทางเคมี ลักษณะโครงสร้างและสมบัติของสตาร์ชถั่วมะแฮะ (*Cajanus cajan* L. Millsp) ที่สกัดด้วยวิธีโม่เปียก พบว่าสตาร์ชดิบ (native starch) จากถั่วมะแฮะประกอบด้วยสารที่ไม่ใช่คาร์โบไฮเดรตน้อยกว่าร้อยละ 2 โดยน้ำหนักแห้ง ปริมาณสตาร์ชและแอมิโลสร้อยละ 96.74 และ 30.74 โดยน้ำหนักแห้ง ตามลำดับ เม็ดสตาร์ชมีรูปร่างทั้งกลมและรี มีขนาดอยู่ในช่วง 4.88-60.87 ไมครอน โดยมีเส้นผ่านศูนย์กลางเฉลี่ยโดยปริมาตร หรือ D[4,3] 27.81 ไมครอน โครงสร้างผลึกเป็นแบบ C และมีปริมาณผลึกสัมพัทธ์ร้อยละ 35.24 อุณหภูมิเริ่มต้นของการเจลาติไนซ์ประมาณ 73 องศาเซลเซียส และพลังงานที่ใช้ในการเจลาติไนซ์ (ΔH_{Gel}) 17.35 จูลต่อกรัม การดูดซับน้ำและน้ำมันที่อุณหภูมิ 70 องศาเซลเซียส เท่ากับ 229 กรัมน้ำ/กรัมสตาร์ชแห้ง และ 51 กรัมน้ำมัน/กรัมสตาร์ชแห้ง ตามลำดับ มีการเพิ่มของกำลังการพองตัว การละลาย และปริมาณแอมิโลสที่ละลายออกตามอุณหภูมิเป็นไปตามแบบแอรีเนียส โดยที่อุณหภูมิ 95 องศาเซลเซียส มีกำลังการพองตัว 35 กรัมต่อกรัม การละลายร้อยละ 23 โดยน้ำหนักแห้ง และปริมาณแอมิโลสที่ละลายออกร้อยละ 72 โดยน้ำหนักแอมิโลสทั้งหมด นอกจากนี้มีปริมาณที่ถูกย่อยด้วยเอนไซม์แอมิโลกลูโคซิเดสร้อยละ 0.36 โดยน้ำหนักแห้ง ความหนืดสูงสุด 3,856 mPa·s ความหนืดเมื่อเย็นตัว 5,604 mPa·s และความหนืดเมื่อคืนตัว 2,791 mPa·s เมื่อเก็บเจลสตาร์ชถั่วมะแฮะที่อุณหภูมิ 4 องศาเซลเซียส นาน 12 วัน พบว่ามีการคืนตัวร้อยละ 56 และเมื่อผ่านการแช่เยือกแข็งและการละลาย 7 รอบมีการสูญเสียน้ำร้อยละ 12 โดยน้ำหนักแป้งเปียก ที่อุณหภูมิ 75 องศาเซลเซียส แป้งเปียกที่ความเข้มข้นร้อยละ 8 โดยน้ำหนักแห้ง มีลักษณะการไหลแบบซูโดพลาสติก มีค่าสัมประสิทธิ์ความคงตัว (K) เท่ากับ 24 $mPa{\cdot}s^{0.44}$ และที่อุณหภูมิ 25 องศาเซลเซียส ความถี่ 0.1-100 rad/s และความเครียดร้อยละ 2 เป็นช่วง rubbery สำหรับเจลสตาร์ชที่ความเข้มข้นร้อยละ 15 โดยน้ำหนักแห้ง โดยมีค่า storage modulus (G´) 38,900 Pa นอกจากนี้เมื่อนำสตาร์ชดิบมาแยกขนาดด้วยตระแกรงร่อนได้ขนาดเล็ก (4.88-52.25 ไมครอน) ขนาดกลาง (4.88-60.87 ไมครอน) และขนาดใหญ่ (9.00-60.87 ไมครอน) ร้อยละ 7, 73 และ 20 โดยน้ำหนักแห้ง ตามลำดับ ค่า D[4,3] ของแต่ละช่วงขนาด เท่ากับ 24.03, 27.06 และ 30.05 ไมครอน ตามลำดับ เมื่อขนาดของเม็ดสตาร์ชใหญ่ขึ้นพบว่ามีปริมาณแอมิโลสสูงขึ้น อุณหภูมิเจลาทิไนเซชัน ΔH_{Gel} สมบัติของแป้งเปียก (ยกเว้น อุณหภูมิที่เริ่มมีการเปลี่ยนค่าความหนืด) และการสูญเสียน้ำมีแนวโน้มเพิ่มขึ้น แต่ปริมาณฟอสฟอรัส ปริมาณผลึกสัมพัทธ์ การดูดซับน้ำ กำลังการพองตัว การละลาย ปริมาณแอมิโลสที่ละลายออกและปริมาณที่ถูกย่อยด้วยเอนไซม์มีแนวโน้มลดลง นอกจากนี้จากการดัดแปรแป้งด้วยวิธีนิกซ์ทามาไลเซชัน โดยต้มและแช่เมล็ดถั่วทั้งเปลือกในสารละลายแคลเซียมไฮดรอกไซด์ ($Ca(OH)_2$) ความเข้มข้นร้อยละ 0-1.0โดยน้ำหนักต่อปริมาตร พบว่าการแช่เมล็ดถั่วมะแฮะแห้งในน้ำก่อนสกัดไม่ทำให้โครงสร้างและสมบัติของสตาร์ชเปลี่ยนแปลง แต่เมื่อแช่เมล็ดถั่วมะแฮะแห้งในสารละลาย $Ca(OH)_2$ มีการสะสมแคลเซียมบนผิวของเม็ดสตาร์ช เป็นผลให้สมบัติแป้งเปียกและ G´เพิ่มขึ้นเล็กน้อย แต่การคืนตัวและการสูญเสียน้ำของเจลสตาร์ชมีแนวโน้มลดลง การต้มถั่วมะแฮะแห้งทำให้เกิดปฏิกิริยาระหว่างสารต่างๆ ที่มีอยู่ในเมล็ดถั่วเป็นผลทำให้องค์ประกอบทางเคมีและสมบัติของแป้งนิกซ์ทามาไลซ์หรือแป้งดัดแปร แตกต่างจากสตาร์ชดิบ การต้มถั่วมะแฮะทำให้เม็ดสตาร์ชบางส่วนเกิดเจลาติไนซ์ เป็นผลทำให้มีปริมาณโปรตีน ไขมัน เยื่อใย เถ้า แคลเซียม และฟอสฟอรัสของแป้งนิกซ์ทามาไลซ์สูงกว่าของสตาร์ชดิบ 6, 2, 2, 2, 2 และ 10 เท่า ตามลำดับ แต่ปริมาณสตาร์ชและแอมิโลสลดลงร้อยละ 30 และ 20 ตามลำดับ เมื่อเปรียบเทียบกับสตาร์ชดิบ เม็ดสตาร์ชของแป้งนิกซ์ทามาไลซ์มีขนาดใหญ่กว่า มีปริมาณผลึกสัมพัทธ์ลดลงร้อยละ 30 ความหนืดเมื่อเย็นตัวมีค่าต่ำกว่า 20 mPa·s ค่า K การคืนตัวและการสูญเสียน้ำต่ำ มีอุณหภูมิเริ่มต้นของการเจลาติไนซ์ การดูดซับน้ำและน้ำมันสูงกว่าสตาร์ชดิบ ที่อุณหภูมิต่ำกว่าอุณหภูมิเริ่มต้นการเจลาติไนซ์ของสตาร์ชดิบ (~70 องศาเซลเซียส) แป้งนิกซ์ทามาไลซ์มีกำลังการพองตัว การละลาย และปริมาณแอมิโลสที่ละลายออกสูงกว่าสตาร์ชดิบ แต่ที่อุณหภูมิสูงกว่าอุณหภูมินี้แป้งนิกซ์ทามาไลซ์กลับมีค่าเหล่านี้ต่ำกว่าสตาร์ชดิบ เมื่อเพิ่มความเข้มข้นของสารละลาย $Ca(OH)_2$ที่ใช้ในการต้มเมล็ดถั่วมะแฮะแห้ง พบว่าปริมาณแคลเซียมและฟอสฟอรัส และปริมาณผลึกสัมพัทธ์เพิ่มขึ้น ส่งผลให้อุณหภูมิการเกิดเจลาติไนซ์ ΔH_{Gel} และการดูดซับน้ำที่ 70 องศาเซลเซียส มีแนวโน้มเพิ่มขึ้น แต่กำลังการพองตัว การละลาย ปริมาณแอมิโลสที่ละลายออก ความหนืดเมื่อเย็นตัว การคืนตัวและการสูญเสียน้ำมีแนวโน้มลดลง

ภาควิชาเทคโนโลยีทางอาหาร ลายมือชื่อนิสิต

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ปีการศึกษา 2553 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม

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4973831123 : MAJOR FOOD TECHNOLOGY
KEYWORDS: CHARACTERIZATION/ NIXTAMALIZATION/ PIGEON PEA/ STARCH

PIYANUCH ROSKHRUA: CHARACTERIZATION AND NIXTAMALIZATION OF PIGEON PEA *Cajanus cajan* L. Millsp. STARCH. ADVISOR: ASST. PROF. PASAWADEE PRADIPPASENA, Sc.D., CO-ADVISOR: ASSC. PROF. SAIWARUN CHAIWANICHSIRI, Ph.D., SASIKAN KUPONGSAK, Ph.D., THIERRY TRAN, Ph.D., 163 p.

Pigeon pea, *Cajanus cajan* L. Millsp, starch was isolated through wet milling and fractionated by sieving. Native starch and its fractions were characterized for their composition, size, shape and crystallinity and properties (thermal properties, water absorption capacity, swelling power, solubility, amylose leaching, pasting properties, freeze-thaw stability and enzymatic digestibility). Nixtamalization, boiling and then steeping grains in 0 – 1.0 % w/v calcium hydroxide ($Ca(OH)_2$) solutions, was used to modify pigeon pea starch granules. In order to evaluate effects of nixtamalization on starch granule structures as well as properties, changes in structures and properties mentioned above (except enzymatic digestibility) were determined. For this purpose, oil absorption capacity, percentage retrogradation and rheological properties were also determined. In dry basis, native pigeon pea starch contained less than 2 % non-carbohydrate compounds, 96.74 % starch and 30.74 % amylose. Starch granules had round, spherical-like, and oval shape. Granule size ranged from 4.88 to 60.87 μm with the volume moment mean diameter, D[4,3], of 27.81 μm. Crystalline pattern was C-type and relative crystallinity was 35.24 %. Through differential scanning calorimeter, granules started to gelatinize (onset temperature, T_o) around 73°C and required enthalpy of gelatinization (ΔH_{Gel}) of 17.35 J/g. At 70°C, water and oil absorption capacities were 229 g water/g dry starch and 51 g oil/g dry starch, respectively. Increases in swelling power, solubility and amylose leaching with temperature followed the Arrhenius-type temperature dependence. At 95°C, swelling power, solubility and amylose leaching were 35 g/g dry sample, 23 % and 72 %, respectively. Enzymatic hydrolysis was 0.36 %. Peak viscosity, final viscosity, and setback were 3,856, 5,604, and 2,791 mPa·s, respectively. At 4°C, retrogradation was 56 %, after 12 day storage. After 7 freeze-thaw cycles, syneresis was 12 %. At 75°C, 8 %w/w paste had pseudoplastic flow with 24 $mPa{\cdot}s^{0.44}$ consistency index. At 25°C, strain of 2 % and frequency range of 0.1 – 100 rad/s were rubbery region for 15 % w/w paste with storage modulus (G´) of 38,900 Pa. By sieving, three granule fractions having D[4,3] of 24.08, 27.07 and 30.05 μm were obtained. As D[4,3] increased, amylose content increased, however phosphorus content and percentage relative crystallinity decreased. Gelatinization temperatures, ΔH_{Gel}, pasting properties with the exception pasting temperature, percentage syneresis increased. Though water absorption capacity, swelling power, solubility, amylose leaching and percentage hydrolysis decreased as D[4,3] increased. Soaking grains in water did not affect structures and properties of pigeon pea starch. Soaking grains in $Ca(OH)_2$ solutions caused deposition of calcium on granule surface resulting in slight increasing in pasting properties and G´, but decrease in percentage retrogradation and syneresis. Interactions among components during boiling altered chemical compositions of starch granule resulting in modification of its physical properties. Boiling grains caused partial gelatinization of starch granules resulting in higher contents of protein, lipid, crude fiber, ash, calcium and phosphorus more than 6, 2, 2, 2, 2 and 10 times, respectively, due to less complete separation of starch and other components. However starch and amylose contents were lower by 30 % and 20 %, respectively. Gelatinized starch granules had D[4,3] higher than native starch by at least 60 %. Boiling grains decreased percentage of relative crystallinity about 30 %. Nixtamalized, or modified, flours had higher gelatinization temperatures as well as water and oil absorption capacities. Below 70°C, swelling power, solubility and amylose leaching of modified flours were higher than those of native starch, the opposite direction was observed at 70°C and higher. Modified flours had RVA final viscosity less than 20 mPa·s. Due to low amylose content and amylose leaching, modified flours had low consistency index. An increase in $Ca(OH)_2$ used for boiling grains increased calcium and phosphorus, as well as percentage of relative crystallinity. This resulted in increases in gelatinization temperatures, ΔH_{Gel} and water absorption capacity at 70°C. Although it decreased swelling power, solubility, amylose leaching, RVA final viscosity, percentage retrogradation and syneresis.

Department: Food Technology
Field of Study: Food Technology
Academic Year: 2010

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ACKNOWLEDGMENTS

I would like to express my deepest gratitude to my advisor, Asst. Prof. Pasawadee Pradipasena, and my co-advisor, Assoc. Prof. Saiwarun Chaiwanichsiri, Dr. Sasikan Kupongsak and Dr. Thierry Tran, for their excellent and valuable guidance, encouragement. I also thank my examining committee, Assoc. Prof. Kalaya Laohasongkram, Assoc. Prof. Klanarong Sriroth and Asst. Prof. Jirarat Tattiyakul, for their valuable suggestions.

I thank all friends at the Department of Food Technology, Chulalongkorn University and my colleagues at the faculty of Agro-Industry, Rajamangala University of Technology Lanna (Nan Campus) for their help and encouragement. I am grateful to all my friends at CIRAD, Montpellier, France, for all their help and support.

I would like to thank Rajamangala University of Technology Lanna (Nan Campus) for providing scholarship for my study and Chulalongkorn University for funding this research.

I dedicate this Thesis to my beloved father, late Phunlop Pangsanit. Finally, I would like to extend my special thanks to my beloved mother, grandmother, sisters (Panutkan and Varaphorn) and husband for their love, encouragement, and emotional support. Without them, I would not have been able to achieve this degree.

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CHAPTER I

INTRODUCTION

Starches are widely used for various applications in many industries. The specific properties are required for each application. To cover the wide range of properties needed for different applications and industries (both food and non-food), starches from different sources are used and modified. Starches from different sources give different properties due to their differences in structures. While cereal and tuber starches (or flours) are widely studied and utilized worldwide; starches from legumes (other than mung bean starch) are limitedly studied and used. With their nutritional values particularly their high protein, vitamin, mineral contents, and antioxidant capacity, legume flours were recently researched to replace wheat flour in many food products such as pasta and cookie. They were also used to develop a high protein snack. Even though they are interesting for their protein content; however, starch is a major and an important structural component, and its properties are important factor to give product texture which is critical for consumer's acceptance. For an economic point of view, starch from local crops should be fully utilized either to replace an expensive (and/or imported) one or to make value added products. In order to do that, structures, properties and their relationships of those starches have to be revealed, so appropriate applications can be evaluated. Rheological properties of starch depend on size and shape of starch granules. Larger starch granule was reported to have higher water absorption and granule swelling rates. Native pigeon pea starch has been reported to have a wide range in granule size from 8 to 69 μm; therefore, it is an excellent sample to determine the effect of granule size on chemical characteristics and functional properties.

There are relationships between starch structures and their properties, and changing in structures alters properties. Since chemical and physical modifications cause changes in starch structures and resulted in property changes, new applications can be deployed. Interesting starch for this research is from pigeon pea. Pigeon pea grain has starch as a major component and is low in fat. Land Developing Department of Thailand has been promoting pigeon pea planting to improve soil quality. Pigeon pea grain is not commonly used for local food and is used for animal feed. The knowledge on structures and properties of its starch will facilitate its utilization for industrial applications, which will benefit farmers and industries in Thailand. Through modification, more applications can be derived from this starch. In this study, pigeon pea starch was modified using nixtamalization. Nixtamalization is a thermal-alkaline process used to prepare dough from corn kernels for making corn chip or tortillas. This process has been proven to detoxify aflatoxin contaminated in corn. Therefore, it is interesting to use this process with legume grains, even though effect of nixtamalization on aflatoxin reduction in pigeon pea grains and its starch/flour was not studied in this research. The knowledge obtained from this research is essential for the assessment on potential industrial applications of pigeon pea starch.

Hypothesis and Objectives: This research has been based upon the hypothesis that the changes in properties are induced by the changes in structures. The objectives of this research are to characterize native pigeon pea starch; to evaluate the effects of granule size on selected structures and properties; to determine the effects of soaking or boiling pigeon pea grains in calcium hydroxide solution of various concentrations on starch structures and properties; and to derive the relationships among these structures and properties.

CHAPTER II

LITERATURE REVIEW

2.1 Legume

2.1.1 Legume grains

Legume grains are from dicotyledonous plant grains belonging to the family *Leguminosae*. The grains are edible. Family *Leguminosae* contains approximately 750 genera and 16,000-19,000 species (Allen and Allen, 1981). The main components of legume grains are carbohydrate and protein, approximately 45-65 % and 15-38 %, respectively (Boulter and Derbyshire, 1978; Longe, 1981; Norton *et al*, 1985). As a result, the grains are a main source of cheap protein in food (Singh *et al*., 2004a). Moreover, legume grains also contain minerals and vitamins (Norton *et al*., 1985).

2.1.2 Pigeon pea (Mahae) grains

The pigeon pea or red gram pea, *Cajanus cajan* or *Cajanus indicus*, is called Tou Mahae (ถั่วมะแฮะ) in Thai. It is a member of the family Fabaceae or Legumimosea (Van der Maesen, 1989). It is a perennial shrub that lives for about 5 years. It can grow under poor soil conditions and tolerate dry weather (Duke, 1981). The seed yield is about 880 to 1,220 kg/rai (Phaikaew *et al*., 1996). Pigeon pea grains have protein, fat, fiber and ash content of 19.34 %, 3.24 %, 5.56 % and 4.05 %, respectively (Nwokolo, 1987). Their starch content is 44.8-53.0 % w/w in which the starch digestibility is 36.2-53.0 % w/w (Wacher, 2003). In a report by Nwokolo (1987), pigeon peas are found to be rich in minerals, especially potassium, calcium,

2.2 Legume flour and starch

Legume flour is defined as finely grind powder from hulled legume grains. Flour contains high content of protein, lipid, fiber and ash (Hoover *et al*., 2010). Native starch is defined as a naturally carbohydrate in flour obtained by purification of flour to remove most of its protein, lipid, fiber and ash. Starches are generally characterized by low protein, lipid, fiber and ash contents. All are lower than 1 %. (Thomas and Atwell, 1999; Preiss, 2004).

2.2.1 Flour and starch composition

The composition of legume flours are shown in Table 2.1. It shows that legume flours also contains 11.2-26.2 % protein, 1.9-6.8 % lipid, 1.0-22.9 % fiber, 2.9-7.9 % ash content, 54.0-57.8 % carbohydrate, 56.0-75.3 % starch and approximately 23.5-49.0 % of amylose content. Legume flours are known to have higher concentration of protein, fiber, lipid and ash content than those from tuber (potato) and root (cassava), while bambarra groundnut and lentil flours have similar protein level as wheat flour. Legume starches are composed of 0.2-0.9 % protein, approximately 0.16 % fiber and 0.04-0.8 % lipid (Lii and Chang, 1981; Duke, 1981). This is similar to corn, potato, wheat and cassava compositions. The low protein, lipid, fiber and ash concentrations of legume starches cause high percentage of carbohydrate, greater than 90 %, and starch concentration, approximately 88.89 % to 92.30 % which starch content and amylose content were higher than barley corn

wheat cassava and potato (Table 2.1). Legume starch was high amylose content resulted in good to make noodle (Sung and Stone, 2004).

Table 2.1 Proximate composition of flours (or starches) from different botanical sources

Composition (% db)	Crude protein (% db)	Crude fiber (% db)	Total lipid (% db)	Ash (% db)	Carbohydrate (% db)	Starch (% db)	Amylose (% db)	Phosphorus	Reference
Flour									
Barley	13.20	-	1.80	1.70	-	67.60	-	-	Eliasson (2006).
Corn	5.18 - 7.82	0.42 - 0.62	1.56 - 2.42	0.19-1.66	87.60-92.50	75.30	9.30	-	Ratnayake *et al.* (1989).
Wheat	10.10 - 13.20	-	0.80 - 1.40	1.90	74.30	-	27.20	-	Huang *et al.* (2007b).
Potato	1.70	-	0.30	1.60	83.20		9.1 -10.80	-	Huang *et al.* (2007b).
Cassava	2.29	1.71	-	1.89		71.50	21.45	-	Aryee *et al.* (2006).
Legume			-	-					
jack bean	26.20	1.07	1.95	6.51	57.83	-	-	-	Olalekan and Bosede (2010).
Cowpea	24.13	0.97	4. 37	4.73	56.60	-	-	-	Olalekan and Bosede (2010).
Chickpea	18.50 - 24.90	9.88 - 22.90	6.69 - 6.80	2.90-3.15	54.00	-	23.10	-	Huang *et al.* (2007a).
Pigeon pea	24.46	1.10	4.78	4.58	56.63	-	-	-	Olalekan and Bosede (2010).
Field pea	25.90	-	1.90	3.07	-	-	-	-	Ratnayake *et al.* (1989).
Bambarra groudnut	11.51	15.48	2.54	7.90	4.19	58.38	-	-	Sirivongpaisal (2008).
Lentil	11.20	20.60	6.83	2.15	2.80	56.40	-	-	Sirivongpaisal (2008).
Starch								-	
Barley	0.10 - 0.20	-	0.60 - 0.80	0.10-0.20	-	96.80	28.80 -45.70	-	Vasanthan and Bhatty (1996).
Corn	1.21	-	1.22	0.36	-	89.27	25.60	0.02-0.03(%)	Mishra and Rai (2006). Galliard and Bowler (1987)
Wheat	0.1-0.6	-	0.7-1.2	0.1-0.4	-	-	27.00-31.00	-	Vandeputte and Delcour (2004)
Potato	0.61	-	0.32	0.19	-	89.44	24.95 -28.10	0.1 (%)	Gunaratne and Hoover (2002); Mishra and Rai (2006); Zaidul *et al.* (2008); Huang *et al.* (2007a).
Cassava	0.51	-	0.51	0.20	-	90.99	16.27	-	Gunaratne and Hoover (2002); Mishra and Rai (2006).
Legume									
Cowpea	0.49	-	0.15	-	-	93.10	25.80 -33.00	-	Huang *et al.* (2007b); Agunbiade and Longe (1999); Chung *et al.* (1998); El-Faki *et al.* (1983).
Chickpea	0.57	-	0.10	-	-	94.00	27.20 -35.00	-	Miao *et al.* (2009); Huang *et al.* (2007a, 2007b); Hoover and Ratnayake (2002)
Yellow pea	0.52	-	0.07	-	-	92.30	31.20	-	Schoch and Maywald (1968).
Field pea	0.19	-	0.28	0.33	-	32.70	49.10	-	Kaur *et al.* (2007b).; Ratnayake *et al.* (1989).
Pigeon pea	0.31	-	0.11	-	-	-	27.00 -46.40	-	Hoover *et al.* (1993); Singh *et al.* (1989); El-Tinay *et al.* (1983); Olalekan and Bosede (2010). .
Jack bean	0.31	-	0.12 - 0.14	-	-	-	37.50	-	Lawal and Adebowale (2005); Betancur *et al.* (2002); Su *et al.* (1997); Hoover and Sosulski (1985a); Kaur *et al.* (2007a).
Mung bean	0.06 - 0.37	-	0.16 - 0.17	0.06-0.11	-	-	27.00-45.30	-	Su *et al.* (1997); Ohwada *et al.* (2003); Schoch and Maywald (1968); Hoover *et al.* (1997)
Lentil	0.19 - 0.56	-	0.01 - 0.09	0.06	-	-	23.50 -32.30	-	Huang *et al.* (2007a); Hoover and Ratnayake (2002); Schoch and Maywald (1968); Hoover *et al.* (2010)
Moth bean	0.37	-	0.16		-	-	27.00	-	Kevate *et al.* (2007).
Navy bean	0.25 - 0.31	-	0.09 - 0.60	0.05	-	-	28.60 -41.40	-	Zhou *et al.* (2004); Hoover and Ratnayake (2002); Su *et al.* (1997); Hoover and Sosulski (1985a); Schoch and Maywald (1968).
Pea (smooth)	0.43	-	0.02 - 0.08	-	-	-	24.00 -49.00	-	Chung *et al.* (2008a); Huang *et al.* (2007a).
Pea (wrinkled)	0.50	-	0.80 - 0.84	-	-	-	-	-	Zhou *et al.* (2004); Ratnayake *et al.* (2001); Schoch and Maywald (1968).
Lima bean	0.24	-	0.10	0.13	-	-	-	-	Schoch and Maywald (1968).
Grass pea	0.18 - 0.68	-	0.04 - 0.05	0.15 - 0.18	-	-	37.95 -38.30	-	Jayakody *et al.* (2007).

On general basis, phosphorous content of tuber and root starches has been reported at 0.089 % db and 0.008 % db, respectively (Table 2.1). Starches also contain lower than 0.4 % of minerals such as calcium, magnesium, phosphorous, potassium and sodium. All these minerals with the exception of phosphorous are of little significance. This is because phosphorous is one of the non-carbohydrate contents which affects the functional properties of starches. About 70 % of total phosphorous content is attached to C-6 of the glucose residue (Noda *et al*., 2005). Phosphorous in starch is presented in the form of phosphate monoesters and phospholipids (Craig *et al*., 1989; Schoch, 1942). The phosphate monoesters are covalently bounded to amylopectin fraction of starch, resulting in its role in increasing starch paste clarity and viscosity. The presence of phospholipids results in less opaque and lower viscosity in pastes (Craig *et al*., 1989; Schoch, 1942). Phosphate groups, which are esterified with amylopectin fraction of potato starch, contribute to high viscosity as well as high transparency, water binding capacity and freeze thaw stability (Craig *et al*., 1989; Swinkels, 1985). The phospholipid content is directly proportional to amylose content in starch (Morrison *et al*., 1993). Additionally, legume starch contains phosphorous in the form of phytic acid (Cheryan, 1980). Phytic acid, principle storage form of phosphorous in plant grains, has been known to interact with specific intracellular proteins (Reddy *et al*., 1982). Salt from of phytic acid is phytate, myoinositol 1,2,3,4,5,6- hexakisphosphate. Phytic acid, with six reactive phosphate groups, is powerfully negatively charged over a broad pH range (Swinkels, 1985), Therefore, it has tremendous potential for binding positively charged molecules (Galliard and Bowler, 1987). It can form complex with minerals and protein and starch in acidic medium (Cheryan, 1980). A structure of this complex was proposed by Thompson in 1986 (Figure 2.1).

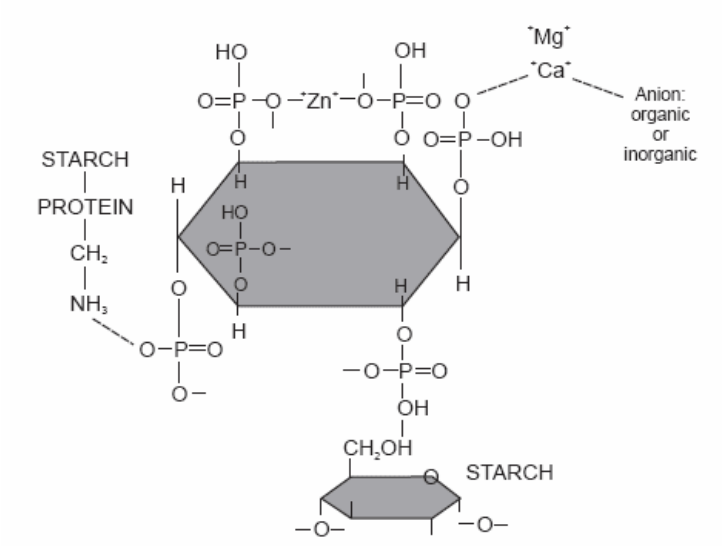


Figure 2.1 A proposed structure of phytate-protein-starch complex. (Thompson, 1986; and Kies, 1998)

2.2.2 Flour and starch structure

Starch is composed of two types of glucose polymers: amylose and amylopectin (Eliasson, 2006). Amylose is usually a linear chain with α-1,4 linkage (Hoover *et al*., 2010). Amylopectin is a highly branched chain with both α-1,4 and α-1,6 linkage (Hoover *et al*., 2010). However there are some amylose with branched chains, though only a small extent with α-1,6-D-glucopyranose (Preiss, 2004; Hoover *et al*., 2010). This only happens one per 170 to 500 glucosyl units (Preiss, 2004). Each branch within amylopectin chain has 15 to 30 α-1,4 linkages (Hutton, 2002), while α-1,6 linkages occur at every 24 to 30 glucose units (Eliasson, 2006). Mainly, amylose consistutes of 840 to 22,000 units of α-D-glucopyranosyl residues linked by α-1,4 bonds. Though, there are variations within published literature pertaining molecular weight of amylose. Amylopectin contains periodic branches which are connected to a backbone by α-D-(1,6)-glucosidic bonds (Wurzburg, 1986), each branch has about 20-30 AGU units. Molecular weight of amylopectin is typically 0.5-5.0 x 10^8 Da and approximately 10^2-10^3 times larger than that of amylose (Zobel, 1988). In normal starches, amylose has a constitution of about 15-30 % of total starch content (Tester and Karkalas, 2002). Amylopectin is the major component of starch, usually within the range of 70% -80%wt. Waxy starches have approximately 0 to 5 % amylose, in comparison to high amylose content starches where the range is 35 to 70 % (Shannon and Garwood, 1984). Legume starch has 21.67 % to 49.0 % amylose content and is classified in normal to high category (Table 2.1).

2.2.2.1 Granule shape and size

Shape and size of granules depend on the source, allowing botanical source identification by microscopic examination of starch (Swinkels, 1985 and Preiss, 2004). Starch granules vary in size (1–100 μm in diameter), shape (oval, round, lenticular, and polygonal, size distribution (uni or bi modal), association as individual or granule clusters and composition (Banks and Greenwood, 1975; Blanshard, 1987; Buléon *et al*., 1998; Fredriksson *et al*., 1998; Gallant and Bouchet, 1986; Galliard and Bowler, 1987; Kent and Evers, 1994; Lineback, 1984; Morrison and Karkalas, 1990; Swinkels, 1985; Tester and Karkalas, 2002; Zobel and Stephen, 1996). The broad range of particle size distribution as well as effects of granule size on composition, properties, structure and applications have gained quite an attention. Small granule fractions of potato, sweet potato and normal maize starches show lower amylose content compared to their larger granule fractions (Jane and Shen, 1993; Chen *et al*., 2003). The phosphorous content in potato starch is inversely proportional to granule size (Jane and Shen, 1993; Chen *et al*., 2003). Granule size varies from tiny granules in rice to large granules in potato starch. Generally, starches from cereals contain smaller granules than those from tuber and root (Swinkels, 1985).

The two populations of granule size distributions in wheat potato rye and barley are classified as A-granules (> 10 mm) and B-granules (< 10 mm) and differ somewhat in their physicochemical characteristics and end-use potential (Anjum and Walker, 1991; Whistler and BeMiller, 1987; Galliard, 1987; Radley, 1976; Morrison and Scott, 1986; Dengate and Meredith, 1984; Vasanthan *et al*, 2001). The proportion of small and large granules differs among genotypes (Eilasson and Larsson, 1983). Raeker *et al*. (1998) reported that wheat starch actually

showed a trimodal rather than a bimodal granule distribution. An intermediate (underdeveloped A-type) granule was mentioned as constituting the third group. However, most of the time wheat endosperm is said to contain just two types of starch granules. A-type granules are 10–35 mm in diameter and account for more than 70 % of the total starch weight but less than 10 % of the granules by number. B-type granules account for over 90% of the granules by number, but less than 30 % of the total starch by weight in wheat endosperm (Raeker *et al*., 1998; Morrison and Scott, 1986; Eilasson and Larsson, 1983; Peng *et al*, 1999).

Table 2.2 shows that most of the granules of legume starch are oval. Although spherical, round, elliptical and irregularly shaped granules are also found in different sources. Round and spherical shaped granules are also found in legume starch granule, though most are composed of simple granules. The granule shape of tuber, root and cereal starches are lenticular, spherical, polyhedral and irregular. Granule size ranges of barley, wheat, potato, cassava and legumes are 2-25 µm, 1-40 µm, 5-100 µm and 5-85 µm accordingly. Flours and starches granule have different shapes and sizes, from Table 2.2 the shape of legume granules are mostly oval to round and spherical. Cereal granules are mostly round to polygonal, while potato granules have large oval shape at the granule size of 5-100 µm. This is considered largest compared to other starch granules. The sizes of legume granules are within 5-85 µm, similarly large to that of potato. Furthermore, granules from barley and wheat have two size distributions, which are large and small with diameter at 2-25 and 1-10 µm, respectively.

Table 2.2 Shape and size of starch.

Starch source	Shape	Size range (μm)	Reference
Barley	Lenticular (A-type), Spherical (B-type)	15-25, 2-5	Tester and Karkalas (2002)
Potato	Lenticular/oval, spherical	5-100	Tester and Karkalas (2002)
Cassava	Oval, round	5-45	Tester and Karkalas (2002); Sriroth *et al.* (1999)
Cereal			
Maize	Round, polygonal	3-26	Gallant and Bouchet (1986)
Wheat	Lenticular (A-type)/ Spherical (B-type)	15-40, 1-10	Tester and Karkalas (2002)
Sorghum	Round, irregular	7-48	Hoover *et al.* (1997)
Rice	Polygonal, angular	3-8	Tester and Karkalas (2002)
Legume			
Cowpea	Oval, spherical	3-64	Okechukwu and Rao (1996); El-Faki *et al.* (1983)
Chickpea	Oval, spherical	-	Huang et al. (2007a), Singh *et al.* (2004b); Hoover and Ratnayake (2002); El-Faki *et al.* (1983)
Jack bean	Oval, round, elliptical	-	Chung *et al.* (2008a); Zhou *et al.* (2004); Hoover and Ratnayake (2002); Hoover and Manuel (1995); Bhatty (1988)
Pigeon pea	Oval, spherical	15-50	Agunbiade and Longe (1999)
Pea (smooth)	Oval, spherical	7-26	Hoover *et al.* (1997); Naivikul and D'Appolonia (1979)
Pea (wrinkled)	Oval, round	6-28	Lawal and Adebowale (2005)
Grass pea	Oval, round, irregular	14-23	Ratnayake *et al.* (2001); Barron *et al.* (2000); Davydova *et al.* (1995); Bertoft *et al.* (1993)
Navy bean	Round	-	Zhou *et al.* (2004); Colonna *et al.* (1982)
Moth bean	Oval, round, elliptical, irregular	34-35	Korus *et al.* (2008); Jayakody *et al.* (2007)
Horse gram	Oval, round	15-85	Hoover and Ratnayake (2002)

Table 2.3 shows chemical compositions, structures and properties of different granule sizes. Large granule has higher amylose content than that of smaller granules, which can be observed from fractionated potato. Large granules have cuboid/irregular shapes, while small granules has spherical/oval, also observed from fractionated potato. In wheat, the granules are lenticular when large

and spherical when small. Different sizes with same type of starch results in variety of chemical properties. Swelling power is decreased while the size of granule increases. However, solubility is directly proportional to granule size.

It was found that different granule size fraction separated from the same source possessed different amylose and phosphorus contents, and percentage relative crystallinity resulting in difference in properties (Table 2.3). For potato, wheat (normal and waxy), normal barley, and chickpea starches, amylose content increase with granule size. The opposite direction was found for yellow pea and cowpea. Phosphorus content of waxy wheat starch increased with granule size, while potato starch was opposite. Swelling power was found to inversely relate to amylose content except for yellow pea. Enthalpy of gelatinization increased with granule size for barley (normal and waxy) and popcorn starches (Table 2.3).

Table 2.3 Composition, structure and properties of starch of different granule size

Sources	Potato			Wheat		Normal barley			Waxy barley		
	Large	Medium	Small	Large	Small	Large	Medium	Small	Large	Medium	Small
Composition											
Amylose content (%)	22.46-28.24[a] 20.70[b]	19.58-26.98[a] 20.00[b]	18.22-19.32[a] 20.20[b]	34[g]	27[g]	25.3-25.9[cf]	24.6-25.7[cf]	20.3-22.4[cf]	2.8[e]	-	3.2[e]
Phosphorus content (ppm)	756[b]	935[b]	1114[b]	-	-	-	-	-	-	-	-
Structure											
Size (%)	-	-	-	-	-	(87.5[i])	(5.5[i])	(7.1[i])	(82.0[i])	(7.6[i])	(10.4[i])
- Range(μm)	40-65[a] 39.9-43.7[b]	20-40[a] 20.3-23.4[b]	1-20[a] 13.4-14[b]	15-40[h]	1-10[h]	7.7-44.9[d]	5.1-26.1[d]	0.9-5.1[d]	7.0-39.2[i]	3.5-7[i]	1.5-3.5[i]
- Average size (μm)	-	-	-	-	-	18.4[d]	12.3[d]	2.2[d]	17.2[i]	9.2[i]	2.0[i]
Shape	Cuboid/ Irregular[a]	Cuboid/ Irregular[a]	Spherical/ Oval[a]	Lenticular[h]	Spherical[h]	Oval/ Round[e]	Oval/ Round[e]	Oval/ Round[e]	Oval/ Round	Oval/ Round	Oval/ Round
Crystallity											
- Relative crystallinity (%)	-	-	-	32.4-36.4[g]	35.5-38.9[g]	25.5-27.4[f]	21.4-22.6[f]	23.3-25.0[f]	36.6[g]	-	33.0[g]
- Crystalline pattern	A[a]	A[a]	A[a]	A[h]	A[h]	A[d]	A[d]	A[d]	A[i]	-	A[i]
Properties											
Swelling power (g/g) at 90°C	22.54-27.29[a]	26.32-30.56[a]	30.43-31.69[a]	8.2[h]	10[h]	13.3-15.3[f]	15.0-16.3[f]	18.6-18.9[f]	32.0[e]	-	43.0[e]
Solubility at 90°C	-	-	-	-	-	-	-	-	-	-	-
Solubility in DMSO(%)	66.0-82.5[a]	64.0-77.0[a]	53.0-66.0[a]	-	-	-	-	-	-	-	-
Hydrolysis rate(%)	0.66-0.83[a]	0.87-1.12[a]	1.35-1.52[a]	-	-	9.1-10.5[f]	9.6-11.2[f]	10.2-11.6[f]	-	-	-
Syneresis (%)	-	-	-	-	-	10.6-13.3[f]	13.5-14.3[f]	17.4-19.4[f]	-	-	-
Thermal properties											
- T_o (°C)	60.2-61.5[b]	60.5-61.0[b]	59.7-60.7[b]	45.93[k]	50.33[k]	54.0.55.6[d]	52.0-53.9[d]	52.2-54.4[d]	59.7[i]	58.9[i]	60.5[i]
- T_p (°C)	64.4-65.6[b]	65.2-66.2[b]	66.7-67.0[b]	60.15[k]	64.61[k]	60.9-62.1[d]	61.2-62.6[d]	63.6-64.5[d]	64.3[i]	64.4[i]	67.6[i]
- T_e (°C)	-	-	-	87.17[k]	89.03[k]	67.0-67.1[d]	68.1-69.1[d]	69.0-69.7[d]	70.5[i]	71.6[i]	77.2[i]
- ΔT (°C)	-	-	-	11.06[k]	8.12[k]	11.0-12.5[d]	14.2-16.9[d]	15.3-17.5[d]	10.8[i]	12.7[i]	16.7[i]
- ΔH (J/g)	19.2-20.0[b]	19.1-20.3[b]	19.4-20.9[b]	-	-	7.5-7.7[d]	5.5-5.8[d]	5.0-5.4[d]	12.1[i]	11.2[i]	10.1[i]
- Regelatinization (%)	-	-	-	35.9[g]	24.8[g]	45.1[g]	-	37.1[g]	-	-	-
Pasting property											
- Peak viscosity (mPa·s)	2828-3384[a]	2685-3109[a]	2516-2663[a]	1677.6[g]	1171.2[g]	1683.6[g]	-	1510.8[g]	-	-	-
- Breakdown (mPa·s)	843-1362[a]	742-115[a]	555-761[a]	648.0[g]	225.6[g]	499.2[g]	-	163.2[g]	-	-	-
- Final viscosity (mPa·s)	2520-3377[a]	2410-2995[a]	2365-2697[a]	2241.6[g]	1863.6[g]	2235.6[g]	-	1922.4[g]	-	-	-
- Setback (mPa·s)	535-1355[a]	467-1049[a]	405-795[a]	1210.8[g]	918.84[g]	1050.0[g]	-	573.6[g]	-	-	-
- Peak temperature (°C)	68.2-72.6[a]	70.4-73.1[a]	74.1-74.4[a]	-	-		-		-	-	-
- Pasting temp. (°C)	-	-	-	85.0[g]	92.0[g]	88.0[g]	-	94.3[g]	-	-	-
Rheological properties											
- G′ (Pa)	3080-4010[a]	2915-3170[a]	2490-2705[a]	-	-	-	-	-	-	-	-
- G″ (Pa)	375-433[a]	370-401[a]	326-356[a]	-	-	-	-	-	-	-	-
- tanδ	0.107-0.122[a]	0.12-0.127[a]	0.131-0.137[a]	-	-	-	-	-	-	-	-
- G* (Pa)	3105-4060[a]	2930-3205[a]	2515-2725[a]	-	-	-	-	-	-	-	-
- η* (Pa s)	32.7-39.7[a]	29.5-32.4[a]	26.0-28.2[a]	-	-	-	-	-	-	-	-
- ή (Pa s)	3.3-4.2[a]	2.9-3.2[a]	2.4-2.7[a]	-	-	-	-	-	-	-	-

[a] Kaur *et al*. (2007); [b] Noda *et al*. (2005); [c] Taketa *et al*. (1999); [d] Tang *et al*. (2001a); [e] Vasanthan and Bhatty (1996); [f] Tang *et al*. (2004); [g] Ao and Jane (2007); [h] Blazek *et al*. (2009); [i] Tang *et al*. (2002).

Table 2.3 Composition, structure and properties of starch of different granule size (continue).

Sources	Waxy wheat		Pop corn			Yellow pea			Cowpea		Chickpea	
	Large	Small	Large	Medium	Small	Large	Medium	Small	Large	Small	Large	Small
Composition												
Amylose content (%)	3.4-3.5[j]	0.6-1.3[j]	24.4[l]	22.4[l]	25.1[l]	25.8[m]	27.9[m]	28.4[m]	22.5[m]	25.0[m]	30.2[m]	25.6[m]
Phosphorus content (ppm)	100.0-130.0[j]	44.0-82.0[j]	-	-	-	-	-	-	-	-	-	-
Structure												
Size (%)	(61.7-75.5[j])	(15.2-28.5[j])	-	-	-	(14.0[m])	(61.0[m])	(25.0[m])	(9.0[m])	(91.0[m])	(27.0[m])	(73.0[m])
- Range(μm)	-	-	-	-	-	-	-	-	-	-	-	-
- Average size (μm)	-	-	13.42[l]	13.64[l]	12.77[l]	32.1[m]	27.3[m]	20.2[m]	16.1[m]	15.7[m]	20.4[m]	17.2[m]
Shape	-	-	Oval/ Polyhedral[l]	Oval/ Polyhedral[l]	Oval/ Polyhedral[l]	-	-	-	-	-	-	-
Crystallity												
- Relative crystallinity (%)	-	-	-	-	-	-	-	-	-	-	-	-
- Crystalline pattern												
Properties												
Swelling power (g/g) at 90°C	-	-	16.4[l]	17.3[l]	16.7[l]	20.4[m]	19.5[m]	22.0[m]	25.3[m]	33.9[m]	24.7[m]	28.6[m]
Solubility at 90°C	-	-	18.2[l]	18.8[l]	18.3[l]	-	-	-	-	-	-	-
Solubility in DMSO(%)	-	-	-	-	-	-	-	-	-	-	-	-
Hydrolysis rate(%)	-	-	-	-	-	-	-	-	-	-	-	-
Syneresis (%)	-	-	-	-	-	-	-	-	-	-	-	-
Thermal properties												
- T_o (°C)	57.0-57.3[j]	55.8-56.4[j]	68.1[l]	68.1[l]	67.9[l]	-	-	-	-	-	-	-
- T_p (°C)	64.7-65.5[j]	65.9-66.5[j]	71.9[l]	71.9[l]	71.9[l]	-	-	-	-	-	-	-
- T_e (°C)	74.6-75.5[j]	77.8-78.5[j]	76.9[l]	76.5[l]	77.2[l]	-	-	-	-	-	-	-
- ΔT (°C)	15.1-15.4[j]	12.9-13.6[j]	7.6[l]	7.6[l]	8.0[l]	-	-	-	-	-	-	-
- ΔH (J/g)	-	-	10.2[l]	9.5[l]	9.7[l]	-	-	-	-	-	-	-
- Regelatinization (%)	-	-	17.3[l]	14.9[l]	16.7[l]	-	-	-	-	-	-	-
Pasting property												
- Peak viscosity (mPa·s)	273.8[j]	163.9[j]	-	-	-	-	-	-	-	-	-	-
- Breakdown (mPa·s)	179.2[j]	88.1[j]	-	-	-	-	-	-	-	-	-	-
- Final viscosity (mPa·s)	139.2[j]	96.0[j]	-	-	-	-	-	-	-	-	-	-
- Setback (mPa·s)	44.5[j]	20.4[j]	-	-	-	-	-	-	-	-	-	-
- Peak temperature(°C)	-	-	-	-	-	-	-	-	-	-	-	-
- Pasting temp. (°C)	-	-	-	-	-	-	-	-	-	-	-	-
Rheological properties												
- G′ (Pa)	-	-	5354[l]	3620[l]	2172[l]	-	-	-	-	-	-	-
- G″ (Pa)	-	-	920[l]	438[l]	393[l]	-	-	-	-	-	-	-
- tanδ	-	-	0.172[l]	0.122[l]	0.181[l]	-	-	-	-	-	-	-
- G* (Pa)	-	-	-	-	-	-	-	-	-	-	-	-
- η* (Pa s)	-	-	-	-	-	-	-	-	-	-	-	-
- ή (Pa s)	-	-	-	-	-	-	-	-	-	-	-	-

[j] Geera *et al*. (2006); [k] Chiotelli a nd Maste, (2002); [l] Sandhu *et al*. (2004); [m] Huang *et al*. (2007a).

2.2.2.2 Crystalline structures

The crystalline structures of starch are identified and classified into A, B, or C type through X-ray diffraction patterns. The differences between A and B crystalline structures are related to packing of double helices unit cells and quantity of water molecules stabilizing them (Figure 2.2). Type C crystalline is a mixture of A and B (Tang *et al*, 2006). Double helices of A-type crystalline forms in a monoclinic lattice with unit cell parameters of 2.124 nm wide, 1.172 nm thick and 1.069 nm high with α and β being 90° while λ equals 123.5°. The crystalline has maltotriose as a repeating unit and four water molecules, 3.6%, per unit cell as shown in Figure 2.2 A. B-type crystalline forms in a hexagonal lattice with unit cell parameters of 1.85 nm wide as well as thick and 1.04nm in height with α, β and λ being 90°, 90° and 120°, respectively. B-crystalline has more "opening" between the packing of double helices and a maltose moiety as a repeating unit as well as 36 water molecules, 25%, per cell unit. This is shown in Figure 2.2 B (Imberty *et al*., 1991).

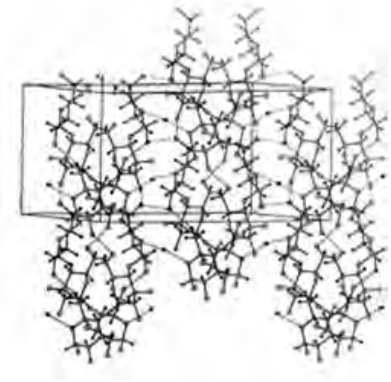
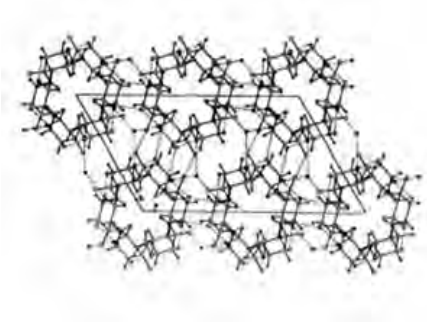
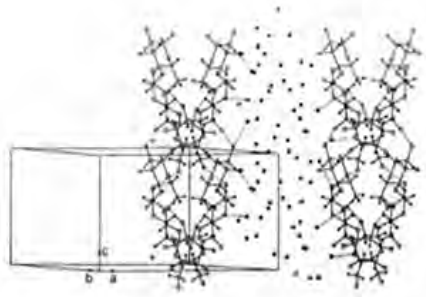
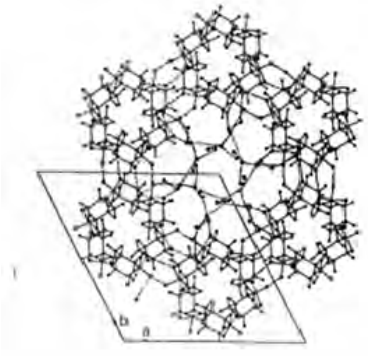
Crystalline type	Dimensional representation along the fiber axis.	The projection of structures onto the A- B plane.
A		
B		

Figure 2.2 Three dimensional crystalline structures for crystalline type A and B.
- - - represents hydrogen bonds between two strands of double helices.
• represents water molecules.
(Imberty *et al*., 1991).

Figure 2.3 shows the X-ray diffraction patterm of cereal starch, such as corn, wheat and rice, gives a typical A type diffraction crystalline with strong diffraction peaks at 2θ with values of 17°, 18° and 23° (Singh *et al*., 2006). Tuber starch, such as canna and potato, shows a typical B crystalline with strong diffraction peak at 17° as well as few small peaks at 20°, 22° and 24° (van Soest, 1995). Crystalline type C gives a diffraction pattern consisting of A and B. Examples of such starches with C type crystalline are from sweet potato and cassava (Zobel, 1988;

Huang, 2007). Table 2.4 shows X-ray diffraction characteristics for these three crystalline types (Hizukuri, 1996b). A diffraction pattern that shows a small reflection at 12.9° and a larger on at 19.88° is corresponding to V-polymorph by lipid amylose complex (Mondragán *et al*., 2004; Bultosa and Taylor, 2003).

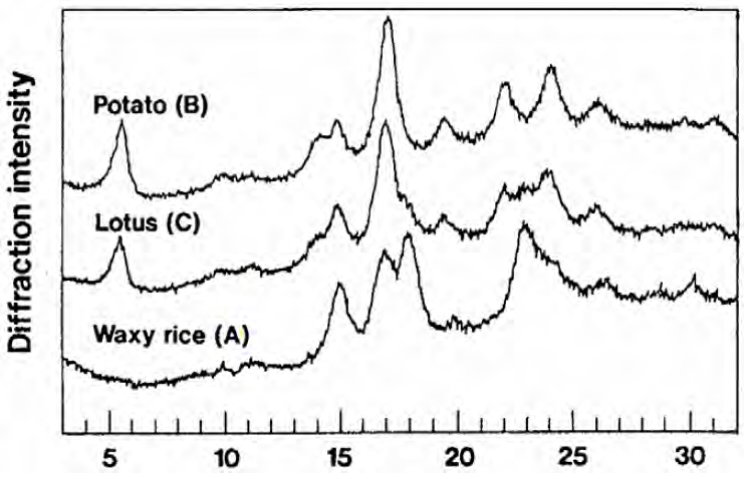


Figure 2.3 X-ray diffraction pattern for crystalline type A, B, and C. (Hizukuri, 1996b).

Table 2.4 X-ray diffraction peak characteristics for crystalline type A, B, and C.

Crystalline type	Peak characteristics	
	Strong peak	Weaker peak
A	15.18°, 17.13°, 18.03°, 22.86° 2θ (5.83, 5.17, 4.91, 3.89 Å)	11.49°, 20.06°, 26.69°, 30.36° 2θ (7.70, 4.42, 3.34, 2.94 Å)
B	6°, 17.16° 2θ (14.71, 5.16 Å)	15°, 21.82°, 24°, 34°, 58.29° 2θ (5.90, 4.07, 3.7, 2.63, 1.58 Å)
C	17.2°, 18.1°, 23.1° 2θ (5.15, 4.98, 3.85 Å)	15.18°, 5.54° 2θ (5.8, 15.7 Å)

(Horng, 2007; Hoover and Sosulski, 1985; Bogracheva *et al*., 1998; Vansteelandt and Delcour, 1999; Protserov *et al*., 2000; Ratnayake *et al*., 2001; Bultosa and Taylor, 2003; Bauer *et al*., 2005).

Typically legume starch has strong intensity peaks at approximately 15°, 17° and 23° 2θ, such similar pattern has been reported in various studies. Black, kidney, navy, northern and pinto beans have strong intensity peaks at 15°, 17° and 23° 2θ (Hoover and Sosulski, 1985). Field pea starch has strong intensity peaks approximately at 15°, 17° and 23° 2θ with a weak intensity peaks at 5° 2θ (Ratnayake *et al*., 2001). According to research by Bogracheva *et al.* (1999), pea starches have a weak intensity peak at 5.6° 2θ, which is characteristically B-type polymorph, strong peak at 17.9° 2θ, characteristically A-type polymorphs, and another peak at 17° 2θ a combination of A and B type polymorphs. The position of small peaks in pea starches could be anywhere between 5 and 6 degree goniometer angle and various 2θ angle locations as per reported by Ratnayake *et al.* (2002). Ratnayake *et al*., (2002) also reported that the variation is affected by the instrumental settings and experimental conditions. There has been a report on crystal sub-types, which are divided into C_a, C_b and C_c (Hizukuri, 1961). This distinction is based on the relative intensity of the peaks referred to as 4a and 4b with 2θ values corresponding to 17 and 18 degrees. There is one relative intensity line at 2θ of 5° as well as the shape or profile of lines on either side of the 6-peaks with values 23° 2θ. With the above, it can be concluded that each type of starch can be classified as 4b/4a > 2/3 (C_a), weak 1-line (C_c) and concave 6-lines (C_b) (Houng, 2007). The relevant data for the legume starches is shown in Table 2.5. The details on crystalline sub-types classification for legume starches is shown in Table 2.5.

Table 2.5 Classification of crystalline sub-types for legume starches according to X-ray diffraction peak characteristics.

Starch	Relative intensity of 4a-and 4b-lines	Intensity of 1-line	Line profile of 6-lines	Crystal type
Chickpea	>2:3	Absent	Convex	C_a
Faba bean	>2:3	Absent	Convex	C_a
Lentil	>2:3	Absent	Convex	C_a
Mung bean	>2:3	Absent	Convex	C_a
Black bean	>2:3	Absent	Convex	C_a
Northern bean	4b-line absent	Absent	Convex	C_b
Navy bean	>2:3	Absent	Convex	C_a
Kidney bean	>2:3	Absent	Convex	C_a
Pinto bean	>2:3	Absent	Convex	C_a

(Horng, 2007; Hoover and Sosulski, 1985)

The amount of crystallinity within starch granules was estimated through intensity of X-ray diffraction and accessibility of deuterium (Horng, 2007). It should be noted that X-ray determinations of crystallinity, although not discussed separately, includes determination of 'absolute' and 'relative' crystallinity (Blanshard, 1987; Buléon *et al.*, 1998; Tester and Karkalas, 2002). Absolute amount of crystallinity differentiates between the amorphous and crystalline components, while relative amount relies on calculating proportion of crystallinity with starch granules as references. Fully amorphous material, example freeze-dried gelatinized material, has 0% crystallinity. The 100% reference is usually generated by extensive acid hydrolysis of starch in which all amorphous but crystalline materials have been eroded. Chiotelli and Le Meste (2002) has shown that with X-ray diffraction A- type

granules are somewhat more crystalline than B-type. Difficulty in interpreting such data lies in the differences in apparent crystallinity of starch determined by X-ray diffraction and can be contributed to varying water content among starch types.

Table 2.6 shows crystalline pattern and relative crystallinity for various starches. The crystal structure of barley, corn and wheat are A-types with crystallinity in the range of 36 % to 40 %. Cassava and potato have B-type crystal structure and crystallinity from 25 % to 44 %. Legume starch mostly has C-type crystal structure and crystallinity from 17.7 % to 45.0 %, while in swrinkled pea has B-type crystal and lowest crystallinity at 17.7 %. Crystallinity differences among legume starches could be influenced by crystallite size, number of crystallites that are arranged in a crystalline array, moisture content, and polymorphic content (Hoover *et al*., 2010).

Table 2.6 Crystalline pattern and relative crystallinity of starches from different botanical sources.

Starch source	Crystalline pattern	Relative crystallinity (%)	Reference
Barley	A	22.0-27.4	Tang *et al*. (2001a)
Corn	A	40.0	Wang *et al*. (2003)
Wheat	A	36.0-39.0	Kim and Huber (2010)
Potato	B	30.0	Gunaratne and Hoover (2002)
Cassava	B	37.0	Gunaratne and Hoover (2002)
Legume			
Cowpea	C	-	Okechukwu and Rao (1996); El-Faki *et al.* (1983).
Chickpea	C	23.0- 27.6	Huang *et al.* (2007a); Singh *et al.* (2004b); Hoover and Ratnayake (2002); El-Faki *et al.* (1983)
Yellow pea	C	-	Huang *et al.* (2007a)
Field pea	C	36.0-45.0	Wang *et al*. (2011)
Pigeon pea	C	33.4	Kaur and Sandhu (2010)
Lentil	C	31.7- 32.3	Chung *et al.* (2008a); Zhou *et al.* (2004); Hoover and Ratnayake (2002); Hoover and Manuel (1995)
Mung bean	A or C	29.1	Hoover et al. (1997); Naivikul and D'Appolonia (1979)...
Jack bean	C	-	Lawal and Adebowale (2005).
Pea (smooth)	C	30.0- 30.3	Ratnayake *et al.* (2001); Barron *et al.* (2000); Davydova *et al*. (1995); Bertoft *et al.* (1993).
Pea (wrinkled)	B	17.7	Zhou *et al.* (2004); Colonna *et al.* (1982).
Grass pea	C	33.0- 34.0	Korus *et al.* (2008); Jayakody *et al.* (2007).
Navy bean	C	27.9	Hoover and Ratnayake (2002).
Pinto bean	C	33.0- 33.4	Zhou *et al.* (2004); Hoover and Ratnayake (2002)

2.3 Flour and starch properties

2.3.1 Starch gelatinization

Starch heated in the presence of excess water undergoes an order-disorder phase transition called gelatinization. The temperature range for this

transition depends on the source of starch (Stevens and Elton, 1971; Donovan, 1979; Biliaderis, 1982; Biliaderis, 1991; Jenkins, 1993). Diffusion of water into granule, water uptake by amorphous phase region, hydration as well as radial swelling of granule, heat uptake, loss of optical birefringence, loss of crystalline order, uncoiling/dissociation of double helices and amylose leaching occur during starch gelatinization (Stevens and Elton, 1971; Donovan, 1979; Biliaderis, 1982; Biliaderis, 1991 and Jenkins, 1993).

Loss of birefringence can be observed under polarized light. Non-gelatinized starch granules show birefringence which results in what refers to as 'maltese cross' pattern (Fitt and Snyder, 1984). When onset temperature (T_o) is reached, the birefringence begins to disappear. One of the most common methods for determining gelatinization temperature range is following the loss of birefringence in excess of water (Moss, 1976). However, this loss occurs over a wide temperature range when water content is decreased.

Loss of crystalline order can be observed during heating through X-ray diffraction. This is because as heated, the diffraction pattern disappears and eventually becomes a pattern indicative of a completely amorphous material (Zobel *et al*., 1988). Temperature range during which crystallinity is lost and rate in which it is lost depend on water content and source of starch (Liu *et al*., 1991; and Svensson and Eliasson, 1995). The temperature range increases with decreasing water content and when water content reaches below 50%, temperature for complete loss crystallinity approaches 100°C. Crystallinity loss seems to occur in two steps. One, the loss occurs at a very low rate. Second, when at a temperature typical of starch the rate increases dramatically (Svensson and Eliasson, 1995).

Endothermic transition is observed using Differential scanning calorimetry, DSC. Starch gelatinization is an endothermic process, which the enthalpy values within the range of 10-20 J/g. DSC has become the most important tool for studying gelatinization of starch to examine the gelatinization transition temperature and the gelatinization enthalpy, ΔH_{Gel}, (Donaovan, 1979; Eliasson, 1980; Atwell *et al*., 1988). DSC parameters are influenced by molecular architecture of the crystalline region (Noda *et al*., 1996). Tester and Morrison (1990) have postulated that ΔH_{Gel} reflects overall crystallinity of amylopectin. Amount of double helical order in native starches should be strongly correlated to the amylopectin content and granule crystallinity should increases with it.

Morphological changes are determined by swelling of starch granules and solubility of macromolecules are the overall effects of gelatinization. The process can be characterized by swelling index and solubility index. High swelling power can possibly relate to sharp consistency increase observed by viscograms within the same temperature range. At the same time, a large amount of soluble material is recovered within the supernatant, indicating high solubility of starch granules. Using light microscopy and electron scanning microscopy, several authors observed morphological changes happening at different temperatures.

Gelatinization properties of starch are related to variety of factors. These factors are size, proportion, crystalline organization and ultra-structure of starch granule. Goering and De Haas (1972) have reported that small granule starch has in general lower pasting temperature than large granule. However, small granule size does not necessarily associated with low pasting temperature. For example dasheen starch has a pasting temperature 20°C higher than that from most other starches' small

granule. Low amylose content of dasheen and the fact that dasheen is a tuber starch make its gelatinization temperature unpredictable. According to Eliasson and Larsson (1983), gelatinization enthalpy of wheat starch is independent of granule size distribution. Though, others have found higher gelatinization enthalpies for A-type than for B-type starch granules in wheat (Peng *et al*., 1999 and Chiotelli and Le Meste, 2002). This lower enthalpy value of B-type gelatinization granules suggests a lower organized structure in B-type than A-type. In another word, it has a lower stability of crystalline regions.

The gelatinization transition temperatures, onset temperature (T_o), peak temperature (T_p) and conclusion temperature (T_c), and gelatinization enthalpy, ΔH_{Gel}, have been shown to be 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 (Noda *et al*., 1996). Cooke and Gidley (1992) have postulated that ΔH_{Gel} reflects the loss of double helical order rather than the loss of crystallinity. However, Tester and Morrison (1990a; 1990b) have postulated that ΔH_{Gel} reflects the overall crystallinity (type and amount of starch crystallites) of amylopectin. In many of the above studies, only a single cultivar has been analyzed. Thus, it is difficult to ascertain whether the DSC parameters truly represent the species in general. Furthermore, due to limited information on amylose chain length and amylopectin branch chain length distribution, it is not possible to discuss the influence of molecular structure on the DSC parameters of legume starches.

Table 2.7 shows gelatinization parameters or thermal properties of starches from various sources. In this case, T_o and T_p of legume starch have higher

gelatinization temperatures than those of barley, corn, wheat, potato and cassava. This is because different types of starch generate different crystalline organization. Barley, corn and wheat have A-type; therefore have higher gelatinization temperature than those of potato and cassava, which are B-type crystalline. However, legume starch, which is a C-type crystalline, has broader ΔH_{Gel} and higher gelatinization temperatures than B-type starches.

Table 2.7 Gelatinization parameters for various starches.

Starch source	Gelatinization parameters (°C)				Reference
	Onset temperature (T_o)	Peak temperature (T_p)	Conclude temperature (T_c)	Gelatinization enthalpy (ΔH_{Gel})	
Barley	50.1–56.1	58.1–64.5	71.0–75.8	9.6–14.2	Li *et al*. (2001)
Corn	62.30	67.70	84.30	14.00	Sandhu and Singh (2007); Singh *et al*. (2003)
Wheat	51.20	56.00	76.00	14.80-17.90	Zhu *et al*. (2009); Singh *et al*. (2003)
Potato	59.72-66.20	62.90-69.60	67.28-75.40	12.55-19.90	Gunaratne and Hoover (2002); Singh *et al*. (2003)
Cassava	64.50	71.00	-	13.00	Gunaratne and Hoover (2002); Sriroth et al. (1999)
Legume					
Cowpea	70.50-72.70	75.40	81.00	10.50-16.90	Huang *et al.* (2007a)
Chickpea	57.90-64.80	63.50-72.50	69.80-81.50	9.20-17.60	Miao *et al.* (2009); Chung *et al.* (2008a); Sandhu and Lim (2008); Huang *et al.* (2007b); Singh *et al*. (2004b); Hoover and Ratnayake (2002)
Yellow pea	58.20	65.10	70.40	12.20	Wang *et al*. (2011)
Field pea	55.90	61.40	66.50	10.60	Kaur and Sandhu (2010)
Pigeon pea	69.30-74.00	75.50-81.10	80.60-87.00	8.80-11.30	Sandhu and Lim (2008); Hoover *et al.* (1993)
Lentil	57.80-65.00	66.80-68.50	71.00-71.50	13.20	Chung *et al.* (2008a); Sandhu and Lim (2008); Zhou *et al.* (2004); Hoover and Ratnayake (2002)
Mung bean	58.00-62.20	67.00-67.40	72.10-82.00	7.90-18.50	Sandhu and Lim (2008); Hoover *et al.* (1997)
Jack bean	76.00	86.00	95.00	-	Lawal and Adebowale (2005)
Pea (smooth)	60.80-63.90	67.40-70.60	73.40-80.10	9.90-13.80	Chung *et al.* (2008b); Zhou *et al.* (2004)
Pea (wrinkled)	117.00	133.00	138.00	2.90	Colonna *et al.* (1982)
Groundnut	71.69	75.33	79.17	11.73	Sirivongpaisal (2008)
Grass pea	66.60-68.30	73.30-75.50	83.20-85.40	14.15-15.32	Jayakody *et al.* (2007)
Navy bean	67.00	69.00	73.50	-	Chung *et al.* (2008b); Hoover and Ratnayake (2002); Su *et al.* (1997)
Pinto bean	70.60-73.30	70.90-76.50	78.40-88.80	12.20-16.20	Zhou *et al.* (2004); Hoover and Ratnayake (2002); Su *et al.* (1997)

2.3.2 Starch retrogrodation

Molecular interactions, hydrogen bonding between starch chains, after cooling of gelatinized starch paste are called retrogadation (Hoover *et al*., 2010). In this phrase, amylose forms double helical associations of 40–70 glucose units, while amylopectin crystallizes through association of the outermost short branches in Figure 2.4 (Jane and Robyt, 1984; Ring *et al*., 1987). Regelatinization enthalphy (ΔH_{Regel}) provides a quantitative meanure for energy required to regelatinize retrograded starch (Karim *et al*., 2000). Endothermic peak for starches after gelatinization and stroge at 4°C appears at a lower transition temperatures. ΔH_{Regel} is usually between 60 – 80% smaller than ΔH_{Gel} because retrograded starch has weaker starch crystallinity (Sasaki *et al*., 2000). Transition temperature during this same phrase is 10-26°C lower than that for gelatinization (White *et al*., 1989; Yuan *et al*., 1993). The crystalline forms are a different nature to those present in native starch granules (Karim *et al*., 2000). Percentage retrogradation is calculated from ΔH_{Regel} to ΔH_{Gel} ratio times 100.

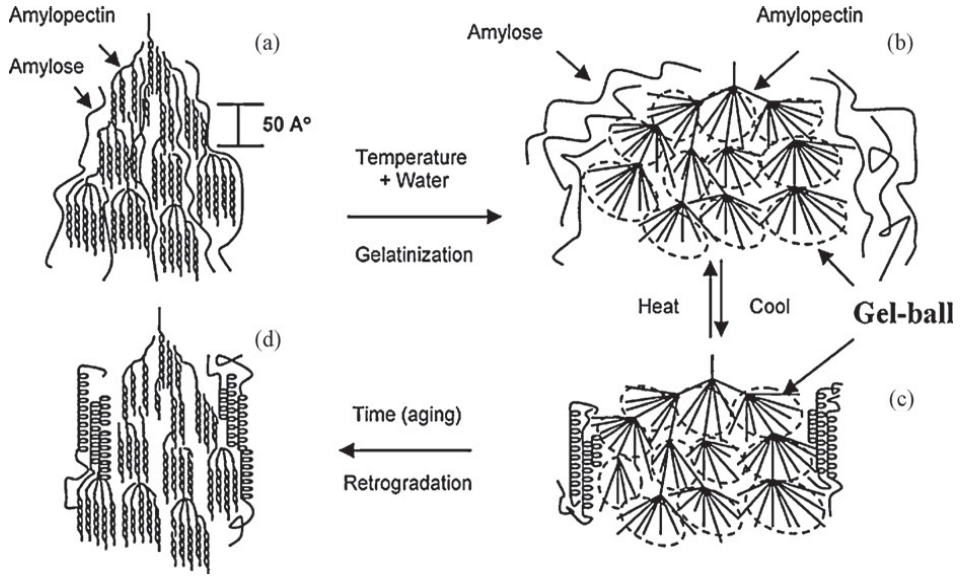


Figure 2.4 Schematic representation for phrase transitions of starch during heating and cooling and aging (Yu and Christie, 2005).

Table 2.8 shows that retrogadation in legume, C-type, is higher than A-type and B-type from corn, wheat, potato and cassava. This is because amylose content of legume starch is higher than other starch sources.

Table 2.8 Percentage retrogradation for various starches.

Starch source	Retrogradation (%)	Reference
Corn	16.70	Sandhu and Singh (2007); Singh *et al*. (2003)
Wheat	24.80	Zhu *et al*. (2009); Singh *et al*. (2003)
Potato	7.10	Gunaratne and Hoover (2002); Singh *et al*. (2003)
Cassava	3.60	Gunaratne and Hoover (2002); Sriroth et al. (1999)
Legume		
Chickpea	50.00	Miao *et al.* (2009); Chung *et al.* (2008a); Sandhu and Lim (2008); Huang *et al.* (2007b); Singh *et al*. (2004b); Hoover and Ratnayake (2002)
Field pea	44.70	Kaur and Sandhu (2010)
Pigeon pea	57.90	Sandhu and Lim (2008); Hoover *et al.* (1993)
Lentil	53.50	Chung *et al*. (2008a); Sandhu and Lim (2008); Zhou *et al*. (2004); Hoover and Ratnayake (2002)
Mung bean	63.20	Sandhu and Lim (2008); Hoover *et al.* (1997)

2.3.3 Water absorption capacity

Starch granules are built up of polymers that are hydrophilic. The granule itself is not soluble in water due to semi-crystalline structure and hydrogen bonds formed between hydroxyl groups within the polymers. This is essential for biological function of starch granule in a plant. This is because if energy is dissolved too early, it cannot be stored as an energy source (Eliasson, 2006). When temperature increases, the amount of water absorption also increases (French, 1984). Chou and Morr (1979) reported that water binding by starches is a function of several parameters such as size, shape**,** conformational characteristics, steric factors**,** hydrophilic – hydrophobic balance within the starch molecule**,** lipid and carbohydrate associated with proteins**,** thermodynamics properties of the system (e.g. bonding

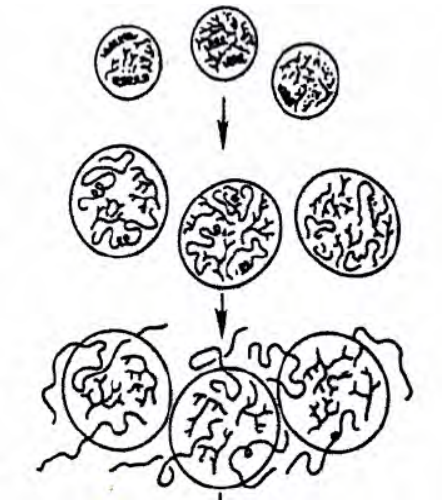
energy and interfacial tension), physicochemical environment (e.g. pH, ionic strength, vapor pressure, temperature, presence or absence of surfactant), and solubility of starch molecules.

The extent of granular swelling has been reported as swelling factor (measures only intragranular water (Tester and Morrison, 1990a) and as swelling power (measures both inter and intragranular water (Leach *et al*., 1959)). Legume starches have been shown to exhibit a single stage restricted swelling and low extent of amylose leaching (Hoover and Sosulski, 1985; Schoch and Maywald, 1968). This is suggestive of strong interactions between starch chains that relax above one temperature and not above multiple temperatures. In most legume starches, no measurable granule swelling or amylose leaching occurs at temperatures below 60°C.

2.3.4 Swelling power, solubility and amylose leaching

Heating starch in the presence of excess water, the crystalline structure is disrupted. This is due to the breakage of hydrogen bonds between the chains; letting water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of amylose and amylopectin. Swelling behavior of starch is the property of its amylopectin content, while amylose acts as a diluent and inhibitor of swelling (Tester and Morrison, 1990). This results in an increase in granule swelling, solubility and amylose leaching as show in Figure 2.5 (Leach *et al*., 1959). Swelling power, solubility and amylose leaching are evidences of interaction magnitude between starch changes within amorphous and crystalline regions. Legume starches have been shown to exhibit a single stage restricted swelling and low amylose leaching extent (Hoover and Sosulski, 1985; Schoch and Maywald, 1968). It is indicative of strong

interactions between starch chains relaxing over one temperature and not over multiple ones. In most legume starches, there are no means of measuring granule swelling or amylose leaching when they occur below 60°C. Increase in temperatures causes swelling and amylose leaching to increase (Table 2.9).



Raw starch granule made up of amylose (helix) and amylopectin (branched).

Addition of water breaks up amylose crystallinity granules and disrupts helices. Granules swell.

Addition of heat and more water causes more swelling. Amylose begins to diffuse out of granule at above gelatinized temperature.

Figure 2.5 Schematic representation of starch gelatinization. (Remsen and Clark, 1978).

Table 2.9 Swelling power, solubility and amylose leaching of flours (or starches) from different botanical sources at different temperature.

Starch source	Swelling power (g/g)				Solubility (%)				Amylose leaching (%)				Reference
	60°C	70°C	80°C	90°C	60°C	70°C	80°C	90°C	60°C	70°C	80°C	90°C	
Potato	37.60	57.40	60.00	54.00	-	13.49	-	-	4.50	18.10	22.00	22.20	Gunaratne and Hoover (2002)
Cassava	4.60	31.00	43.00	36.50	-	14.36	-	-	7.00	15.00 - 7.00	16.60 - 2.50	17.20 - 27.00	Gunaratne and Hoover (2002)
Legume													
Cowpea	4.80	7.20	21.00	27.00	-	-	-	-	-	-	-	-	Huang *et al.* (2007a).
Chickpea	2.90	5.90	8.70	9.80	2.70	4.80	2.50	1.20	1.20 - 5.60	-	4.50- 28.90	5.50 - 36.10	Miao *et al.* (2009); Sandhu and Lim (2008); Huang *et al*. (2007a); Singh *et al.* (2004b); Hoover and Ratnayake (2002).
Yellow pea	-	-	-	-	-	2.00	13.40	20.50	-	-	-	-	Sandhu and Lim (2008); Hoover *et al.* (1993); Singh *et al.* (1989).
Field pea	8.50	13.70	19.40	23.50	-	-	-	-	10.50	16.30	19.60	26.30	Hoover and Ratnayake (2002); Hoover and Manuel (1995); Sandhu and Lim (2008); Chung *et al*. (2008a); Ratnayake *et al.* (2001)
Pigeon pea	1.50	2.50	8.10	11.80	-	-	-	-	-	1.00 - 6.90	11.00 - 17.00	17.50 - 22.50	Sandhu and Lim (2008); Liu *et al.* (2006); Ohwada *et al*. (2003).
Lentil	3.70- 5.00	14.00 - 15.40	22.20 - 23.70	22.50- 24.50	-	6.00	13.90	20.00	4.30 - 4.40	7.40 - 7.80	9.70 - 10.50	10.90 - 11.90	Lawal and Adebowale (2005).
Mung bean	2.20	2.80	6.80	10.40	0.30	0.70	5.00	1.10	3.60	26.70	32.30	35.10	Sandhu and Lim (2008); Huang *et al.* (2007a); Hoover and Ratnayake (2002); Ratnayake *et al.* (2001).
Jack bean	-	-	-	33.90	-	-	-	-	-	-	-	27.62	Korus *et al.* (2008) and Jayakody *et al*. (2007).
Pea (wrinkled)	-	-	-	-	-	2.80	7.80	15.50	-	-	-	-	Chung *et al.* (2008c); Hoover and Ratnayake (2002); Gujska *et al*. (1994); Hoover and Sosulski (1985).
Grass pea	2.20- 2.40	4.20 - 4.80	12.50 - 14.50	22.10 - 27.00	-	-	-	-	-	3.00	22.50 - 20.00	27.50 - 30.50	Jayakody *et al*. (2007)
Navy bean	5.60	10.80	23.30	24.40		0.60	7.50	12.60	1.00- 3.50	7.50- 8.30	8.60 - 18.50	9.00 - 21.90	Gujska *et al*. (1994); Hoover and Ratnayake (2002)
Pinto bean	2.30	2.70	4.50	6.90	0.90	1.10	2.90	5.90	2.00	2.60	7.50	8.00	Anton *et al*. (2008)

It has been reported that amylose acts as both diluents and inhibitor of swelling. This is especially true in the presence of lipids, which can form insoluble complexes with some amylose during swelling and gelatinization (Leach *et al*., 1959; Zeleznak and Hoseney, 1986). Molecule starches are held together by hydrogen bonding in the form of crystalline bundles. Hence, swelling power and solubility patterns of starches have been used as evidence for associative binding force within granules (Leach *et al.*, 1959. When aqueous suspension of starch granules is heated, these structures are hydrated and the swelling takes place. According to research done by Schoch and Maywald in 1968, starches have been classified into high swelling, moderate swelling, restricted swelling or high restricted swelling. High swelling starches have swelling power of approximately 30 g wet gel/g dry gel or higher at 95°C. Their granules enormously swelled and internal bonds become fragile towards shearing when starch is cooked in water.

Table 2.9 shows swelling power and solubility of potato, cassava and legume starches. It is evident that swelling power of potato starch is higher than those of cassava and legume. When comparing the starch compositions, it is found that potato starch has higher phosphorous (Chou and Morr, 1979). Furthermore, legume starch has lower swelling power to both potato and cassava because it contains high level of amylose. This causes stronger structure between the granules resulting in lower swelling as well as lower solubility. At 60-70°C, swelling, solubility and amylose leaching are low because the temperatures are lower than gelatinization of all starches. When increases the temperature, swelling power, solubility and amylose leaching increase. This is because the temperatures become higher than that of gelatinization.

2.3.5 Pasting properties

A paste is a viscous mass with a continuous phase of solubilized amylose and/or amylopectin as well as a discontinuous phase of granule ghosts and fragments (Hoover *et al*., 2010). Pasting specifically refers to changes in starch upon further heating after gelatinization. This results in further swelling and leaching of polysaccharides from the starch granules and increase in viscosity due to application of shear forces (Atwell *et al*., 1988; Tester and Morrison, 1990a, 1990b). Most legume starches demonstrate highest pasting temperature, absence of peak viscosity, increased viscosity during holding period and high set-back in comparison to tuber root and cereals. Legume pasting properties possibly reflects their high amylose content, trace presence of lipid complex amylose chains, strong interaction between starch chains with native granules and orientation of amylose chains relative to another (Hoover and Sosulski, 1985). It is probable that pasting properties are influenced by amylose and amylopectin chain length.

The understanding of legume starches rheology has come mainly from using Rapid Visco Analyzer (RVA, Newport Scientific) in which measurements are made under non-laminar flow conditions and starch paste is subjected to both thermal and mechanical treatments. This, however, makes it difficult to relate its viscous behavior to either one of the parameters. There is a need to extend the use of rheometers, by which thermal treatments are separated from mechanical one, helping to determine rheological characteristics of legume starches under well-defined flow regimes (Hoover *et al*., 2010). Corn, and wheat starches are rich in lipids and show lower breakdown values.

Table 2.10 Pasting properties of starches from different botanical sources.

Starch source	Pasting characteristics					Reference
	Pasting temperature (°C)	Peak viscosity (mPa•s)	Breakdown viscosity (mPa•s)	Final viscosity (mPa•s)	Set back (mPa•s)	
Barley	79.20	1533	254	2183	931	Li *et al*. (2001)
Corn	78.25	2609	818	2530	739	Sandhu and Singh (2007); Singh *et al*. (2003)
Wheat	75.00	1122	372	1902	1152	Zhu *et al*. (2009); Singh *et al*. (2003)
Potato	70.00-73.50	3196-3467	3112	2227	412	Kaur *et al*. (2007); Gunaratne and Hoover (2002); Singh *et al*. (2003)
Cassava	66.20	1769	177	2451	859	Gunaratne and Hoover (2002); Sriroth et al. (1999)
Legume						
Cowpea	80.70	1440	300	-	2535	Huang *et al.* (2007a).
Chickpea	51.40	3942…	897	-	2496	Miao *et al.* (2009); Sandhu and Lim (2008); Huang *et al*. (2007a); Singh *et al.* (2004b); and Hoover and Ratnayake (2002).
Pigeon pea	50.90	4025	967	-	2882	Sandhu and Lim (2008); Hoover *et al*. (1993); and Singh *et al.* (1989).
Lentil	50.30	4637	-	-	605-662	Hoover and Ratnayake (2002); Hoover and Manuel (1995); Sandhu and Lim (2008); and Chung *et al*. (2008a).
Mung bean	80	6107	2523	-	1195	Sandhu and Lim (2008); Liu *et al.* (2006); and Ohwada *et al*. (2003).
Jack bean	84.00	6450	950	-	875	Lawal and Adebowale (2005).
Pea (smooth)	52.50	4398	1159	2752	-	Sandhu and Lim (2008); Huang *et al.* (2007a); Hoover and Ratnayake (2002); and Ratnayake *et al.* (2001).
Grass pea	74.14-74.30	3074-3226	491-806	5004-5622	2269-3349	Korus *et al.* (2008) and Jayakody *et al*. (2007).
Groundnut	77.3	570	175	600	205	Sirivongpaisal (2008)
Navy bean	73.90	2746	891	-	3488	Chung *et al.* (2008c); Hoover and Ratnayake (2002); Gujska *et al.* (1994); and Hoover and Sosulski (1985a).

2.3.6 Rheological properties

Rheological properties involve the flow and deformation characteristics of materials under stress. They are important to understanding structures, handling, processing, masticating and utilizing of foods. Therefore, rheological methods are the appropriate tools for studying the functionality of hydrocolloids (Walter, 1998). By applying rheological measurements and concepts to polysaccharide solutions facilitate acquiring information on molecular size and shape, polymer structure, solvent – polysaccharide interactions as well as inter-molecular networks (Be Miller and Whistler, 1996, Walter, 1998). Information obtained depends on the analysis and solution concentration. Behavior of diluted and concentrated polysaccharide solutions yields different information.

2.3.6.1 Steady shear viscosity

Viscosity is ability to resist flow when force is applied to liquid. For Newtonian liquid, viscosity is defined as ratio of shear stress to shear rate as shown in Equation 2.1 (Ferry, 1980).

$$\eta = \tau / \dot{\gamma} \qquad [2.1]$$

where η = viscosity (Pa.s)

τ = shear stress (Pa)

$\dot{\gamma}$ = shear rate (s^{-1})

Equation 2.2 is general equation for non-Newtonian liquid (Okechukwu and Rao, 1996).

.

$$\tau = \tau_0 + K\dot{\gamma}^n \qquad [2.2]$$

where τ_0 = yield stress (Pa)

K = consistency coefficient ($Pa \cdot s^n$)

n = flow behavior index

2.3.6.2 Viscoelastic properties

Dynamic or oscillatory rheological measurement is the best technique to separate solid-like and liquid-like characteristics of materials. (Steffe, 1996, Ferry, 1980). The results are very responsive to chemical synthesis and physical structure. Behavior of most polysaccharide solutions can be described as viscoelastic, which is somewhere between two extremes of behavior, in phase and out of phase. Value of solid–like and liquid–like behavior in polysaccharide systems are depended upon their relative amounts of elastic and viscous behaviors. For elastic solid, energy used in deformation of elastic solid is recovered as the sample springs back into its original state. For liquid, there is no such recovery and the energy is lost (Walter, 1998 and Steffe, 1996). The ration of in phase stress to applied strain is modulus.

During gelatinization, starch granules swell to several times their initial size and rupture. At that moment, amylose leaches out from inside each granule and forms a three dimensional network (Eliasson, 1985; Tester and Morrison 1990b). Starch exhibits unique viscosity behavior with a change of temperature, concentration and shear rate (Nurul *et al*., 1999). The viscosity can be measured by rheometer pasting curves. Starches which are capable of swelling to high degree are less resistant to breakdown upon cooking and have viscosity decreased significantly

after reaching their maximum value. Dynamic rheometer allows continuous assessment of starch suspension dynamic moduli during temperature and frequency sweep testing. The storage dynamic modulus (G′) is a measure of energy stored in materials, which recovers per cycle. The lost modulus (G″) is a measure of energy dissipated or lost per cycle of sinusoidal deformation (Ferry, 1980). Equations 2.3 and 2.4 define storage modulus and loss modulus, respectively.

$$G' = (\sigma_0 / \gamma_0) \cos(\delta) \quad [2.3]$$

$$G'' = (\sigma_0 / \gamma_0) \sin(\delta) \quad [2.4]$$

where G' = storage modulus
G'' = loss modulus
σ_0 = sheer stress amplitude
γ_0 = strain amplitude
δ = phase shift or phase angle relative to strain

The ratio of energy lost to energy stored for each cycle is defined by tan(δ), which is another parameter which indicates physical behavior of a system (Ferry, 1980).

The effect of granular structure of starch on pasting behavior is reported by Tsai *et al*. (1997) by using a dynamic rheometer. Large and cuboidal or irregular shaped granules in potato starch have higher storage and loss modulus and lower tan(δ) than small and oval ones (Singh and Singh, 2001). Presence of high phosphate monomer content and absence of lipids as well as phospholipids in potato starch can be responsible for high value of G′ and G″. Corn starch has lower G′ and G″ than potato starch. Phospholipids and more rigid granules present in corn starch could be responsible for lower G′. The formation of amylose – lipid complex in corn

starch during gelatinization is the reason behind lower G′ and G″ (Singh *et al*., 2002). Potato starches show higher breakdown in G′ than corn and wheat (Kaur *et al*., 2002). Different breakdown values in starch are possibly affected by granule rigidity, lipid content and peak G′ values. Amylose content is another factor that significantly affects rheological properties of starches. It has been reported there are increase in G′ and G″ of rice starch with increase in amylose content during temperature sweep testing (Lii *et al*., 1996). Higher G′ values for potato starches have higher amylose content (Kaur *et al*., 2002). It has also been reported that starches isolated from waxy potatoes show both lower G′ and G″, while has higher tan(δ) value (Kaur *et al*., 2002).

2.4 Nixtamalization

Nixtamalization, the thermal-alkaline process, is an ancient Mesoamerican, process for preparation of maize/corn flour, in which the whole grain is cooked and then soaked for 12-16 hr in a saturated alkaline solution from either lime, calcium hydroxide or $Ca(OH)_2$, or ash, potassium hydroxide, prior to milling (González *et al*., 2004; Bryant and Hamaker, 1997; Serna-Saldivar *et al*., 1990). This process is also used for pericarp removal from corn and sorghum (Vivas *et al*., 1987; González *et al*., 2004; Laria *et al*., 2007). During nixtamalization, these phenomena occur in the following order (Trejo-Gonzalez *et al*., 1982; Robles *et al*., 1988; Gómez *et al*., 1989; Serna-Saldivar *et al*., 1990; Stylianopoulos *et al*., 1991; Gómez *et al*., 1991; Hernández-Urizar and Bressani, 1997; Martínez-Flores *et al*., 2002; Mondragón *et al*., 2004; Sefa-Dedeh *et al*., 2004; Laria *et al*., 2007):

- softening of the hull,
- diffusion of water and $Ca(OH)_2$, in the forms of OH^- ions and Ca^{2+} ions, (or potassium hydroxide) into the kernel,
- swelling and partial gelatinization of starch granules losing of crystallinity, leaching of amylose and reaction between the leached amylose chains and calcium ions (Figure 2.6),
- solubilization and denaturation of protein, as well as reaction among denatured proteins and reaction between denatured protein and other components (such as lipid, starch and calcium ions),
- saponification of lipid (formation of calcium salt of fatty acids), and

- formation of calcium phytate, amylose-lipid complex, amylose-lipid-Ca complex, phytate-amylose complex, phytate-protein complex.

Granule size of the nixtamalized corn starch was larger than that of the native starch due to the partial swelling and gelatinization of starch granule during nixtamalization process (Mendez-Montealvo *et al*., 2006). Changing in starch crystallinity was observed upon cooking whole corn in the presence of lime (Rodríguez *et al*., 1996). Crystallinity of the nixtamalized corn starch was higher than that of the native starch when 0.2 % lime was used. However, crystallinity of the nixtamalized corn starch decreased as lime concentration increased when lime of concentration higher than 0.2 % was used (Mondragón *et al*., 2004 and 2006).

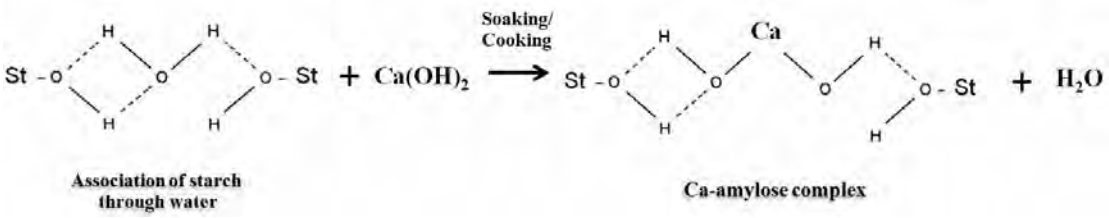


Figure 2.6 Scheme of calcium-amylose interaction (Adapt from Robles *et al.*,1988; González *et al*., 2004; Reguera *et at*., 2000 and Gonzalez *et al*., 2004).

The calcium-amylose, Ca-amylose, interactions inhibit transferring of water into starch granules, and thus limit granule swelling and gelatinization of the middle part of starch granules (Robles *et al*., 1988; Laria *et al*., 2007; Rodríguez *et al*., 1996; Mondragón *et al*., 2006). This Ca-amylose interaction may contribute to the starch crosslinking through calcium bridge formation (Rodríguez *et al*., 1996). Increase in lime concentration increase calcium uptake which results in the stronger

calcium-amylose interaction (Laria *et al*., 2007; Rodríguez *et al*., 1996). This Ca-amylose interactions result in higher granule rigidity and lower granule degradation (Laria *et al*., 2007). The Ca-amylose, amylose-lipid and amylose-lipid-Ca complexes, which from during nixtamalization, affect water absorption capacity and rheolgical properties of nixtamalized flour and masa dough (Nierle and El Baya, 1990; Kaur, and Singh, 2000; Mondragón *et al*., 2004; Sefa-Dedeh *et al*., 2004). These complexes affect leaching of amylose and subsequently affect rheological properties (Tester and Morrison, 1990; Mondragón *et al*., 2004). Nixtamalization causes an increase in water absorption capacity of corn starch. Effect of [$Ca(OH)_2$] on water absorption capacity of nixtamalized corn flour could be due to pre-gelatinization of granule, Ca^{2+} ions and Ca-starch interaction (Sefa-Dedeh *et al*., 2004). The nixtamalized corn flour had lower setback viscosity than the native one (Sefa-Dedeh *et al*., 2004). Setback viscosity of corn flours obtained from the cooked whole corn in 0-1 % lime increased as lime concentration increased (Sefa-Dedeh *et al*., 2004). Paste of nixtamalized corn flour exhibited shear thinning with lower consistency index than paste of native corn starch (Méndez-Montealvo *et al*., 2008). Though it had flow behavior index with higher tendency to behave like Newtonian lipid (Méndez-Montealvo *et al*., 2007). From dynamic rheological measurement, nixtamalized corn flour gave low G′, indicating a softer gel that native corn starch (Méndez-Montealvo *et al*., 2007 and 2008). The internal organization of nixtamalized granule had higher flexibility in amylopectin branching points which limited gel formation (Méndez-Montealvo *et al*., 2008). The dynamic rheological properties, G′ and G″, of nixtamalized corn flour gel were affected mainly by Ca-starch interactions (Mondragón *et al*., 2006). Nixtamalization was

reported to cause annealing inside starch granules and nixtamalized granule structure was stabilized by Ca^{2+} ions, so nixtamalized corn flour had higher gelatinization temperature but lower retrogradation than native corn starch (Méndez-Montealvo *et al*., 2006).

Nixtamalization increases nutritional value of corn-based food. Since, this treatment converts corn bound niacin to free niacin, increases calcium content by 400-750 % with 85 % available for adsorption, reduces aflatoxin by 50-75 % and reduces fumonisin (FAO, 1992; Serna-Saldivar *et al*., 1990; Dombrink-Kurtzaman *et al*., 2000). Nixtamalization was reported to be better process to reduce aflatoxin than the high shear-high heat process, extrusion (Elías-Orozco *et al*., 2002). Nixtamalization was also reported to improve protein digestibility, increase calcium to phosphorus ratio and reduce ability of phytic acid to interact with minerals which in turn increase bioavaibility of minerals (Robles *et al*., 1988; Gómez *et al*., 1991 and 1992; Mondragón *et al*., 2006).

CHAPTER III

MATERIALS AND METHODS

3.1 Sample preparation

3.1.1 Starch isolation

Pigeon pea grains (obtained from Nan Land Developing Department, Nan, Thailand in the year of 2007) were hulled to remove pericarp (Hull miller, SB-4, Sombupan company, Pisanulok, Thailand). The hulled grains were soaked in water (weight ratio of hulled grain to water = 1:5) at 6±1°C for 16 hr. After draining, they were washed twice. Then, they were wet milled with stone mill using weight ratio of dry hulled grain to water of 1:5. The slurry was filtered through 50, and then 100 mesh sieves (Lavallab Inc., Canada). The cake was washed twice and filtered through 50 and 100 mesh sieves again. After sedimentation of starch at 6±1°C for 3 hr, the supernatant was drained and the yellow upper layer was removed. The cake was suspended in water (weight ratio of wet cake to water = 1:2). For protein removal, the pH of the suspension was adjusted to 8.5 with 0.1 M NaOH (analytical grade, Ajax Finechem, NZ), stirred for 30 min, and then allowed to sediment at 6±1 °C for 5 hr. After draining, the precipitate (starch granule) was washed twice (weight ratio of precipitate to water = 1:1). The pH of starch suspension was adjusted to pH 7 with 1.0 M HCl (analytical grade, Ajax Finechem, NZ), and then allowed to sediment at 6±1°C for 5 hr. The cake was suspended in distilled water (weight ratio of precipitate to water = 1:2) and filtered through a 100 mesh sieve. The filtrate containing starch was collected and precipitated at 6±1°C for 3 hr. The starch precipitate was dried in

hot air oven (HA – 100S, Yeo Heng Co., Ltd., Bangkok, Thailand) at 65±1°C for 14 hr. The dried starch was milled with blender (HR2001, Philips, Belgium) and sieved through 200 mesh wire sieve, packed in aluminum foil bags and stored at 5±1°C. The moisture content of dried pigeon pea starch was determined using AOAC (2005) to be 9±1 % w/w (dry basis, db).

3.1.2 Starch fractionation

Pigeon pea starch was separated into large, medium and small granule fractions using the method of Yamazaki and Wilson (1964) and Kaur *et al*. (2007) with minor modifications. Starch dispersions (5 % w/w) were filtered sequentially through 200 mesh and 500 mesh sieves. The granules retained on the 200 mesh sieve were resuspended in distilled water and considered as the large granule fraction. The granules passed through the 200 mesh sieve were filtered further through the 500 mesh sieve. The granules passed through the 500 mesh sieve were the small granule fraction. The granules retained on the 500 mesh sieve were the medium granule fraction. The medium and small granule fractions were resuspended in distilled water. All starch suspensions were allowed to settle at 6±1°C for 5 hr. All starch precipitations were dried to a moisture content of 8 % w/w (db) in a cabinet hot air drier (HA–100S, Yeo Heng Co., Ltd., Bangkok, Thailand) at 50±1°C for 14 hr.

3.1.3 Starch modification

Pigeon pea grains were soaked at 6±1°C in 0-1.0 % w/v calcium hydroxide solution, $Ca(OH)_2$, (Ajax Finechem, NZ) for 16 hr (weight ratio of grains to water or $Ca(OH)_2$ solution = 1:10), or nixtamalized by boiling at 98±1°C for 30 min in

$Ca(OH)_2$ solution in the range of 0-1.0 % w/v and then steeped in the same cooking vessel at 6±1°C for 15.5 hr. After steeping, they were washed twice with tap water to remove bran and excess lime. Then, the grains were dried at 70±1°C for 14 hr. Starches were isolated from these treated grains using the method described in 3.1.1.

3.2 Chemical composition analysis

Moisture, protein, lipid, crude fiber, ash, carbohydrate, starch, amylose, phosphorus and calcium content of the starch samples prepared in 3.1 were analyzed as following.

3.2.1 Moisture, protein, lipid, crude fiber, ash and carbohydrate contents

Standard AOAC methods (2005) were used to determine moisture AOAC 925.10 (convection oven, OF-02G, Montreal-Biotech Inc., Canada), total nitrogen AOAC 920.87 (Kjeldahl analysis, Vapodest 10, Pauley Equioment Solution, UK), total lipid AOAC 920.39 (Soxhlet extraction, AV6AII/16, C. Gerhardt UK Ltd., UK), ash AOAC 942.05 (Muffle furnace, GF-03, Luoyang Gefei Co., Ltd, China) and crude fiber AOAC 920.86 (enzymatic–gravimetric method). Crude protein content was calculated from total nitrogen content using 6.25 as conversion factor. Total carbohydrate content was calculated from the difference between 100 and the sum of moisture, crude protein, total lipid, crude fiber and ash content. The analysis was performed in triplicate and mean values were reported.

3.2.2 Starch content

Starch sample (50 mg, weighed with an accuracy of 10^{-3} mg) was dispersed in deionized water (100 ml). The dispersion was vortexed (ZX_3 vortex mixer, Alfa Medical, NY, USA) for about 1 min. The α-amylase solution in 25% propylene glycol (3 ml) from *Bacillus licheniformis* (α-amylase for Starch Assay Kit, A3403, Sigma-Aldrich, St. Louis, MO, USA) was immediately added to the sample suspension. This α-amylase contained 21 mg protein per ml and had activity of 786 units/mg protein (1 unit liberated 1.0 mg of maltose from starch in 3 minutes at pH 6.9 and 20°C). The pH of sample suspension was adjusted to pH 7 with 1M NaOH, incubated in shaking water bath (WB 22, Memmert, Germany) at 85±1°C for 30 min and cooled to 10°C with cooling ice-water. The resulting dextrin solution was centrifuged at 18,000g and 10°C for 10 min (Sigma 3K10, Bioblock Scientific, Illkirch, France). The supernatant (1 ml) was transferred and diluted with deionized water in volumetric flask (100 ml). To determine glucose concentration in the supernatant, the diluted dextrin solution (800 µl) was mixed with amyloglucosidase solution (200 µl) from *Aspergillus niger* (Sigma Chemical Co., St. Louis, MO, USA), incubated at 55±1°C for 1 hr 30 min in a controlled shaking water bath, and then centrifuged at 3,000g and 10°C for 10 min (Bergmeyer and Bernet, 1974). The glucose oxidase/peroxidase/2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) or GOD-POD-ABTS reagent (2 ml) was added to the supernatant (0.8 ml). The solution was allowed to stand in the dark room at 25±2°C for 30 min, then absorbance (or optical density, OD) was measured at 560 nm using a spectrophotometer (JENWAY 6300 spectrophotometer, Bariworld Scientific Ltd., Dunmow, UK) to determine the glucose concentration in supernatant. Blank solution was a mixture of water (0.8 ml)

and GOD-POD-ABTS reagent (2 ml). A standard curve was prepared using pure glucose from potato (Fluka Chemie GmbH, Buchs, Switzerland). The procedures for preparing amyloglucosidase solution, GOD-POD-ABTS reagent and glucose standard curve were described in Appendix A. The glucose concentration in supernatant was calculated using Equation A in Appendix A. The analysis was performed in triplicate, and mean values were calculated using Equation 3.1.

$$s_t = [C_G(0.9)(100)(100)(1/10)]/[(w_t)(m_d/100)] \quad [3.1]$$

where s_t = dried total starch (% w/w db)

C_G = glucose concentration (μg/ml) determined from Equation A

0.9 = conversion factor of glucose in starch sample

100 = correction for dilution of initial solution

1/10 = conversion from glucose concentration from g/ml to % starch w/w on wet basis (wb)

w_t = weight of total starch sample in mg

m_d = dry matter in starch sample (% w/w wb)

3.2.3 Amylose content

Amylose content was determined by the calorimetric method (Mestres *et al.*, 1996) using a differential scanning calorimeter **(**DSC 7, Perkin-Elmer, Norwalk, VA, USA). Pure indium (part no. 03190033, Perkin- Elmer, Norwalk, CA) was used for heat flow and temperature calibration at the rate of 10°C/min. Pure potato amylose (Fluka Chemie GmbH, Buchs, Switzerland) was used as standard. Samples (≈ 10 mg, weighed with an accuracy of 10^{-3} mg) or pure potato amylose (5 mg, weighed with an accuracy of 10^{-3} mg) was placed in a stainless-steel pan (part

no. 03190218, Perkin-Elmer, Norwalk, CA). Then, 50 µl of a 2 % w/v lyso-phospholipid solution in distilled water (L4129 Sigma Type I from egg yolk, LPC, Sigma Chemicals GmbH, Deisenhofen, Germany) were added into the sample pan. The sample pan was hermetically sealed and stored for one hr at room temperature (25±1°C). The sample pan and the empty reference pan were heated from 25 to 160 °C at 10°C min^{-1}, held at 160°C for 2 min, and then cooled to 60±1°C at 10°C min^{-1}. The enthalpy (ΔH) of amylose-lysophospholipid complex formation during the cooling step was used to calculate amylose content in percentage using Equation 3.2. The analysis was performed in triplicate and the mean value was reported.

$$A = (\Delta H_S/\Delta H_A)(100) \quad [3.2]$$

where A = amylose content (%)
ΔH_S = enthalpy of amylose-lysophospholipid complex formation per dry weight for starch sample
ΔH_A = enthalpy of amylose-lysophospholipid complex formation per dry weight for pure potato amylose

3.2.4 Calcium content

Calcium content (ppm) was determined following the standard AOAC method 999.10 (2000) using the Inductively Coupled Plasma-Optical Emission Spectroscopy or ICP-OES (Ultima 2C, HORIBA Jobin Yvon S.A.S., Longjumeau Cedex, France). The analysis was performed in duplicate and mean values were reported.

3.2.5 Phosphorus content

Phosphorus content (ppm) was determined using the method described by Heyns (1959). A sample (≈ 2.5 g) was weighed with an accuracy of 10^{-3} g in a crucible, mixed with calcium carbonate (1.0 g) and burned in muffle furnace (Muffle furnace, GF-03, Luoyang Gefei Co., Ltd, China) at 550±1°C for 5 hr or until getting a white to light grey ash. Ash from starch sample was transferred to a volumetric flask (250 ml) and added with pure water (20 ml). The crucible was washed with HCl until no carbon dioxide was liberated (~ 20 ml). The washed ash solution in HCl was transferred into the same volumetric flask containing ash from starch sample, diluted with pure water to 250 ml and filtered (filter paper no. 1, Whatman, Whatman International Ltd., England). The filtrate (10 ml for native starch and starch obtained from soaked grain and 5 ml for flour obtained from boiled grain) was transferred into a test tube. Deionized water (5 ml) was added to filtrate of flour obtained from boiled grain. Then, Vanadate-Molybdate reagent (2 ml) obtained from Ajax Finechem, NZ was added. The solution was left at room temperature (25±1°C) for 45 min. Then, the OD of the solution was measured with a spectrophotometer (JENWAY 6300 spectrophotometer, Bariworld Scientific Ltd., Dunmow, UK) at 435 nm against a blank [a mixture of deionized water (10 ml) and the Vanadate-Molybdate reagent (2 ml)]. Solutions of potassium dihydrogen phosphate standard (CAS No. 7778-77-0, Fisher Scientific, UK) of concentration of 1.66, 3.33, 6.66, 9.99 and 13.33 mg/ml were used to obtain phosphorus standard curve as described in Appendix B. The phosphorus concentration in solution was calculated using Equation B in Appendix B. Then phosphorus content in the sample was calculated using Equation 3.3. The analysis was performed in triplicate and mean value was reported.

$$P = [C_P(250)(10)]/[(w_d)(V_f)] \quad [3.3]$$

where P = phosphorus content (ppm)

C_P = phosphorus concentration (ppm) determined from Equation B

250 = correction for dilution of ash

10 = volume (ml) of filtrate or diluted filtrate reacted with the Vanadate-Molybdate reagent

$w_{sam_{db}}$ = weight of sample on dry basis (g)

V_f = filtrate volume (10 ml for native starch and starch obtained from soaked grain and 5 ml for flour obtained from boiled grain)

3.3 Structure characterization

3.3.1 Granule shape and birefringence

Starch sample was suspended in distilled water to obtain starch concentration of 1 % w/w. The suspension was dropped on a glass slide and covered with glass slip. The starch granule image was taken under an optical microscope (BX 51TF, Olympus, Japan) at a magnification of 400X, then a polarized film (U-POT, Olympus, Japan) was placed to cover the light source of a microscope and granule birefringence image was taken at a magnification of 400X. The microscopic image of starch granule was recorded using Pinnacle Media Center (PCTV USB2, Pinnacle System GmbH, Germany).

Starch sample was spread on a 0.5 cm^2 carbon sheet, placed on an aluminum stub, and then coated with gold-palladium using an ion sputter (Balzers Union SCD 040, Balzers Union Ltd., Balzers, Liechtenstein). Starch granule shape

was determined using Scanning Electron Microscope (JSM-5410 LV, JEOL Ltd., Tokyo, Japan) at a magnification of 1,000X, 2,500X and 6,000X.

3.3.2 Granule size and size distribution

The starch granule size and size distribution were determined using a Laser Diffraction Particle Size Analyzer (Mastersizer 2000, Malvern Instruments, Ltd., Malvern, UK). The starch (0.113 g dry weight) was dispersed in distilled water (150 ml) and agitated at a very slow speed using a magnetic stirrer for 1 hr at room temperature (28±2°C). The suspension was filled in the small volume sample presentation unit of the Mastersizer to obtain an obscuration value of ~20 %. The refractive index of water and starch granule relative to water used were 1.330 and 1.529, respectively. The absorption of starch granule used was 0.1. A He-Ne laser was used to provide a light at a wavelength of 630 nm. A background reading was taken using distilled water (100 ml). The analysis was performed in triplicate. Range of granule diameter and mean value of volume moment mean diameter (D[4,3]) were reported.

3.3.3 Crystallinity pattern and relative crystallinity

To obtain sample with moisture content about 15 % w/w (wet basis, wb) prior to crystallinity determination, sample was kept over a saturated NaCl solution (relative humidity about 75 %) in a desiccator under vacuum for 2 weeks. Crystallinity of the starch granule was analyzed using a wide angle X-ray diffractometer (D5005, Siemens AXS, Germany) equipped with a copper source operating at 40 kV and 50 mA, producing X-rays as monochromatic copper K_{α} radiation with a wavelength of 0.154 nm. The diffraction data were collected over an

angular range from 5 to 30° (2θ) at 0.1° intervals with a scanning rate of 60 s/°. The x-ray pattern was compared with the peak characteristic of theoretical diffractogram given by Zobel (1964). The relative crystallinity was determined quantitatively from the ratio of the sharp peak area to the total peak area (Nara and Komiya, 1983; Kaur and Sandhu, 2010), using a peak-fitting software (Origin-version 8.0, Microcal Inc., Northampton, MA, USA.).

3.4 Functional properties determination

3.4.1 Thermal properties and retrogradation

Thermal properties were determined using DSC. Pure indium was used for heat flow and temperature calibration at the rate of 10°C/min. Sample (≈ 10 mg, weighed with an accuracy of 10^{-3} mg) was placed in preweighed stainless-steel pan. Deionized water (50 μl) was added to sample. After hermetic sealing, the sample pan was kept at room temperature (25±1°C) for one hour. The sealed sample and empty reference pans were heated from 20 to 120°C at the rate of 10°C/min. The onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy of gelatinization (ΔH_{Gel}) were determined from the endotherm. For sample obtained from boiled grain, two endotherms were observed and the thermal properties for gelatinization were obtained from the second endotherm (over the temperature above 80°C), because the first endotherm likely corresponded to pre-gelatinized and retrograded starch.

To determine retrogradation tendency following gelatinization, sample was gelatinized in DSC pans as described above, then stored at 4°C for 1, 2, 3, 4, 8 and 12 days. Then, the sample was taken out from a refrigerator and kept at room

temperature for 1 hr and heated again in DSC using the same conditions described above. The enthalpy of regelatinization of the sample was determined from the endotherm. The percentage of retrogradation was calculated from Equation 3.4.

$$R = (\Delta H_{Regel}/\Delta H_{Gel})(100) \quad [3.4]$$

where R = retrogradation (%)

ΔH_{Regel} = enthalpy of regelatinization ($J/g_{dry\ starch}$)

ΔH_{Gel} = enthalpy of gelatinization ($J/g_{dry\ starch}$)

The analysis was performed in duplicate and mean values of T_o, T_p, T_c, ΔH_{Gel} and percentage of retrogradation were reported.

3.4.2 Water and oil absorption capacities

Water absorption capacity of starch was measured by the centrifugation method (Sosulski, 1962). The sample (5 g, weighed with an accuracy of 10^{-3} g) was suspended in distilled water (30 ml) in preweighed centrifuge tube. The suspension was kept at 25 or 70°C for 30 min (stirred occasionally), then centrifuged at 6,000g for 10 min (Sigma 3K10, Bioblock Scientific, Illkirch, France). The supernatant was decanted and sediment was weighed. The water absorption capacity was calculated using Equation 3.5.

$$AC_w = 100(w_s - w_{sam_{wb}})/w_{sam_{db}} \quad [3.5]$$

where AC_w = water absorption capacity (g water per 100 g dry sample)

w_s = weight of sediment (g)

$w_{sam_{wb}}$ = weight of sample on wet basis (g)

$w_{sam_{db}}$ = weight of sample on dry basis (g)

Oil absorption capacity was determined by the method of Lin *et al*. (1974). Sample (0.5 g, weighed with an accuracy of 10^{-3} g) was mixed with corn oil (6 ml) in preweighed centrifuge tube. The corn oil used was Morakot (Morakot Industries PCL., Thailand). The suspension was stirred for 1 min with a thin brass wire to disperse sample in oil, kept at 25 or 70°C for 30 min, and centrifuged at 3000g for 25 min. The supernatant (oil) was removed with a pipette. Then, the centrifuge tube was inverted upside down for 25 min to drain the oil, and then weighed. The oil absorption capacity was calculated using Equation 3.6.

$$AC_{oil} = 100(w_i - w_f)/w_{sam_{db}} \quad [3.6]$$

where AC_{oil} = oil absorption capacity (g oil per 100 g dry sample)

w_i = initial weight of sample and centrifuge tube (g)

w_f = final weight of sample and centrifuge tube after centrifugation and oil draining (g)

$w_{sam_{db}}$ = weight of sample on dry basis (g)

The analysis was performed in triplicate, and mean values of water absorption capacity and oil absorption capacity were reported.

3.4.3 Swelling power, solubility and amylose leaching

Swelling power, solubility and amylose leaching were determined according to the method described by Mestres *et al*. (1996). Starch was weighed to obtain 0.42 g dry weight. Starch suspension in water of 1.5 % w/w (db) was prepared and heated in a Rapid Visco Analyzer (RVA series 4, Newport Scientific NSW, Australia). The temperature-time profile used for heating the suspension at 160 rpm was:

- holding at 35°C for 1 min,
- heating to final temperature at 6°C/min, and
- holding at final temperature for 30 min

The final temperature was 50, 60, 70, 80, 85, 90 or 95°C. The heated starch suspension was immediately transferred to a centrifuge tube and centrifuged at 8,000g at 25°C for 5 min. The supernatant and sediment were separated and weighed.

For swelling power and solubility determination, the supernatant and sediment gel were dried at 100°C (24 hr for supernatant and 48 hr for sediment), and then weighed. The swelling power and solubility were calculated using Equation 3.7 or Equation 3.8, respectively.

$$SP = (w_{sed} - w_{sed_d}/w_{sed_d}) \quad [3.7]$$

$$S = (w_{sup_d}/w_{sam_{db}})(100) \quad [3.8]$$

where SP = swelling power (g wet gel/g dry gel)

S = solubility (%)

w_{sed} = weight of wet sediment (g)

w_{sed_d} = weight of dried sediment (g)

w_{sup_d} = weight of dry solid in supernatant (g)

$w_{sam_{db}}$ = weight of sample on dry basis = 0.42 g

For amylose leaching, amylose content in supernatant was analyzed using iodine-binding ratio (Zhu *et al*., 2008). The supernatant (500 µl for supernatant from native starch and starch obtained from soaked grain and 3000 µl for supernatant from flour obtained from boiled grain) was diluted to 100 ml in volumetric flask and added with 0.1 M acetic acid (1 ml) and 2 % w/v iodine solution (2 ml). The solution was

kept in the dark room at room temperature (25±1°C) for 20 and 25 min, and the OD of the solution was measured with a spectrophotometer (JENWAY 6300 spectrophotometer, Bariworld Scientific Ltd., Dunmow, UK) at 620 and 545 nm, respectively. The preparations of iodine solution, amylose and amylopectin standard curve were described in Appendix C. The concentration of amylose in supernatant (or melt phase) was determined from Equation C in Appendix C (Zhu *et al*., 2008). Then, percentage amylose leaching was calculated and expressed in mg of amylose leached per 100 mg of original amylose content in dry sample using Equation 3.9.

$$AL = 100[(10^{-3}C_A)(10^{3}D_S)T_s]/(w_{sam_{db}}A/100) \quad [3.9]$$

where
- AL = amylose leaching (%)
- C_A = amylose concentration (μg of amylose per ml solution) in supernatant determined from Equation C
- 10^{-3} = conversion factor from μg to mg or from μl to ml
- D_S = correction for dilution of supernatant (ml of final solution per μl of supernatant)
- T_S = total volume of supernatant (ml)
- $w_{sam_{db}}$ = weight of sample on dry basis (mg)
- A = amylose content in dry sample (%)

The analysis was performed in duplicate, and mean values of swelling power, percentage solubility and amylase leaching were reported.

3.4.4 Pasting properties

The pasting properties of starches were determined using a Rapid Visco Analyzer (RVA-4, Newport Scientific, Warriewood, Australien). Starch was weighed to obtain 2.6 g dry weight and mixed with distilled water in RVA can to get the starch suspension (25 g) of 10.4 % w/w (db). The suspension was stirred rapidly at 960 rpm for 10 s. Then, the stirring speed was decreased to 160 rpm and held constant at 160 rpm. The suspension was subjected to the following heating and cooling cycles at 160 rpm:

- For native starch and its fractions of different size
  - holding 50°C for 1 min,
  - heating to 95°C at 12°C/min**,**
  - holding at 95°C for 2.5 min**,**
  - cooling to 50°C at 12°C/min, and
  - holding at 50°C for 2 min.
- For native starch, starch obtained from soaked grain and flour obtained from boiled grain
  - holding 25°C for 1 min,
  - heating to 95°C at 12°C/min**,**
  - holding at 95°C for 2.5 min**,**
  - cooling to 50°C at 12°C/min, and
  - holding at 50°C for 2 min.
- For flour obtained from boiled grain
  - holding 25°C for 1 min,
  - heating to 95°C at 12°C/min**,**
  - holding at 95°C for 30 min**,**
  - cooling to 50°C at 12°C/min, and
  - holding at 50°C for 2 min.

The pasting properties, namely pasting temperature (T_p), peak viscosity (η_P), final viscosity (η_F), setback (η_S) and breakdown (η_B), were determined from pasting curve. The analysis was performed in triplicate, and mean values of pasting properties were reported.

3.4.5 Enzymatic digestibility

Enzymatic digestibility was determined using method described by Noda *et al*. (2005). Samples (1.0 g dry basis, weighed with an accuracy of 10^{-3} g) was suspended in deionized water (50 ml). The sample suspension (500 µl) was mixed with 0.1 M acetate buffer pH 5.0 (250 µl) and 5 units/ml amyloglucosidase solution (250 µl), incubated at 40°C for 4 hr in a controlled shaking water bath, cooled with cool water, and then centrifuged at 8,000 g for 10 min. The free glucose in the supernatant was analyzed by glucose oxidase, peroxidase and ABTS assay as described in section 3.2.2. The percentage of hydrolysis was calculated using Equation 3.10. The analysis was performed in triplicate, and mean value was reported.

$$R_H = (100)[C_{GH}(0.9)(1/10)]/[(w_t)(m_d/100)] \qquad [3.10]$$

where R_H = hydrolysis (%)
C_{GH} = glucose concentration in supernatant obtained from hydrolyzed sample
0.9 = conversion factor of glucose in starch
1/10 = conversion from glucose concentration from g/ml to % starch w/w
w_t = weight of total starch (mg)
m_d = dry matter in starch (% w/w wb)

3.4.6 Freeze-thaw stability

Freeze-thaw stability of sample was determined in term of percentage of syneresis described by Schoch (1968). Five sets of starch suspension in deionized water were prepared to obtain the starch concentration of 2 % w/w db. The suspension was heated at 90°C for 30 min, then cooled to room temperature within 6 min in an bath containing ice. The paste (20 g) was transferred, weighed with an accuracy of 10^{-3} g in the preweighed centrifuge tube and stored at -20°C. Each set of paste was subjected to 7 freeze-thaw cycles with different duration of frozen storage. The duration of frozen storage at -20°C for each cycle was 1, 2, 3, 4 or 7 days.

To determine percentage of syneresis for each cycle, the paste was taken out after 1, 2, 3, 4 or 7 day storage, and kept at room temperature (25±1°C) for 1 hr, then centrifuged at 3000g for 15 min. The supernatant was drained. The sediment was weighed and stored again at -20°C for 1, 2, 3, 4 or 7 days to determine percentage of syneresis for next cycle. The percentage of syneresis was calculated using Equation 3.11. To determine percentage of syneresis for 7 freeze-thaw cycles, and the above procedure was repeated for each cycle.

$$S_y = (100)(w_p - w_{sed})/(w_{sed}) \qquad [3.11]$$

where S_y = syneresis (%)

w_p = weight of initial paste for each (g)

w_{sed} = weight of sediment for each cycle (g)

The analysis was performed in triplicate, and mean percentage of syneresis was reported.

3.4.7 Rheological properties

3.4.7.1 Steady shear behavior

Starch suspensions in deionized water were prepared to obtain the starch concentrations in 8 % w/w db. The suspension was heated at 95°C for 20 min (RC, VELP Scientifica srl., Italy). During heating, the suspension was stirred at 1,200 rpm (T-50, IKA Laboratory Equipment, Illinois, USA). The paste was loaded on the plate of the rheometer (Physica MCR 301, Anton Paar GmbH, Germany) and allowed to cool down to 75°C. Paraffin oil was used to cover paste surface to prevent moisture loss. The flow behavior of the paste was determined with parallel plate geometry (PP50) at 75°C, in the shear rate range between 50-1000 s^{-1}. Consistency coefficient and flow behavior index of paste were determined according to the power law as described in Equation 3.12 (Okechukwu and Rao, 1996).

$$\tau = K\dot{\gamma}^{n} \qquad [3.12]$$

where τ = shear stress (Pa)

$\dot{\gamma}$ = shear rate (s^{-1})

K = consistency coefficient ($Pa \cdot s^{n}$)

n = flow behavior index

The analysis was performed in triplicate, and mean values of consistency coefficient and flow behavior index were reported.

3.4.7.2 Viscoelasticity

Starch suspensions in deionized water were prepared to obtain the starch concentrations in 15 % w/w db. The suspension was heated at 95°C for 20

min. During heating, the suspension was stirred at 1,200 rpm. The paste/gel was kept at 4°C for 15 hr. Then, the paste/gel was allowed to warm up to 25°C and dynamic viscoelastic behavior of paste/gel was determined using a rheometer (Physica MCR 301, Anton Paar GmbH, Germany) with plate geometry (PP50) at 25°C. Paraffin oil was used to cover paste surface to prevent moisture loss. The strain giving the linear viscoelastic region in the range of frequency of 0.1-100 Hz was determined to be 2 %. Then, the storage modulus (G′), loss modulus (G″) and phase angle (δ) of the paste/gel were determined at 25°C, 2 % strain and 0.1-100 rad/s. The analysis was performed in triplicate, and mean values of G′, G″ and tan δ were reported.

3.5 Statistical Analysis

Analysis of variance (ANOVA) and Duncan's multiple range tests of the obtained data was analyzed SPSS software (version 17, SPSS Inc., Chicago, USA).

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Characteristics and properties of pigeon pea starch

Whole pigeon pea starch, which will be called native pigeon pea starch later in nixtanalization part, had a broad unimodal distribution of granule (particle) diameter (Figure 4.1), which was determined as equivalent volume diameter (D_v). Its D_v ranged from 4.88 to 60.87 μm and its volume moment mean diameter (D[4,3]) was 27.81 μm (Figures 4.1 and 4.2). The starch granules were fractionated into three fractions by sieving. The fractions that retained on 200- or 500-mesh sieves were called large granule fraction (LGF) or medium granule fraction (MGF), respectively. The fraction that passed through 500-mesh sieve was called small granule fraction (SGF). There was 8.91 %w/w db lost during fractionation. From total weight of these three fractions, the weight fractions on dry basis of SGF, MGF and LGF were 0.07, 0.73 and 0.20, respectively. Each fraction also had a broad unimodule distribution size similar to that of whole starch (Figure 4.1). The ranges of D_v and D[4,3] for SGF, MGF and LGF were 4.88-52.25, 4.88-60.87 and 9.00-60.87 μm, and 24.03, 27.06 and 30.05 μm, respectively (Figures 4.1 and 4.2).

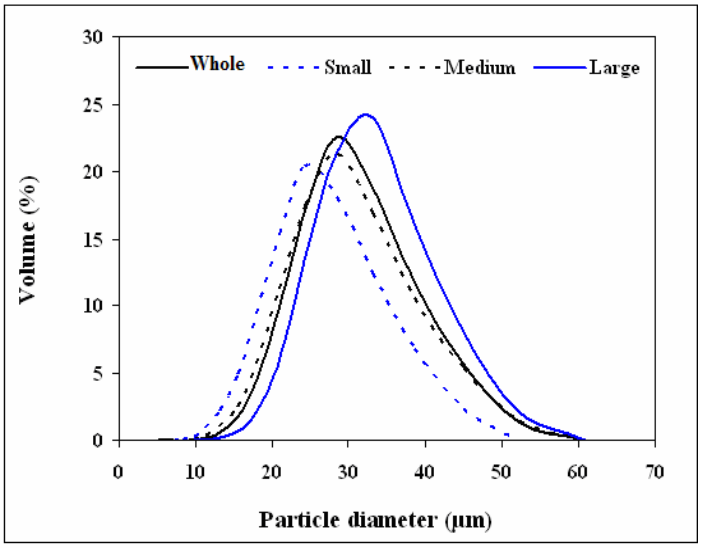


Figure 4.1 Particle size distribution of pigeon pea starch granules.

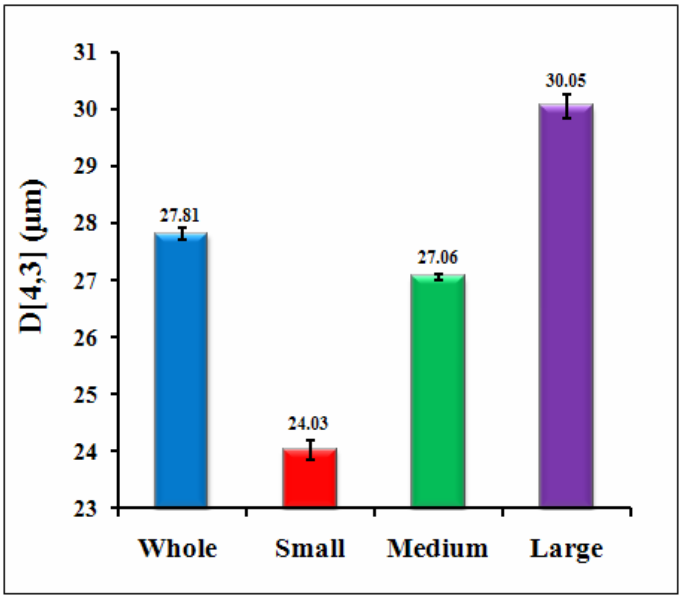


Figure 4.2 Volume moment mean diameter (D[4,3]) of pigeon pea starch granules.

SEM image (Figure 4.3) shows that granules of whole starch had round (spherical-like) and oval shape. The shapes of granules in SGF were mostly round (spherical-like). Numbers of oval shape granules were higher in MGF. Granules in LGF were mostly oval shape. All granules in the SEM images had smooth surface.

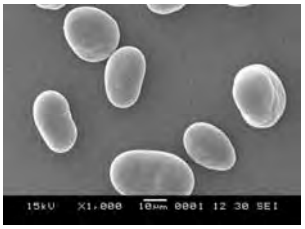
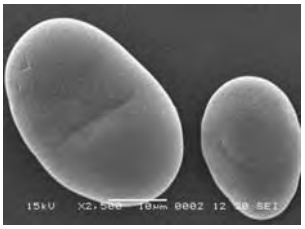
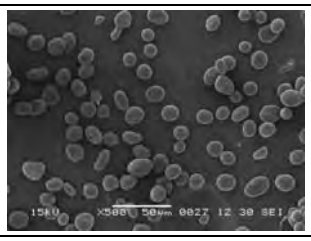
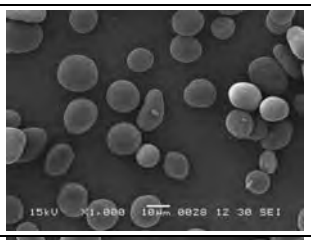
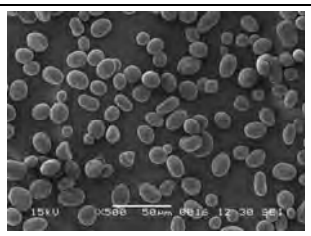
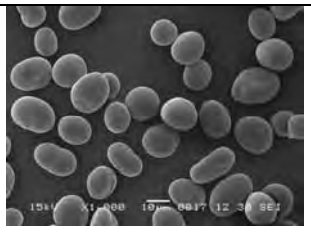
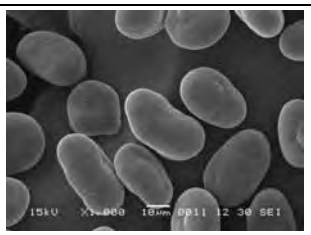
Sample	× 500	× 1,000	× 2,500
Whole			
Small			
Medium			
Large			

Figure 4.3 SEM images of pigeon pea starch granules.

Granules of legume starches varied in size and size distribution. Size ranges of starch granules were found to be 34-35, 14-23, 7-26, 6-28, 3-64 and 15-85 μm for moth bean, grass pea, smooth pea, wrinkled pea, cow pea and horse gram, respectively as shown in Table 2.2 (Tester and Karkalas, 2002; Okechukwu and Rao, 1996; El-Faki *et al*., 1983; Huang *et al*., 2007a; Singh *et al*., 2004b; Hoover and Ratnayake, 2002; El-Faki *et al*., 1983; Chung *et al*., 2008a; Zhou *et al*., 2004; Hoover and Ratnayake, 2002; Hoover and Manuel, 1995; Bhatty, 1988; Hoover *et al*., 1997; Naivikukul and D'Appolonia 1979; Lawal and Adebowale, 2005; Ratnayake *et al*., 2001; Barron *et al*., 2000; Davydova *et al*., 1995; Bertoft *et al*., 1993a; Zhou *et al*.,

2004; Colonna *et al*., 1982; Korus *et al*., 2008; Jayakody *et al*., 2007; Hoover and Ratnayake, 2002). Shape and surface of pigeon pea starch granules were similar to those legume starch granules, which were reported to have round, spherical, oval and elliptical shapes and smooth surface as shown in Table 2.2. Hoover and Ratnayake (2002) and Agunbiade and Longe (1999) had earlier reported that legume starches are irregular in shape, oval and kidney shaped. The biochemistry of the chloroplast or amyloplast, as well as the physiology of the plant, mainly dictates the morphology of starch granules (Badenhuizen, 1969). The membranes and physical characteristics of the plastids may also be responsible for providing a particular shape or morphology to starch granules during granule development (Jane *et al*., 1994; Lindeboom *et al*., 2004).

Effects of granule size on amylose content and relative crystallinity were reported that amylose content was inverse function, while relative crystallinity was direct function of granule size for potato starch (Hizakuzi, 1996; Chen *et al*., 2003). Starch granule size may affect its physicochemical properties, such as gelatinization and pasting, enzyme digestibility, swelling power and solubility (Lindeboom *et al*., 2004). Therefore, the chemical compositions, granule shape, crystallinity and selected properties (thermal, water absorption capacity, swelling power, solubility, amylose leaching, pasting, steady shear viscosity, viscoelasticity and enzymatic digestibility) of these three granule fractions of pigeon pea starch were also determined and compared to those of whole starch. Then, the analyzed chemical composition and properties of whole starch will be compared with the calculated one which calculated from Equation 4.1.

$$\chi_{\text{whole (calculated)}} = w_{SGF}\chi_{SGF} + w_{MGF}\chi_{MGF} + w_{LGF}\chi_{LGF} \quad [4.1]$$

where

$\chi_{\text{whole (calculated)}}$ = calculated value for composition/property of whole starch

w_{SGF} = weight fraction of SGF

w_{MGF} = weight fraction of MGF

w_{LGF} = weight fraction of LGF

χ_{SGF} = value for composition/property of SGF

χ_{MGF} = value for composition/property of MGF

χ_{LGF} = value for composition/property of LGF

4.1.1 Chemical compositions

Figure 4.4 show that the main component in whole starch and its three fractions was carbohydrate (~98 % w/w db). Whole starch and its three fractions contained crude protein, lipid, crude fiber and ash less than 0.7 % w/w db individually (Figure 4.4 A). While the starch, amylose and phosphorus contents in whole starch were approximately 96.74 % w/w db, 30.74 % w/w db and 155.26 ppm, respectively (Figures 4.4 B, C and D). Differences in carbohydrate and starch contents among these four samples were less than 1 % (Figures 4.4 B). Figures 4.4 C and D show an increase in amylose content and a decrease in phosphorus content as a function of D[4,3]. There was no significant difference in amylose content between whole starch and LGF.

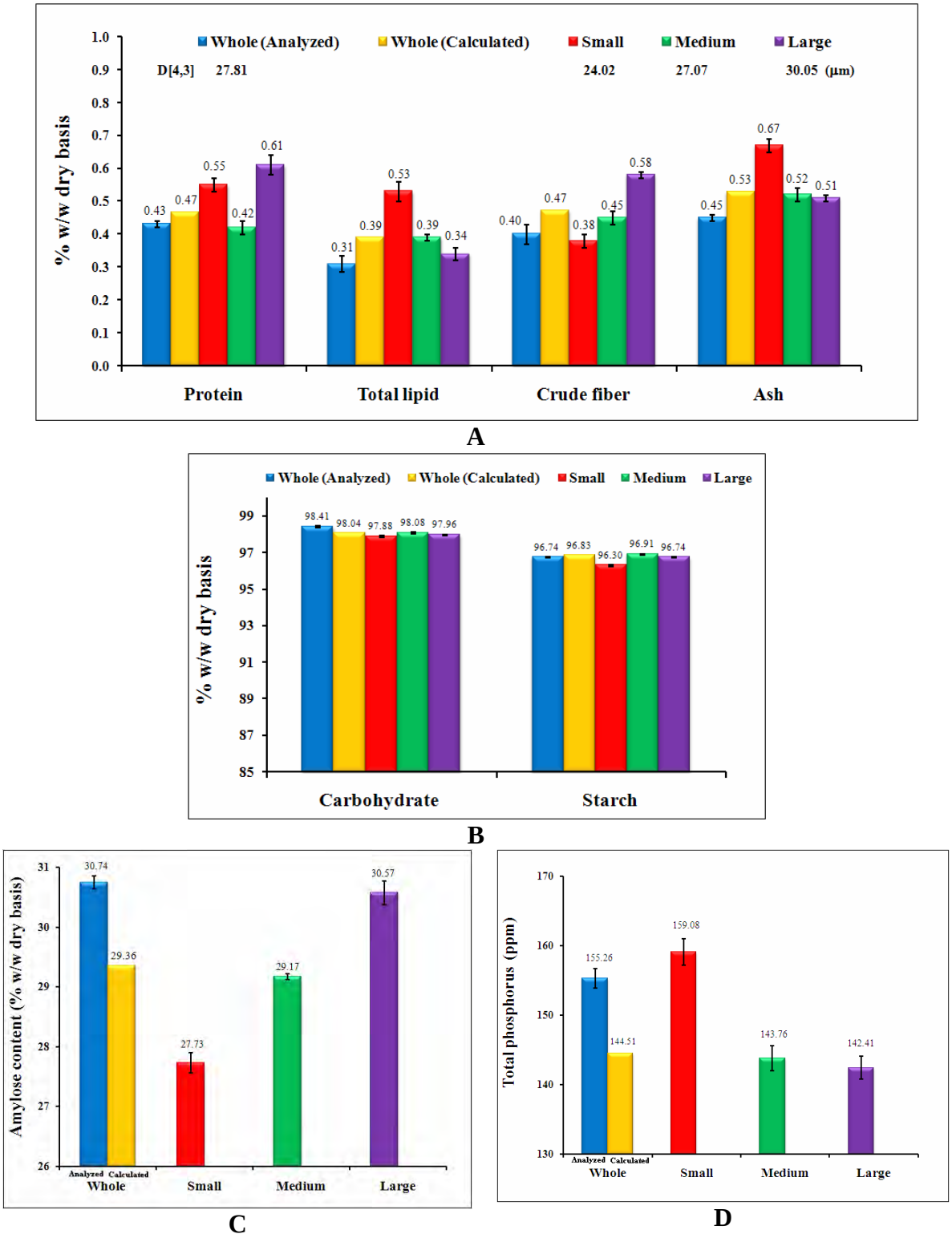


Figure 4.4 Chemical composition of pigeon pea starches.

Non-mutant legume starches have been reported to have higher amylose content than cereals, often in the range of 30-40 % (Madhusudhan and Tharanathan, 1995). The difference in amylose content may be attributed to differences in the activities of enzymes (soluble starch synthase and starch branching enzymes) involved in the biosynthesis of linear and branched components within starch granules (Krossmann and Lloyd, 2000). The amylose content of the starch granules has also been reported to be affected by climatic conditions and soil type during grown, and to vary with granule size (Singh *et al*., 2003; Cottrell *et al*., 2004; Morrison and Nasir, 1987; Asaoka *et al*., 1985). Chen *et al*. (2003) and Kaur *et al*. (2007) found a lower amylose content in the smaller granule size fraction for potato starch. Thus the SGF, which would have been at the initial stages of growth, may have the lowest amylose content because of the highest activity of soluble starch synthase and starch branching enzymes (Sidebottom *et al*., 1998). On the other hand, Noda *et al*., (2005) have reported that there was no difference in amylose content among different granule size fractions for potato starch. Thus, it is hard to draw a conclusion on relationship between amylose content and granule size. In this study, phosphorus contents in nonhusked whole pigeon pea grains and husked pigeon pea grains were also determined and were 2,660.33 and 3,122.29 ppm, respectively. This result suggested that phosphorus in pigeon pea grains was mainly in cotyledon. The presence of phosphorus in starch granules was in the form of phospholipid and phosphate monoester (Sing *et al*., 2003). Phospholipids in starch usually form complex with amylose, while phosphate monoesters are covalently bound to the C-6 (about 70%), C-3 and C-2 of the amylopectin glucose unit (Schoch, 1942; Hizukuri *et al*., 1970; Craig *et al*., 1989; Noda *et al*., 2005). An inverse relationship between

amylose content and phosphorus content suggested that the major form of phosphorus in pigeon pea starch was phosphate monoester which covalently bound to amylopectin. It has been reported that the phosphorus group is attached to longer unit-chains with DP > 20 of amylopectin (Takedes and Hizukuri, 1982). Similarly, Kainuma *et al*., (1978); Chen *et al*., (2003); Noda *et al*., (2005) have reported that the small starch granules fractionated from potato starch have higher phosphorus content than large granules. Phosphate groups, esterified to the amylopectin fraction of potato starch, contribute to high viscosity, water binding capacity and freeze thaw stability (Swinkels, 1985; Craig *et al*., 1989). Phospholipid content is proportional to amylose content (Morrison *et al*., 1984; Morrison *et al*., 1993). Phospholipids present in starch have a tendency to form a complex with amylose and long branched chains of amylopectin, which results in limited swelling. Pigeon pea grains had phosphorus content about 20 times higher than pigeon pea starches. This result indicated that most of phosphorus in grains was not bound to starch and was removed during starch extraction. It was found that the main storage form of phosphorus in many plant seeds and grains was phytic acid and phytate (Reddy *et al*., 1982). Phytic acid may be the major form of storage phosphorus in pigeon pea grain, because it is water soluble and can be removed by water during starch extraction.

4.1.2 Crystallinity

Figure 4.5 shows that whole starch, SGF, MGF and LGF had large peaks at 17.2°, 18.1° and 23.1° 2θ and small peaks at 5.5° and 15.2° 2θ in indicating the C-type diffraction pattern, which is mixture of A-type and B-type polymorphs (Hoover and Sosulski, 1985; Rathnayake *et al*., 2001). These four samples had the

relative intensity of the peaks at 18° to 17° 2θ of 0.9 (>2/3), absence of 5° 2θ peak and convex line profile at 23° 2θ indicating C_a-type polymorph (Hizakuzi, 1996; Horng, 2007). Relative crystallinity of whole pigeon pea starch was 35.24%. Among three granule fractions, the percentage of relative crystallinity slightly decreased as D[4,3] increased (Figure 4.6).

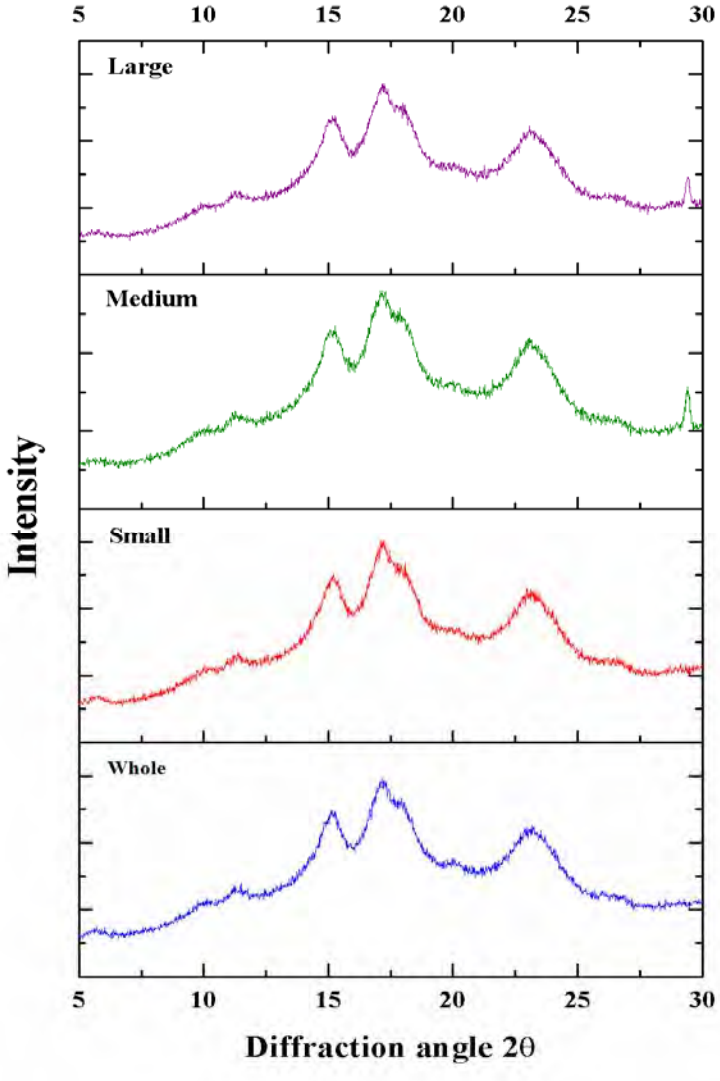


Figure 4.5 X-ray diffraction pattern of pigeon pea starches.

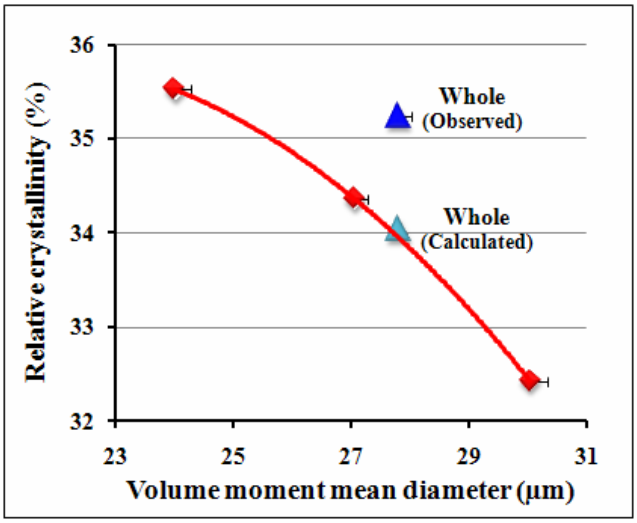


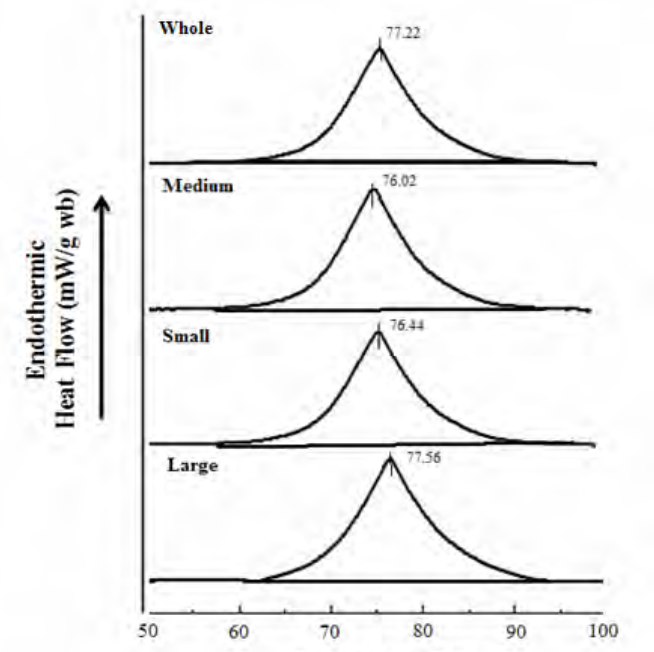
Figure 4.6 Relative crystallinity of pigeon pea starches versus volume moment mean diameter.

The C-type polymorph is typical polymorph for legume starch, with the exception of the A-type polymorph in commercial mung bean starch and the B-type polymorph in wrinkled and grass pea starches (Hoover and Rorke, 1991; Hoover and Swamidas, 1993; Bogracheva *et al*., 1998; Zhou *et.al*, 2004; Jayakody *et.al*, 2007; Horng, 2007; Hoover *et.al*, 2010). The C_a-subtype polymorph was also common subtype polymorph among the legume starches (Horng, 2007). It was found that percentage of B-type polymorph was around 22-49, confirming that A-type polymorph was higher than B-type polymorph, for many legume starches as shown in Table 2.3 (El-Faki *et al.*, 1983; Bhatty, 1988; Hoover and Manuel, 1995; Okechukwu and Rao, 1996; Naivikukul and D'Appolonia, 1997; Hoover *et al*., 1997; Hoover and Ratnayake, 2002; Zhou *et al.*, 2004; Singh *et al.*, 2004b; Lawal and Adebowale, 2005; Huang *et al.*, 2007a; Chung *et al.*, 2008a). Relative crystallinity of legume starches was around 30% except for wrinkled pea starch which was 17.7% (El-Faki *et al.*, 1983; Bhatty, 1988; Hoover and Manuel, 1995; Okechukwu and Rao, 1996; Hoover

et al., 1997; Naivikukul and D'Appolonia, 1997; Hoover and Ratnayake, 2002; Zhou *et al.,* 2004; Singh *et al.,* 2004b; Lawal and Adebowale, 2005; Huang *et al.,* 2007a; Chung *et al.,* 2008a). Relative crystallinities found for these four pigeon pea starch samples were comparable to those of other C-type legume starches reported to be between 27.1% and 33.5% (Zhou *et al*., 2004). Among three granule fractions of pigeon pea starch, the higher the amylose content the lower the relative crystallinity, as crystalline lamellae are made up of packed double helices of amylopectin branch chains (Ratnayake *et al*., 2001). Therefore, it is expected that relative crystallinity will be inversely related to amylose content (Sandhu and Lim, 2008). However, relative crystallinity of whole starch was higher than the calculated one and that of MGF (Figure 4.6), indicating the small granules was lost during size fractionation.

4.1.3 Thermal properties

The difference in T_o, T_p and T_c among whole starch and its three granule size fractions were ≈ 1 $^{\circ}$C (Figure 4.7). However, these temperatures tended to increase with D[4,3]. The difference in ΔH_{Gel} among these four samples was within 1 J/g of dry starch (Figure 4.8). Among three granule fraction, ΔH_{Gel} also tended to increase with D[4,3]. As expected the values of T_o, T_p and T_c of whole starch lined between these of MGF and LGF. Since the weight fraction of MGF was largest and the size distribution profile of whole starch lined between these two granule fractions. However, ΔH_{Gel} of whole starch determined from DSC, $\Delta H_{Gel_{whole\ (observed)}}$ in (Figure 4.8) was lower than that of MGF and the calculated one.



	T_o	T_p	T_c
Whole	72.69^{a} ± 0.13	77.22^{b} ± 0.32	82.62^{b} ± 0.14
Small	71.96^{b} ± 0.10	76.02^{d} ± 0.42	82.02^{d} ± 0.28
Medium	72.56^{a} ± 0.26	76.44^{c} ± 0.42	82.43^{c} ± 0.25
Large	73.08^{a} ± 0.74	77.56^{a} ± 0.20	83.49^{a} ± 0.18

Figure 4.7 Thermograms for pigeon pea starches.

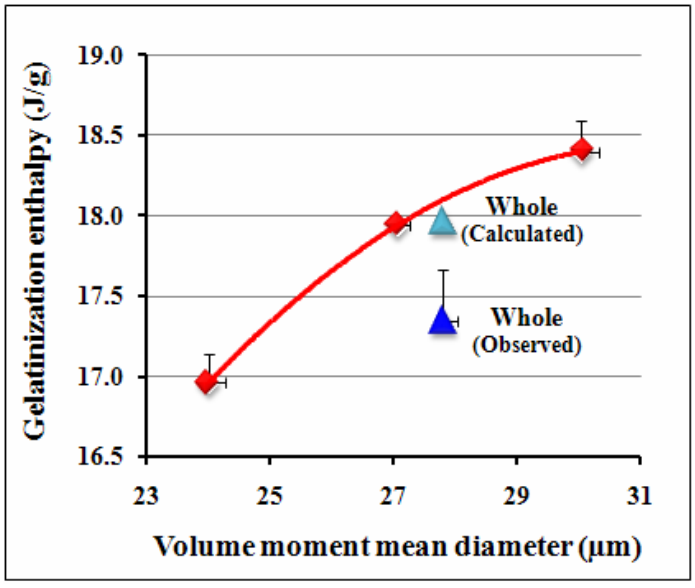


Figure 4.8 Gelatinization enthalpy of pigeon pea starches versus volume moment mean diameter.

The ΔH_{Gel} has been reported to be influenced by the degree of crystallinity of the starches (Eliasson and Gudmunsson, 1996). Tester and Morrison (1990) have postulated that ΔH_{Gel} reflects the overall crystallinity (quality and amount of crystallites) of amylopectin. Since the percentage crystallinity decreased with D[4,3] for pigeon pea starch. Therefore, an increase in ΔH_{Gel} with D[4,3] was un-expected and could not explain by the loss of double helical order in amylopectin crystalline region. Whereas, Cooke and Gidley (1992) have reported that ΔH_{Gel} reflects the loss of double helical order. Therefore, ΔH_{Gel} gives an overall measure of crystallinity (quality and quantity) and is an indicator of the loss of any molecular order within the granule, both in crystalline (double helices formed between the branches of amylopectin) and amorphous (double helices formed between amylose chains and between amylose and the exterior branched chains of amylopectin) regions, that occurs during gelatinization (Tester and Morrison, 1990; Cooke and Gidley, 1992; Hoover and Vasanthan, 1994; Ratnayake *et al*., 2001; Tester *et al*., 2004). For this study, the higher amylose content in the larger granules might result in the higher amylose-amylose interactions in starch granules, which might require higher thermal energy and gelatinization temperatures to break down this interaction in order to gelatinize.

4.1.4 Water absorption capacity, swelling power, solubility and amylose leaching

Water absorption capacity, swelling power, solubility and amylose leaching decreased as D[4,3] increased (Figure 4.9 and 4.10). An increase in temperature resulted an increase in water absorption capacity, swelling power,

solubility and amylose leaching (Figure 4.9, 4.11, 4.12 and 4.13). For each sample, water absorption capacity increased about two-fold as temperature increased from 25°C and 70°C. The Arrhenius-type temperature dependence of swelling power, solubility and amylose leaching was found for all starches with R^2 ~0.90 (Figure 4.11, 4.12 and 4.13) indicating a thermally activated process of swelling, solubilization and amylose leaching. Amylose leaching was not detected at 50°C for any sample. For water absorption capacity, the observed and calculated values were almost the same (Figure 4.9). For swelling power, solubility and amylose leaching, the observed values were higher than the calculated of values and those of MGF (Figure 4.11).

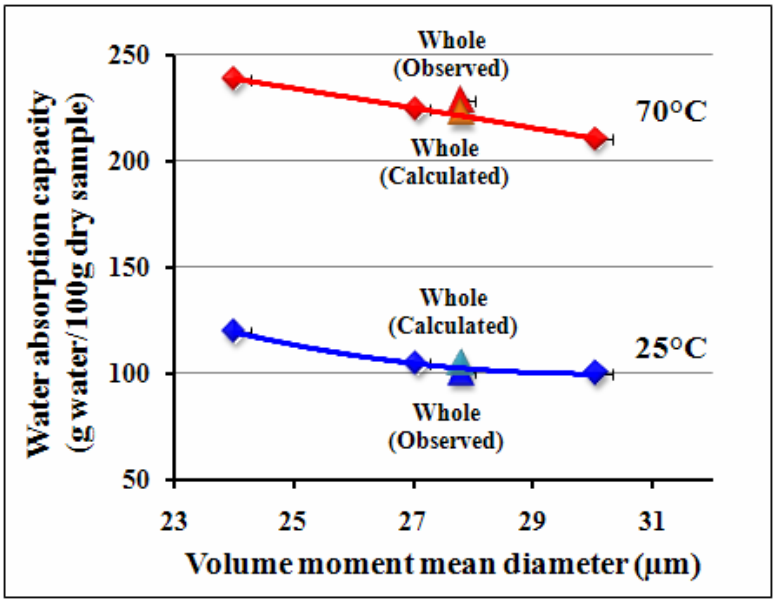


Figure 4.9 Water absorption capacity of pigeon pea starches versus volume moment mean diameter.

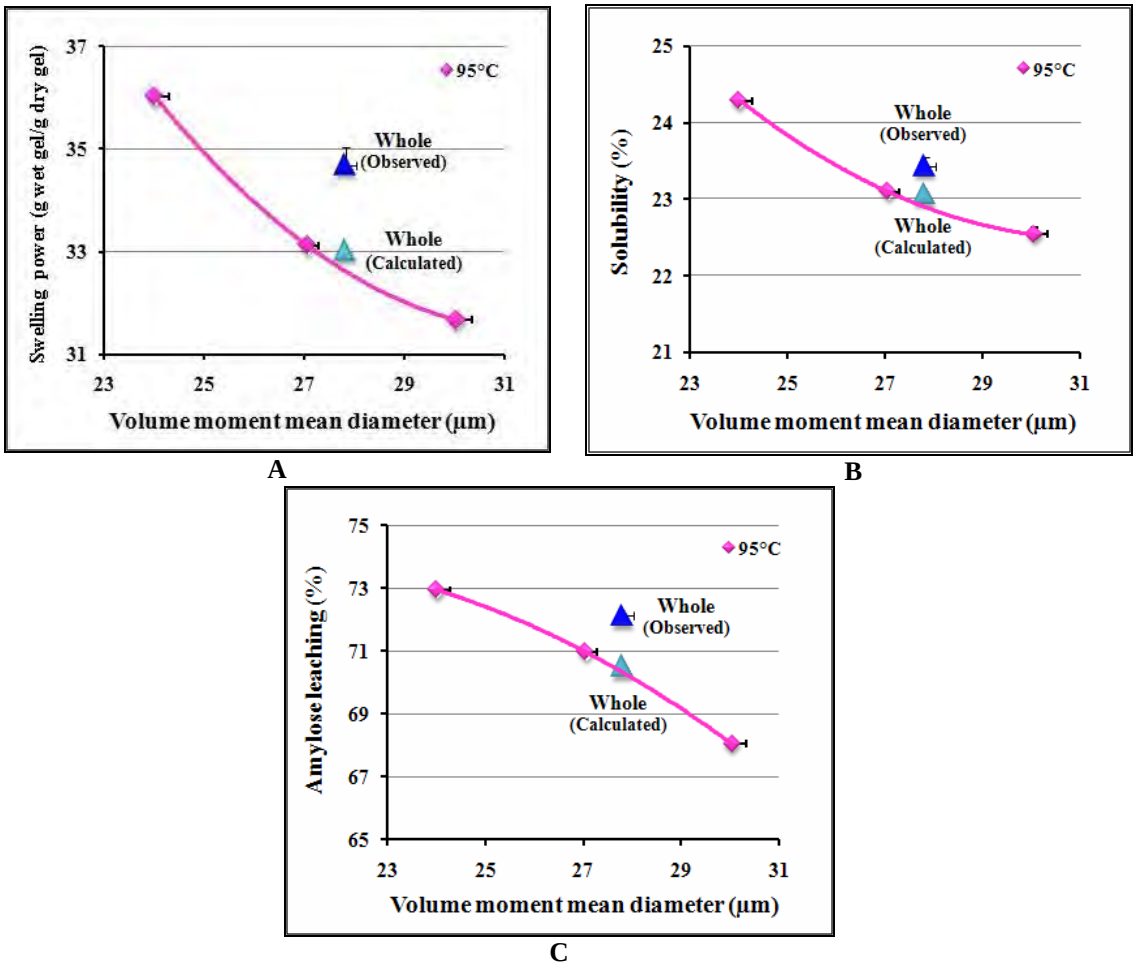


Figure 4.10 Effects of volume moment mean diameter on swelling power (A), solubility (B) and amylose leaching (C) of pigeon pea starches at 95°C.

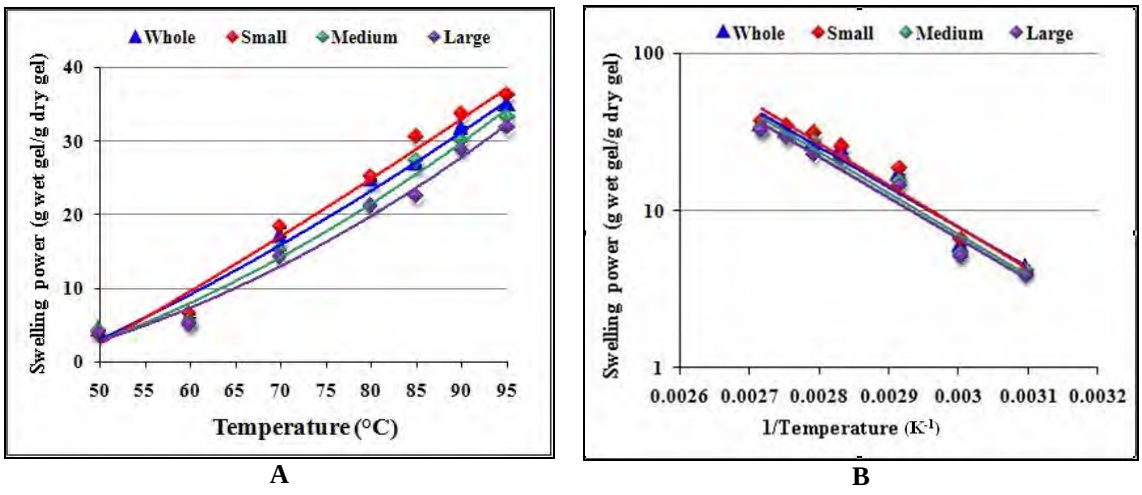
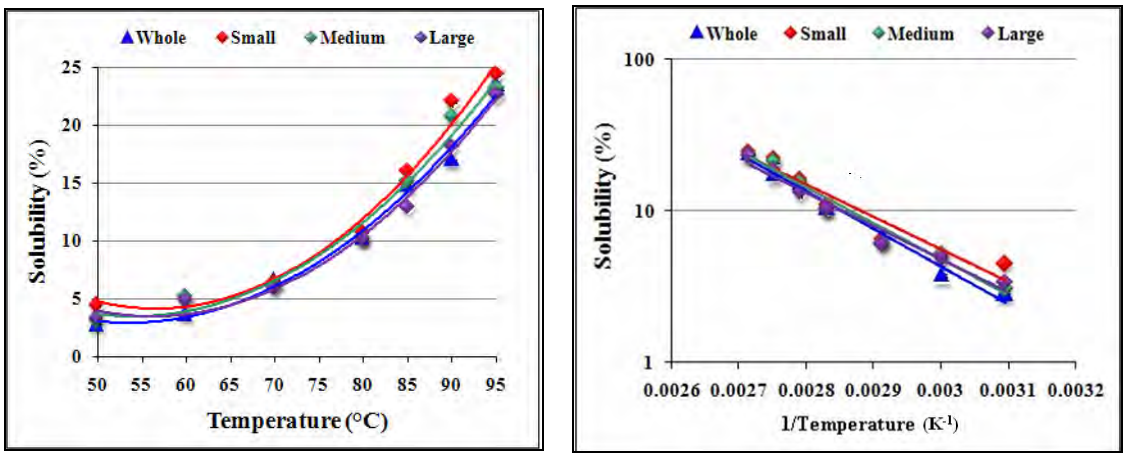
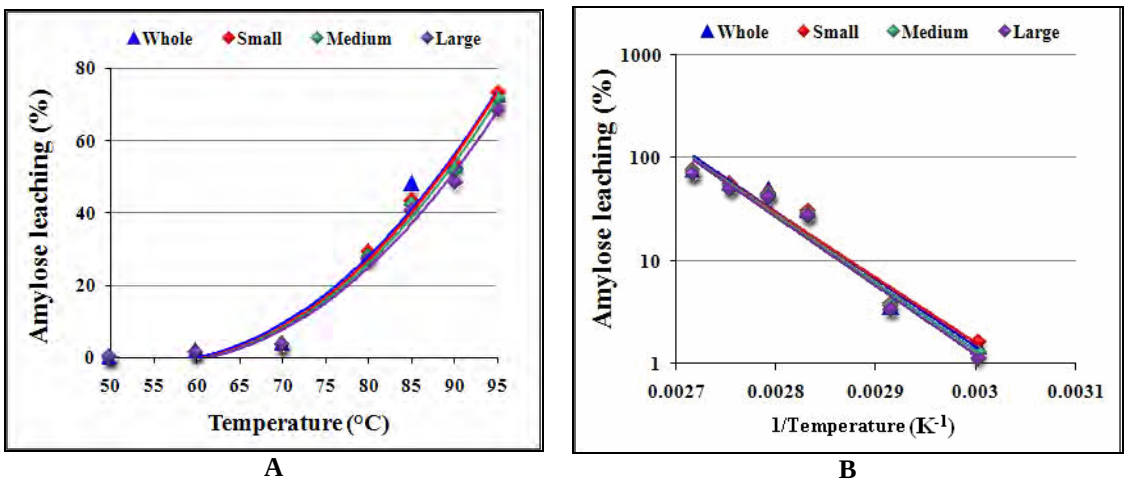


Figure 4.11 Swelling power of pigeon pea starches as a function of temperature (A) or 1/temperature (B).



A B

Figure 4.12 Solubility of pigeon pea starches as a function of temperature (A) or 1/temperature (B).



A B

Figure 4.13 Amylose leaching of pigeon pea starches as a function of temperature (A) or 1/temperature (B).

The SGF have higher surface to volume ratio than MGF and LGF, which can accelerate water penetration into the granules resulting in higher water absorption capacity. At both temperatures, the observed values of water absorption capacity lined on the regression lines drawn from the values of three granule fractions and were very close to the calculated values (Figure 4.9). This may indicate the size (diameter) and surface area dependence nature of water absorption capacity for pigeon pea starch granules. Furthermore, phosphorus content in pigeon pea starch

decreased as D[4,3] increased. This result suggests that the SGF was more hydrophilic due to higher phosphorus content, which the repulsion between adjacent starch molecules caused by negatively charged phosphate groups in small granules can apparently reduced interchain associations and increased levels of hydrated molecules (Noda *et al*., 2005; Lim and Seib, 1993). The higher the amylose content the higher the starch granule rigidity and the lower the water absorption capacity. Decrease in lipid content did not agree with a decrease in water absorption capacity as D[4,3] increased. This suggested that the combine effects of surface to volume ratio, amylose content and phosphorus content over came effect of lipid content for water absorption capacity.

Sasaki and Matsuki (1998) found that swelling power inversely correlated with amylose content, which increased with D[4,3] for pigeon pea starch. Starch swelling is a property of amylopectin and amylose acts as a diluents and a swelling inhibitor (Tester and Morrison, 1990; Tester, 1992; Sasaki and Matsuki, 1998; Lii *et al*., 1996). When starch granules are heated in excess water, the crystalline structure is disrupted and hydrogen bonds form between water molecules and the exposed hydroxyl groups of amylose and amylopectin, causing an increase in granule swelling and solubility (Leach *et al*., 1959; Hoover *et al*., 2010). For each sample, the rapid increase in swelling power, solubility and amylose leaching between 70 and 95°C (Figure 4.11, 4.12 and 4.13), may be due to an increase in molecular mobility of the amorphous region, which breaks the double helices existing within the amorphous and crystalline domains (Ratnayake *et al*., 2001; Tester *et al*., 2004). Specially, SGF have the highest swelling power, solubility and amylose leaching, which confirmed by the gelatinization data the lowest T_o and ΔH_{Gel}). Sandhyarani

and Bhattacharya (1989) suggested that low-amylose starch granules were fewer firms and tended to disintegrate easily while swollen and extensively overcrowded. The higher swelling power indicated the higher extent of granule disintegration resulting in higher solubilization of soluble solids and amylose out of starch granules.

4.1.5 Pasting properties

Whole pigeon pea starch and its three fractions had pasting temperature, peak viscosity and final viscosity ranging from 84.00 to 86.44°C, 3,063 to 3,856 mPa·s and 4,044 to 5,604 mPa·s, respectively (Figure 4.14). Among three granule pasting temperature decreased, while peak, trough and final viscosities, breakdown, and setback increased with D[4,3] increased. Moreover, the observed values of peak and setback were higher than the calculated values and highest among four samples.

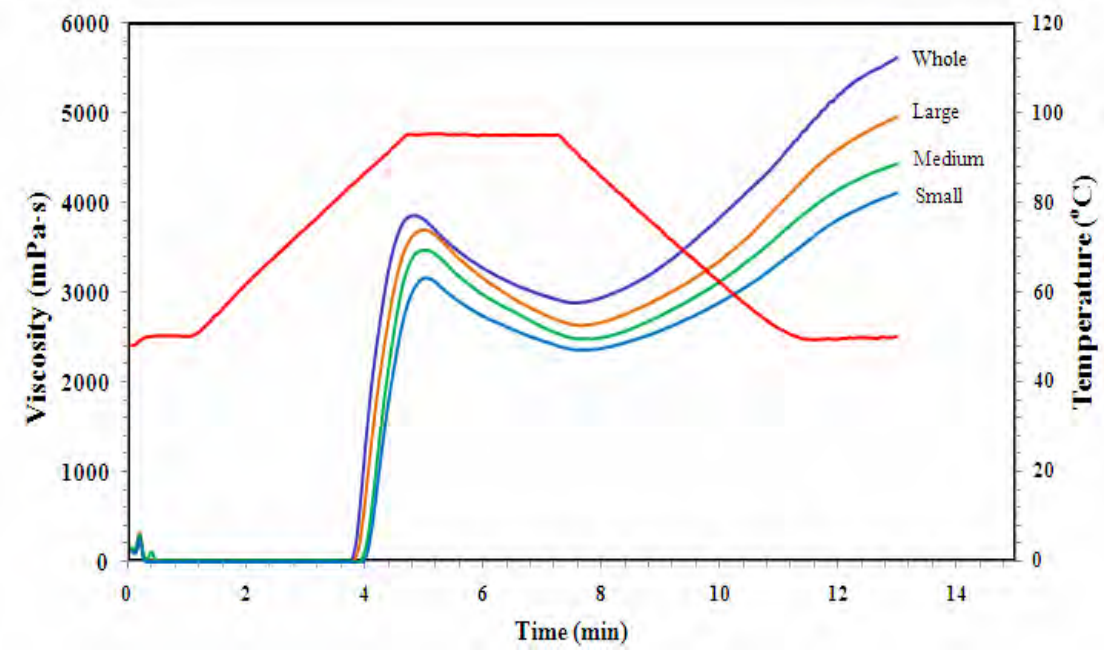


Figure 4.14 RVA profiles of pigeon pea starches.

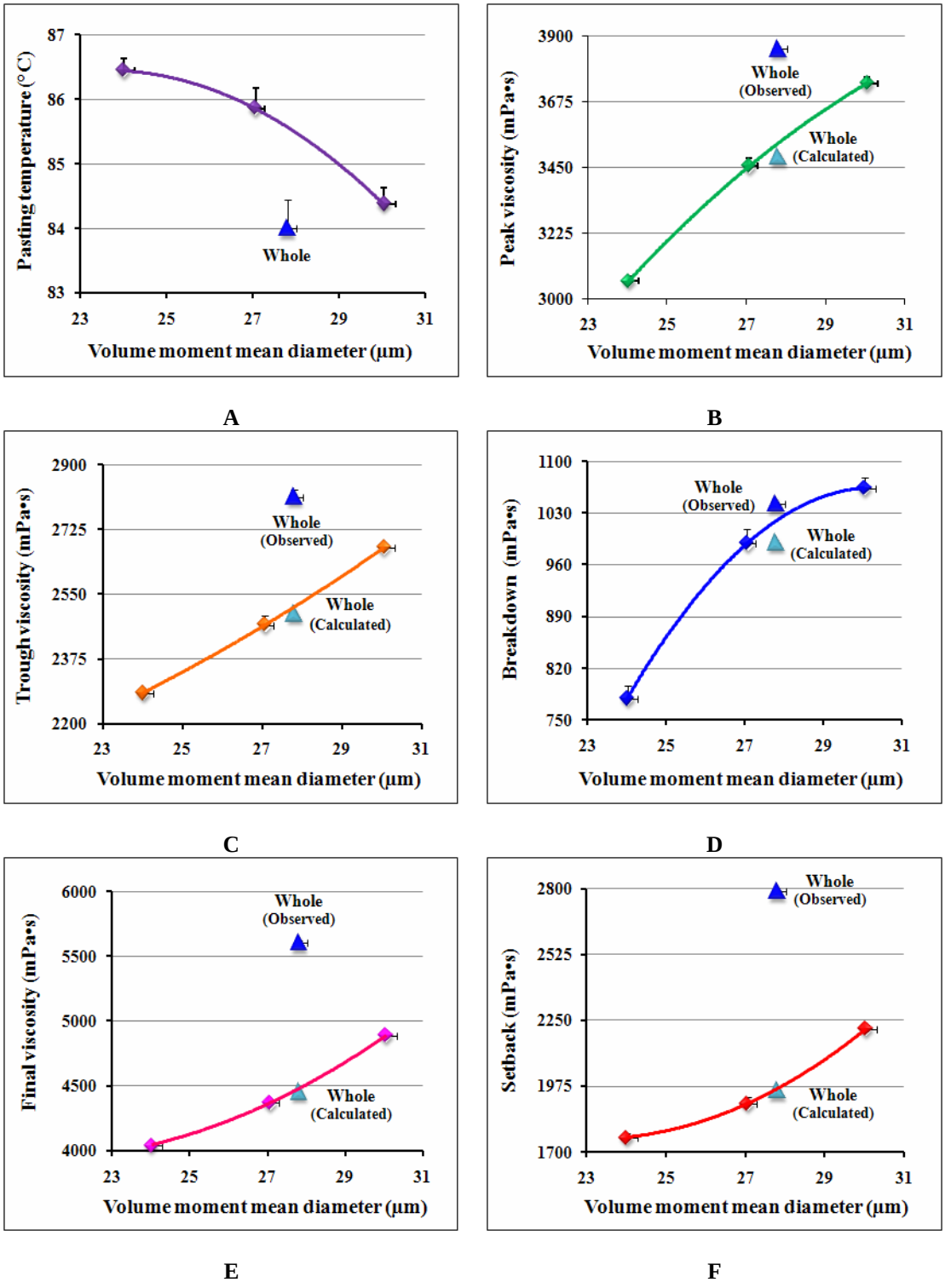


Figure 4.15 Pasting temperature (A), peak viscosity (B), trough viscosity (C), breakdown (D), final viscosity (E) and setback (F) of pigeon pea starches versus volume moment mean diameter.

Even though the amylose content was higher for higher D[4,3], the paste peak, trough, and final viscosities remained high. This was due to size and shape (axial ratio) effects. The viscosity of dispersion or paste depends on size and shape of solute which in this case was swollen starch granules. Even though, the swelling power decreased with D[4,3], but the total size of swollen granules might still directly relate to D[4,3]. SEM images in Figure 4.3 show axial ratio of starch granule increased with D[4,3]. Therefore, the increase in peak, trough and final viscosity with D[4,3] was followed the basic viscosity principle, that viscosity of dispersion is solute size and shape dependence (Simha, 1940; Tanford, 1961). Pasting temperature is defined as the temperature when the derivative of viscosity in function of time is equal to 2 RVU/s or 24 mPa.s/s (RVA, Newport Scientific, Warriewood, Australien). Since, the larger granule started to touch each other earlier during swelling causing increase in viscosity earlier, therefore pasting temperature was lower. Among four samples, the highest values of these viscosities of whole starch may be due to the fact that whole starch has the broadest size distribution, therefore, the small granules might pack in the voids between the larger granules. This resulted in shorter (mean) spatial distance between the first neighbors of swollen starch granules which increased the granule-granule interactions. The breakdown value increased with D[4,3], which meant the swollen granules were easily disintegrated. Granules with high D[4,3] had high amylose content and should be rigid, decreasing the breakdown value. The effect of granule size on the breakdown is more important than the effect of amylose content in this case. Larger granules can be expected to develop a higher peak viscosity and to have a bigger breakdown (Tran *et al.*, 2009). Setback was a value for both ability of gelation and gel strength. When paste cooled, the strength of gel network depended on

amylose interaction in melt phase. The high setback value of paste was due to high amylose content in melt phase. In this study, amylose content in melt phase was determined (for amylose leaching determination) for whole, SGF, MGF and LGF and they were 20.36, 13.94, 14.42 and 16.39 μg/ml.

4.1.6 Freeze-thaw stability

Figure 4.16 represented the syneresis percentage of gelatinized starch pastes. Each starch paste had an increasing syneresis tendency as the freeze-thaw cycle increased. For each cycle, LGF paste showed higher percentage syneresis than MGF, whole and SGF pastes. The linear relationship between percentage syneresis and freeze-thaw cycle was observed for SGF. For each sample, slope of the line drawn for the first four cycles was about the same. But the slope of the line drawn from the 4th cycle to the 7th cycle decreased as D[4,3] increased.

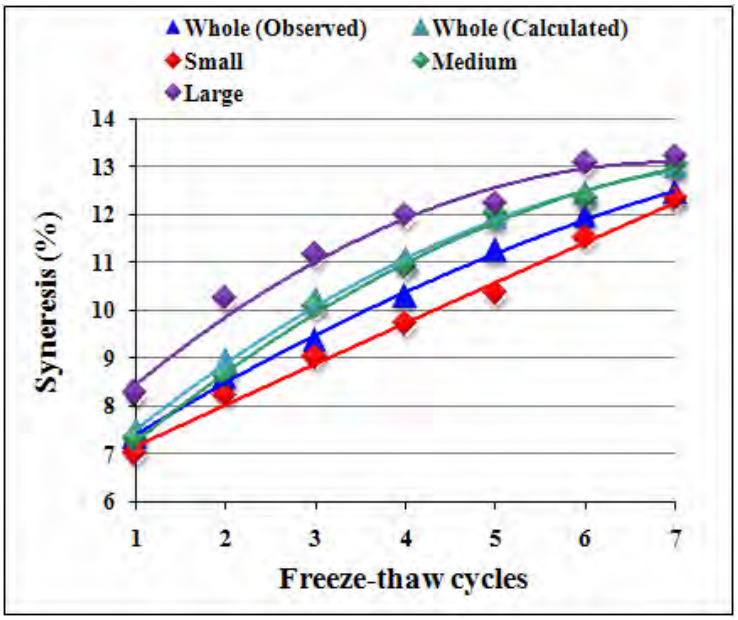


Figure 4.16 Freeze-thaw stability of pigeon pea starches.

Amylose aggregation and crystallization have been reported to be complete within the first few hours of storage while amylopectin aggregation and crystallization occur at later stages (Miles *et al*., 1985). Therefore, percentage syneresis of the 1st cycle reflected amylose aggregation and crystallization. Among three fractions, the higher the amylose content in starch granules and melt phase, the higher the percentage syneresis. These were confirmed by higher amylose content in starch granule and melt phase of LGF than those of MGF and SGF (Figure 4.4). The difference in slope of the line drawn from the 4th cycle to the 7th cycle reflected the extent of amylopectin aggregation. Therefore, the higher the amylopectin content the higher the slope. However, this section could not explain for whole starch which had the highest amylose content in melt phase. The syneresis of starches are indirectly influenced by the structural arrangement of the starch chains within amorphous and crystalline regions of the ungelatinized granule as this structural arrangement influences the degree of granule breakdown during gelatinization and also influences the interactions that occur among starch chains during gel storage (Perera and Hoover, 1999).

4.1.7 Enzymatic digestibility

The percentage hydrolysis of whole, SGE, MGF and LGF starches were 0.36, 0.64, 0.43, and 0.38, respectively (Figure 4.17). Enzymatic digestibility decreased as D[4,3] increased, even though larger granule had higher amylose content and α-amylase predominantly hydrolyzes amylose and exterior branched chain of amylopectin in amorphous region (Marsden and Gray, 1986; Franco *et al*., 1988). Moreover, smaller granule had higher phosphorus content indicating higher phosphate monoester bound to starch molecules, and thus exhibited α-amylase activity (Zhou *et*

al., 2004). Therefore, the main reason for the lower susceptibility to α-amylase hydrolysis of the larger granule was the lower surface area to volume ratio resulting in the smaller contact area between substrate and enzyme (Duffus *et al*., 1995; Cottrell *et al*., 2004; Tester *et al*., 2004; Sandhu and Lim, 2008). The value of the calculated hydrolysis was lower than the observed value point by approximately 0.07 % (Figure 4.17).

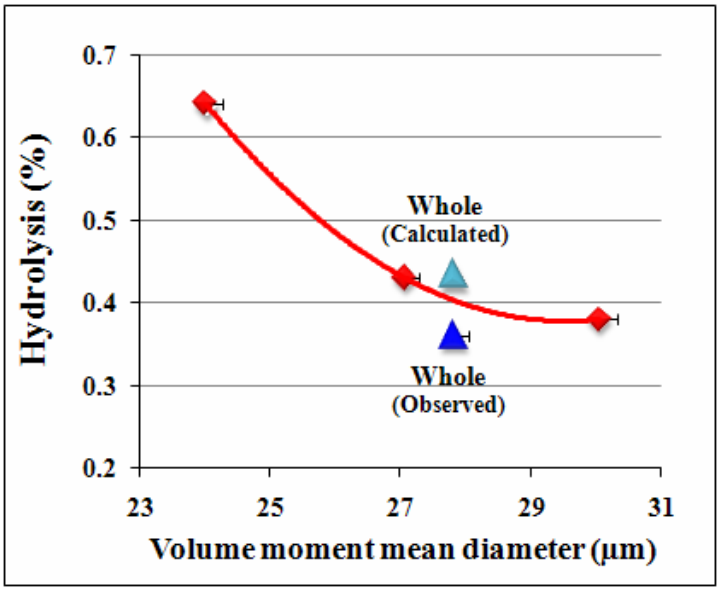


Figure 4.17 Percentage hydrolysis of pigeon pea starches versus volume moment mean diameter.

Since D[4,3] of whole was in between that of MGF and LGF, so it was expected that its properties should be in between those of MGF and LGF. However, the observed properties (except water absorption capacity, pasting properties and percentage hydrolysis) of whole were between those of SGF and MGF. There was no explanation for these behaviors.

4.2 Nixtanalization of pigeon pea

In this section, effects of soaking or boiling grains in water or $Ca(OH)_2$ solution and $Ca(OH)_2$ concentration, $[Ca(OH)_2]$, on chemical composition, structure and properties of pigeon pea starch were determined and evaluated. The pH of $Ca(OH)_2$ solutions for 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 % w/v were 6.86, 7.98, 8.56, 9.27, 10.01, and 10.42, respectively.

4.2.1 Effects of soaking or boiling grains in 0-1.0 % w/v $Ca(OH)_2$ solution on chemical composition

Soaking pigeon pea grains in water or $Ca(OH)_2$ at any concentrations did not give major change in the content of crude protein, lipid, crude fiber, ash, carbohydrate, starch and amylose in comparison to that of native starch (Tables 4.1 and 4.2). However, when soaking grains in $\geq$ 0.4 % w/v $Ca(OH)_2$ solutions, chemical analysis showed that both calcium and phosphorous in starches from soaked grains were of higher concentrations than those presented in native starch by 40-70 ppm and 10-48 ppm, respectively (Table 4.3). This indicated that there were transference of calcium into pigeon pea grains and slight effect on ridding of phosphorous in the extracted starch.

Boiling pigeon peas in water or $Ca(OH)_2$ increased crude protein, crude fiber, lipid, ash, calcium and phosphorous (Tables 4.1 and 4.3). However, there were decreases in carbohydrate, starch and amylose contents at 95 % significant level (Tables 4.1 and 4.2). Furthermore, there was a tendency that crude protein, lipid, ash, starch, amylose, calcium and phosphorous increased in accordance with $[Ca(OH)_2]$. The boiled samples prior to extraction contained less than 95 %

carbohydrate; therefore these obtained samples would now be referred as modified flour.

Table 4.1 Proximate composition of native starch, starches from soaked grains and modified flours.

Boiling time (hr)	Soaking time (hr)	$[Ca(OH)_2]$ (% w/v)	Proximate composition (% dry basis)					
			Moisture content	Crude protein	Total lipid	Crude fiber	Ash	Carbohydrate
-	-	-	9.88 ±0.04	0.43±0.03	0.31± 0.03	0.40± 0.03	0.45 ±0.04	98.42 ±0.05
-	16.0	0.0	8.64 ±0.04	0.51±0.03	0.30± 0.03	0.38± 0.03	0.32 ±0.03	98.49 ±0.05
-	16.0	0.2	8.58 ±0.02	0.46±0.03	0.23± 0.03	0.32± 0.03	0.41 ±0.03	98.58 ±0.03
-	16.0	0.4	8.67 ±0.02	0.52±0.04	0.32± 0.03	0.28± 0.02	0.44 ±0.03	98.44 ±0.03
-	16.0	0.6	8.47 ±0.03	0.55±0.03	0.36± 0.03	0.34± 0.03	0.50 ±0.02	98.25 ±0.03
-	16.0	0.8	8.13 ±0.02	0.62±0.04	0.38± 0.02	0.33± 0.03	0.57 ±0.03	98.10 ±0.11
-	16.0	1.0	8.85 ±0.03	0.73±0.03	0.45± 0.03	0.38± 0.02	0.66 ±0.03	97.78 ±0.05
0.5	15.5	0.0	5.57 ±0.04	5.58±0.03	1.58± 0.04	0.72± 0.02	0.55 ±0.03	91.57 ±0.07
0.5	15.5	0.2	7.83 ±0.03	6.23±0.03	2.28± 0.03	0.63± 0.04	1.04 ±0.03	89.82 ±0.10
0.5	15.5	0.4	6.73 ±0.04	6.45±0.04	2.23± 0.04	0.66± 0.03	1.78 ±0.04	88.89 ±0.10
0.5	15.5	0.6	6.62 ±0.03	6.16±0.03	2.29± 0.09	0.72± 0.02	1.82 ±0.03	88.31 ±0.05
0.5	15.5	0.8	6.20 ±0.02	6.62±0.04	2.76± 0.03	0.64± 0.04	1.95 ±0.06	88.03 ±0.06
0.5	15.5	1.0	7.30 ±0.02	6.38±0.04	2.81± 0.02	0.71± 0.02	1.98 ±0.02	88.12 ±0.06

Data represent (mean ± SD, n = 3).

Table 4.2 Starch and amylose contents in native starch, starches from soaked grains and modified flours.

Boiling time (hr)	Soaking time (hr)	$[Ca(OH)_2]$ (% w/v)	Starch content (% dry basis)	Amylose content (% dry basis)
-	-	-	96.74 ± 0.02	30.74 ± 0.11
-	16.0	0.0	96.52 ±0.05	30.22 ± 0.46
-	16.0	0.2	96.29 ±0.03	31.01 ± 0.24
-	16.0	0.4	96.35 ±0.02	30.66 ± 0.46
-	16.0	0.6	95.99 ±0.02	30.78 ± 0.44
-	16.0	0.8	96.30 ±0.03	30.91 ± 0.50
-	16.0	1.0	95.94 ±0.02	30.96 ± 0.41
0.5	15.5	0.0	62.62 ± 0.08	20.08 ± 0.28
0.5	15.5	0.2	64.58 ±0.04	20.97 ± 0.28
0.5	15.5	0.4	63.88 ±0.08	21.41 ± 0.34
0.5	15.5	0.6	64.79 ±0.08	21.75 ± 0.37
0.5	15.5	0.8	65.32 ±0.01	23.37 ± 0.37
0.5	15.5	1.0	64.05 ±0.08	23.74 ± 0.42

Data represent (mean ± SD, n = 3).

Table 4.3 Calcium and phosphorus contents in native starch, starches from soaked grains and modified flours.

Boiling time (hr)	Soaking time (hr)	$[Ca(OH)_2]$ (% w/v)	Calcium content (ppm)	Phosphorus content (ppm)
-	-	-	478.50±1.95	155.26 ± 1.37
-	16.0	0.0	488.25±2.38	158.38 ± 1.10
-	16.0	0.2	462.75±0.42	161.54 ± 0.52
-	16.0	0.4	548.75±1.48	171.38 ± 0.86
-	16.0	0.6	535.00±2.71	177.77 ± 0.45
-	16.0	0.8	539.25±1.03	184.09 ± 0.67
-	16.0	1.0	558.00±0.37	194.84 ± 0.57
0.5	15.5	0.0	1260.25±0.86	1599.16 ± 1.65
0.5	15.5	0.2	1920.25±1.02	2413.14± 1.66
0.5	15.5	0.4	4268.75±0.57	4091.02 ± 1.60
0.5	15.5	0.6	5665.75±0.86	5324.91 ± 0.96
0.5	15.5	0.8	5257.75±1.21	5580.46 ± 1.22
0.5	15.5	1.0	6093.50±1.67	5963.81 ± 1.64

Data represent (mean ± SD, n = 3).

The effects of boiling pigeon peas in water or $Ca(OH)_2$ on chemical analysis are as following.

- Both water and $Ca(OH)_2$ defused into pigeon peas during soaking process in $Ca(OH)_2$ (Gómez *et al.*, 1991). This resulted in more hydrated starch granules.
- Starch is partially gelatinized, which resulted in starch granular size increase. Diffusion of starch and especially amylose out from starch granules caused both to dissolve in water during starch extraction (Gómez *et al*., 1992 and Mondragón *et al*., 2004). This resulted in lower contents of carbohydrate, starch and amylose in modified flour versus native starch.
- Protein denaturation results in inability of protein to dissolve (Gómez *et al*., 1989; Paredes-López and Saharópulos, 1992; Gómez *et al*., 1989; Serna-Saldivar *et al.*, 1990). Therefore, denatured protein did not dissolve out with water during starch extraction leading to higher concentration versus that of native starch.
- Lipid is saponification. During nixtamalization endosperm free fatty acids, which form complex with helical amylose, are neutralized giving calcium salts (Ca^{2+} salts of aliphatic acids), and thus remain as complex with amylose (Reguera *et at*., 2003 and Gonzalez *et al*., 2004; Gómez *et al*., 1991; Carmen, 2003). This lipid-amylose complex was difficult to remove during extraction.

- Chelating between calcium and phytic acid produces calcium phytate that did not dissolve in water (Hernández-Urizar and Bressani, 1997; Rojas-Molina *et al*., 2009). Hence calcium phytate remained after starch extraction causing higher calcium and phosphorous contents in modified flour versus native starch (Hernández-Urizar and Bressani,, 1997; Bressani *et al*., 2004).
- There are interactions between starch-protein, protein-protein, protein-lipid, amylose-lipid, calcium-starch, calcium-protein, phytate-protein and phytate-amylose (Laria *et al*., 2007; Paredes-López and Saharópulos, 1992; Gómez *et al*., 1989; Serna-Saldivar *et al.*, 1990; Gómez *et al*., 1991; Carmen, 2003; Rickard and Thompson, 1997; Thompson, 1986; Kies, 1998). Hence protein, lipid, calcium, starch and amylose were trapped in the gelatinized starch granules. As a result protein, lipid, calcium and phosphorus in modified flour were high in concentration. These components were difficult to extract or separate, so that the proportion of starch and amylose in modified flour decreased.

4.2.2 Effects of soaking or boiling grains in 0-1.0 % w/v $Ca(OH)_2$ solution on granule shape and birefringence.

Figure 4.18 shows microscopic pictures of starch granules from native starch, starches from soaked pigeon pea grains and modified flours. Native starch granules were round and oval similar to those from green peas and potatoes. Hilum

position can be seen through the microscopic pictures, while through polarized light microscope shows birefringence of starch granules. The characteristic of birefringence indicated that there was no damage from soaking in water or $Ca(OH)_2$ and did not result in amylopectin crystalline dissolution. While granules of modified flours showed partial granule breakdown and loss of birefringence. The loss of birefringence was in correlation with $Ca(OH)_2$ concentration in which the peas were boiled (Figure 4.18). This was the result of gelatinized starch from boiling; hence there was partial dissolution in amylopectin crystalline. Modified flour contained both starch granules that maintain natural shape and those with evident destruction of birefringence. The surface area of modified flour starch granule is not smooth due to dissolution and gelatinization; causing the loss of natural granule shape and crystallization of amylopectin. Further observations also indicate clumping of modified flour from dissolution and gelatinization. Starch granules from modified flour have physical properties similar to paste or glue, resulting in clumping.

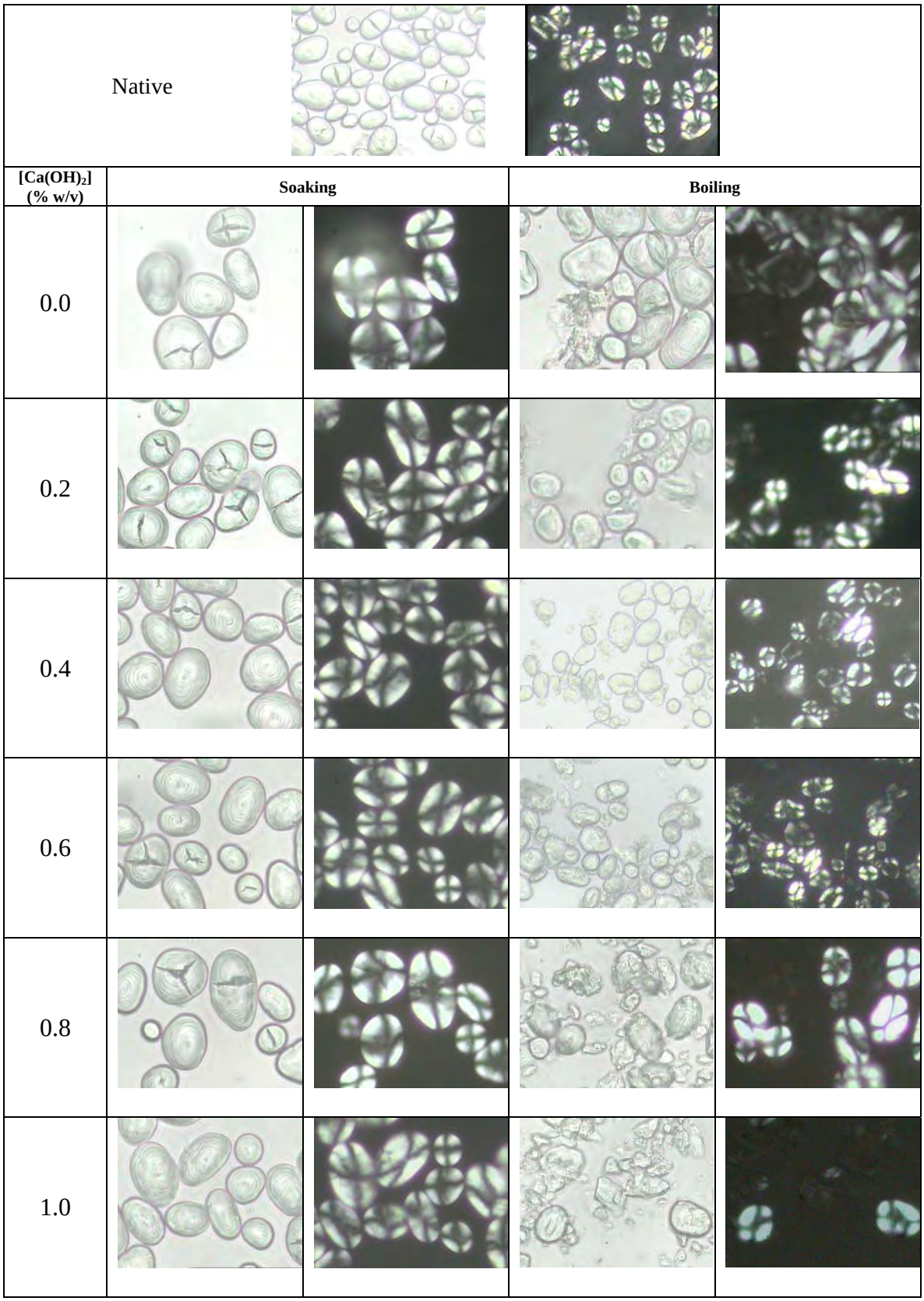


Figure 4.18 Microscope and polarized light microscope images of starch granules from native starch, starches from soaked grains and modified flours.

SEM analysis showed that granules of native starch and granules from soaked grains had smooth surface and oval in shape (Figure 4.19). Even though starch granules from soaked grains in 0.2 % w/w $Ca(OH)_2$ did not indication granule destruction (smooth surface area), the granules from soaked grains in 0.4 % w/w $Ca(OH)_2$ and higher concentration had calcium deposition on the surface. As with the loss of birefringence, the concentration of surface calcium deposition increased with the concentration of $Ca(OH)_2$. This demonstrated there was diffusion of calcium during soaking which were not eliminated through starch extraction. While SEM analysis from modified flour at any $Ca(OH)_2$ concentrations demonstrated rough surface area, indicating surface granule dissolution from gelatinization. Furthermore, surface calcium deposition was also observed.

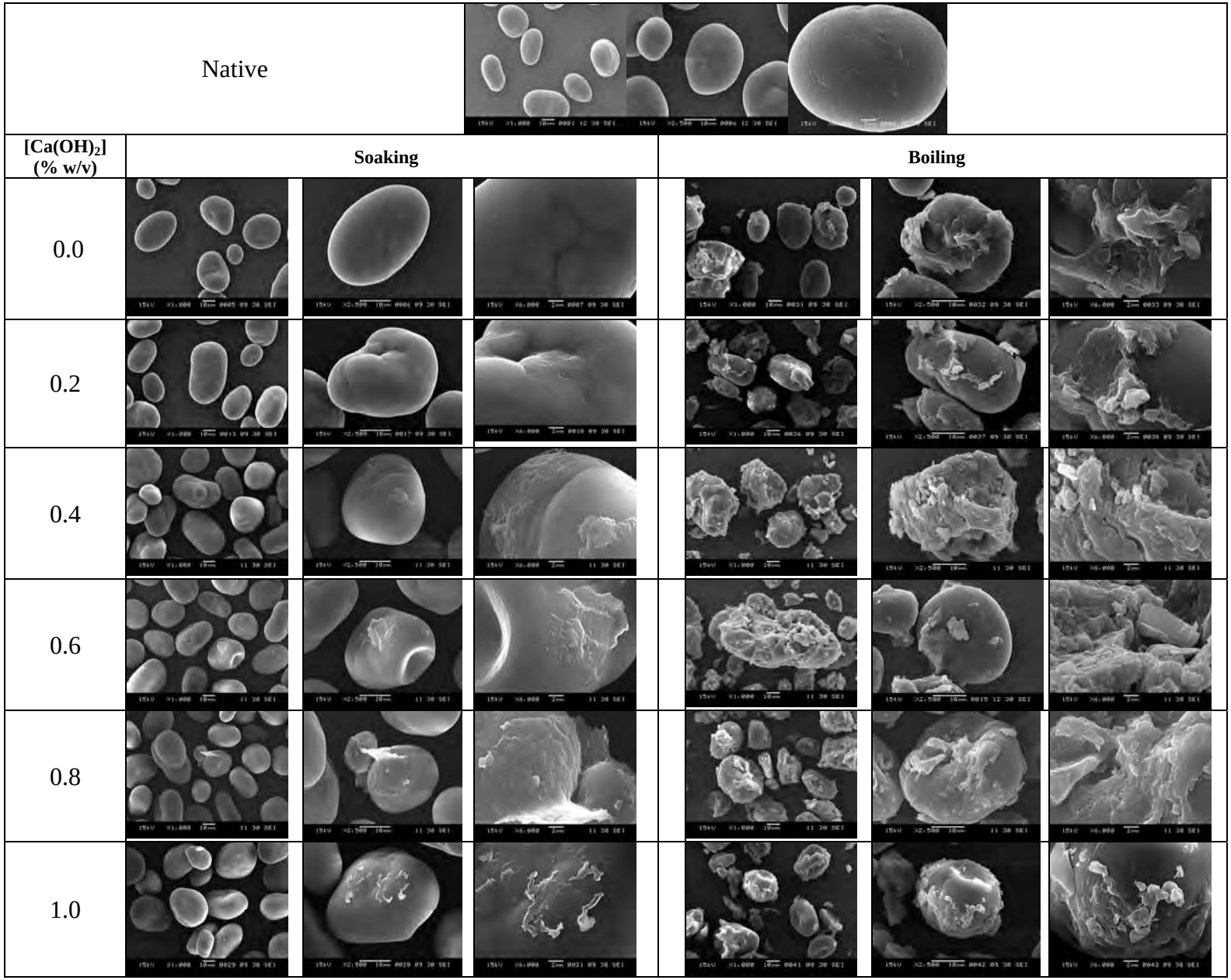


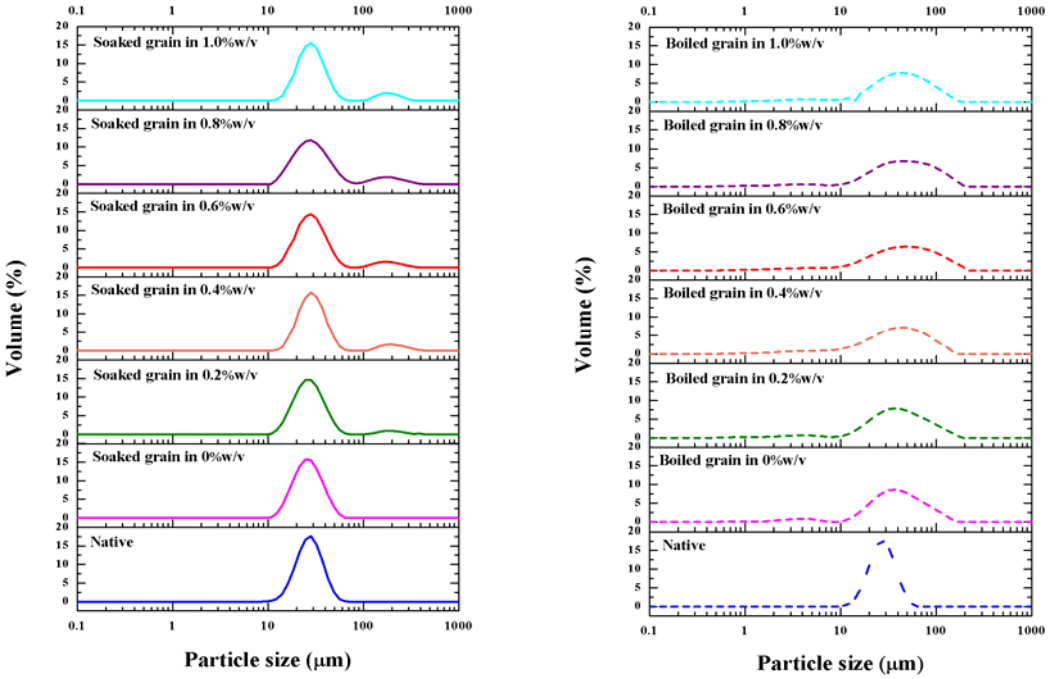
Figure 4.19 SEM images of granules from native starch, starches from soaked grains and modified flours.

4.2.3 Effects of soaking or boiling grains in 0-1.0 % w/v $Ca(OH)_2$ solution on granule size and size distribution

Soaking grains in water did not affect D[4,3] and size distribution pattern (Figures 4.20). Soaking grains in 0.2-1.0 % w/v $Ca(OH)_2$ solutions gave bimodal size distribution and wider size range (Figure 4.20). The second peak, accounting for ≈ 5-15 % v/v, ranged from ≈100-400 μm and caused an increase in D[4,3] ≈ 30-78 % (Figure 4.21). However, D_v at the mode for each sample from soaked grains and native starch was about the same ≈ 28 μm (Figure 4.20 A). Boiling grains in water or 0.2-1.0 % w/v $Ca(OH)_2$ solutions gave bimodal granule size distribution with < 10% v/v of particles having size between 0.5-10 μm (1^{st} peak), and caused a wider range of granule size and an increase in D[4,3] higher than 60 % (Figures 4.20 and 4.21). However, D_v at the mode for each modified flour was about the same, ≈ 40 μm (Figure 4.20 B). There was no specific trend in changing D[4,3] with $Ca(OH)_2$ concentration (Figure 4.21).

Since reversible swelling occurred when starch granules were soaked in water at > 40°C (French, 1984), therefore, soaking grains in water did not affect granule size and size distribution. For soaking grains in $Ca(OH)_2$ solution, the second peak reflected the size of granule aggregates as the granules were bound together by the deposited calcium on granule surface, which was evidenced by SEM image (Figure 4.22). Starch gelatinization occurred during boiling grains caused irreversible swelling of starch granules (French, 1984). Aggregate of swollen gelatinized granules was promoted in boiling grains as granules contacted with each other and gelatinized starch has strong binding and adhesive properties (Mentzer, 1984; Kennedy and

Fischer, 1984). The swollen granules and their aggregate as evidence in SEM images (Figure 4.19) resulted in a larger particle size comparing to that of the native starch.



A **B**

Figure 4.20 Effect of soaking or boiling pigeon pea grains in 0-1.0 % w/v calcium hydroxide solution on granule size distribution.

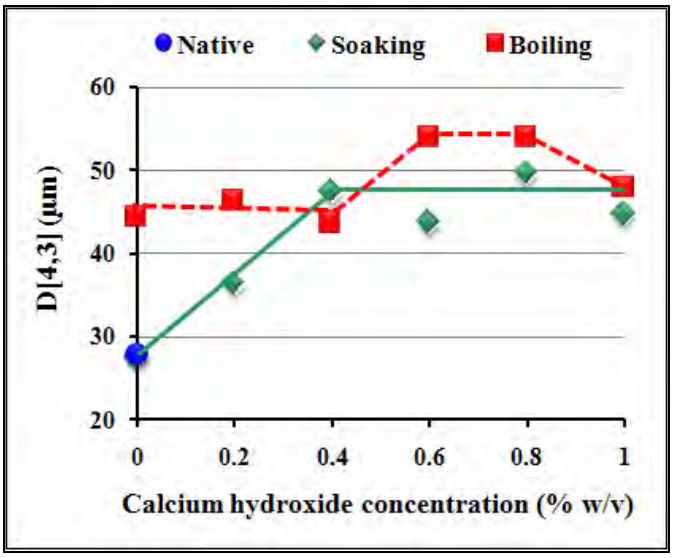


Figure 4.21 Effect of soaking or boiling pigeon pea grains in 0-1.0 % w/v calcium hydroxide solution on volume moment mean diameter (D[4,3]).

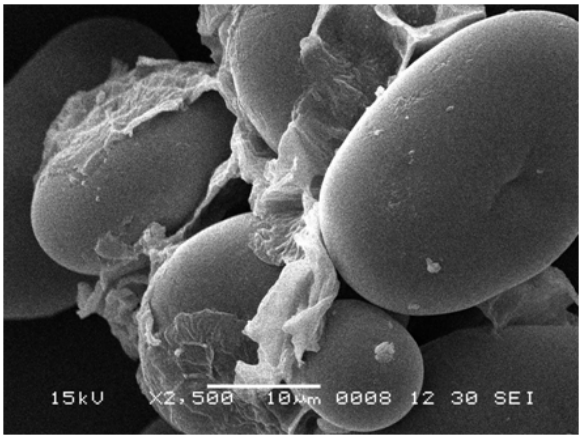
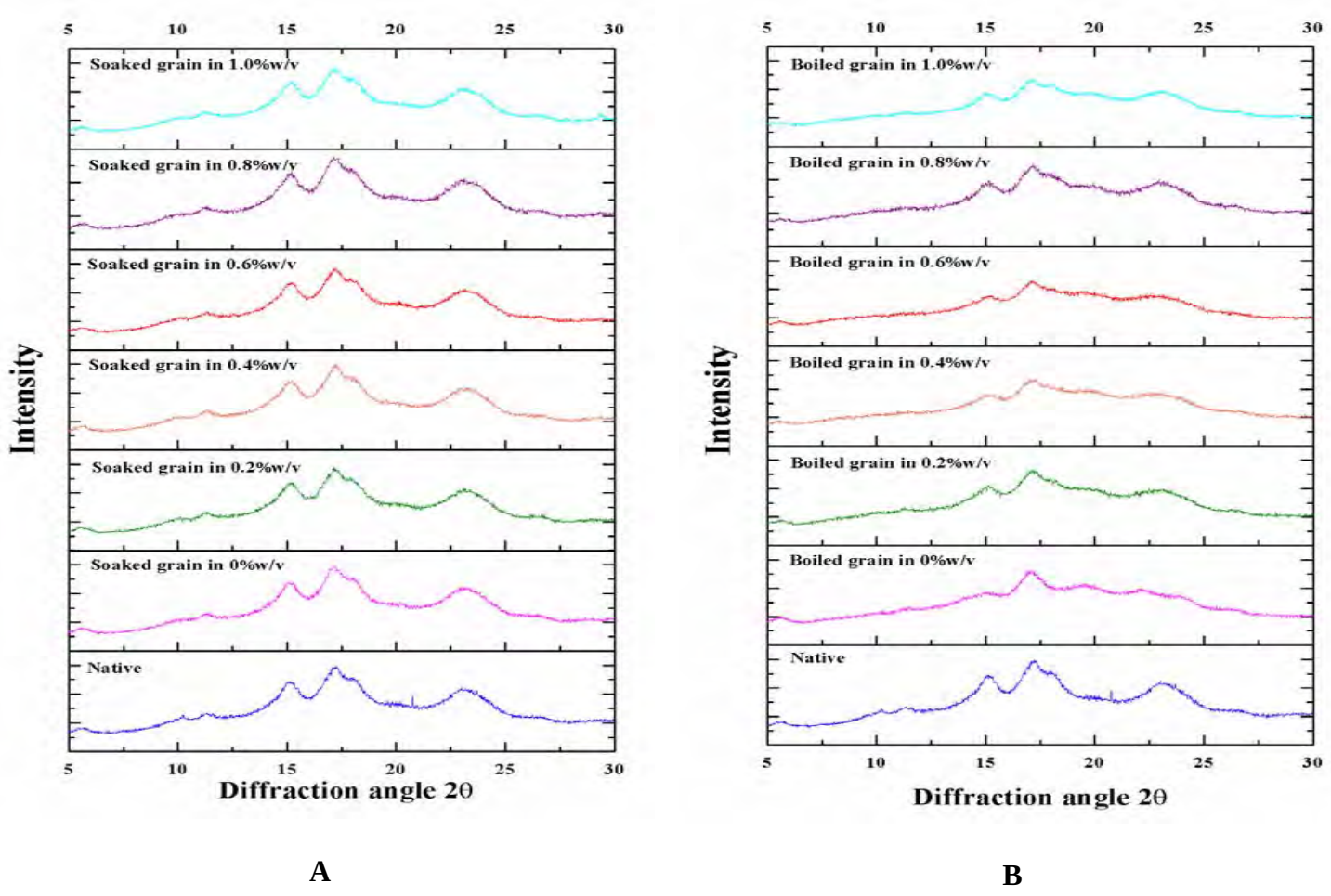


Figure 4.22 SEM images of starch granules from soaked grains.

The small particle fraction (1st peak) contained broken particles of swollen granules, which were weaker than the native one and were broken during starch isolation. Particle size and size distribution of corn flour obtained from boiling corn kernel in $Ca(OH)_2$ solution (nixtamalized corn flour or NCF) were reported to be the most critical parameter for texture of tortilla chip, taco shell and soft tortilla (Gómez *et al*., 1989; Gómez *et al*., 1991). Small NCF particles gave higher water uptake, viscosity, cohesiveness, plasticity and smoothness than large NCF particles (Gómez *et al*., 1989; Gómez *et al*., 1991). Large NCF particles (> 250 μm) disrupted the dough network, reduced blistering, decreased oil uptake and gave crispiness, therefore, they gave good textural characteristics for fried products (Gómez *et al*., 1991). Pigeon pea flours from boiled grains (modified pigeon pea flours) can be fractionated into specific size range for different applications.

4.2.4 Effects of soaking or boiling grains in 0-1.0 % w/v $Ca(OH)_2$ solution on crystallinity.

Figure 4.23 shows large peaks at 17.2, 18.1 and 23.1 2θ and small peaks at 5.54 and 15.18 2θ in every sample indicating the C_a-type diffraction pattern (Horng, 2007). Therefore, soaking and boiling grain in water or 0.2-1.0 % w/v $Ca(OH)_2$ solutions did not alter the amylopectin crystalline pattern in starch granules. However, the X-ray pattern of all modified flours also showed the presence of the characteristic peak of the V polymorph, a shoulder at 12.9 2θ and a small peak at 19.8 2θ (Mondragón *et al*., 2004). Soaking grains in water or 0.2-1.0 % w/v $Ca(OH)_2$ solutions did not affect percentage relative crystallinity (Figure 4.24). Comparing to the native starch, percentage relative crystallinity of modified flours was lower (Figure 4.24). Percentage relative crystallinity increased linearly with an increase in $[Ca(OH)_2]$ (Figure 4.24).



A **B**

Figure 4.23 Effect of soaking or boiling pigeon pea grains in 0-1.0 % w/v calcium hydroxide solution on crystalline pattern.

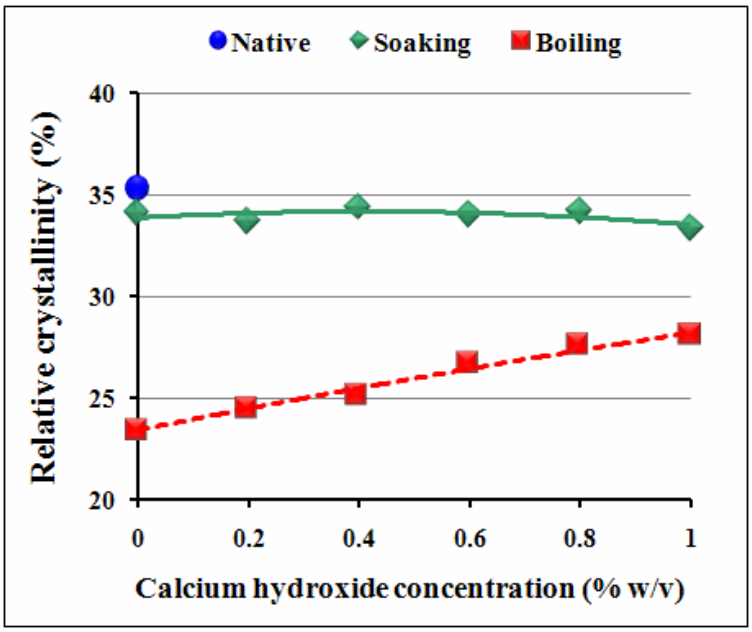


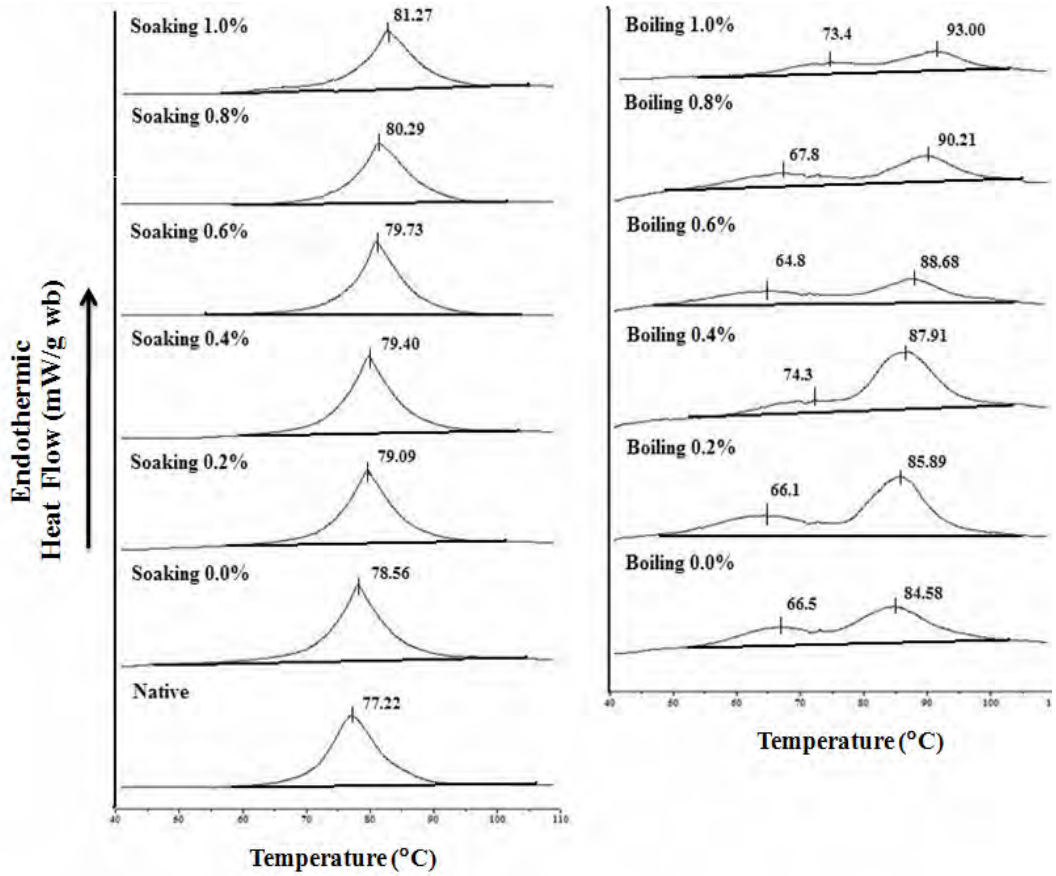
Figure 4.24 Effect of soaking or boiling pigeon pea grains in 0-1.0 % w/v calcium hydroxide solution on percentage relative crystallinity.

Gelatinization of starch occurring during boiling grains in water or $Ca(OH)_2$ solutions partially destroyed the crystalline organization of starch granules (French, 1984; Gómez *et al*., 1992; Mondragón *et al*., 2004). After boiling, recrystallization could occur during steeping and cooling grains (Gómez *et al*., 1992; Mondragón *et al*., 2004). The V polymorph confirmed the amylose-lipid interactions occurred during boiling grains (Mondragón *et al*., 2004).

4.2.5 Effects of soaking or boiling grains in 0-1.0 % w/v $Ca(OH)_2$ solution on thermal properties

Soaking grains in water did not significantly affect gelatinization temperatures and ΔH_{Gel} (Figures 4.25 and 4.26). For soaking grains in $Ca(OH)_2$ solutions, gelatinization temperatures slightly increased with $[Ca(OH)_2]$ used for soaking (Figure 4.25). However, ΔH_{Gel} did not affect by $[Ca(OH)_2]$ used (Figure

4.26). Boiling grains caused partial gelatinization of starch granules resulting in two endotherm thermograms of resulting flours (modified flours). The first endotherm (under 80ºC) corresponded to regelatinize of retrograded starch which previously gelatinized during boiling grains. The second endotherm corresponded to gelatinize of modified flour. T_o, T_p and T_c of modified flour from boiled grains were > 7, > 7 and > 10 ºC higher than those of native starch, respectively (Figure 4.25). Their values increased with [$Ca(OH)_2$] used for boiling. Figure 4.26 shows that modified flours had lower ΔH_{Gel} than that of native starch, but ΔH_{Gel} increased with [$Ca(OH)_2$] used.



	$[Ca(OH)_2]$ (% w/v)	T_o (°C)	T_p (°C)	T_c (°C)
Native	-	72.69±0.13	77.22±0.32	82.62±0.14
Soaking	0.0	72.11±0.32	78.56±0.13	82.62±0.15
	0.2	73.00±0.15	79.09±0.23	84.61±0.20
	0.4	73.53±0.14	79.40±0.15	85.23±0.22
	0.6	73.70±0.19	79.73±0.46	85.97±0.32
	0.8	74.12±0.22	80.29±0.29	86.77±0.20
	1.0	74.69±0.46	81.27±0.32	87.55±0.15
Boiling	0.0	80.25±0.23	84.58±0.36	92.64±0.22
	0.2	80.64±0.14	85.89±0.29	93.80±0.26
	0.4	83.09±0.38	87.91±0.22	95.86±0.15
	0.6	83.55±0.46	88.68±0.32	97.02±0.29
	0.8	84.23±0.14	90.21±0.29	97.62±0.22
	1.0	84.92±0.32	93.00±0.22	98.63±0.19

Figure 4.25 Effect of soaking or boiling pigeon pea grains in 0-1.0 % w/v calcium hydroxide solution on thermal properties.

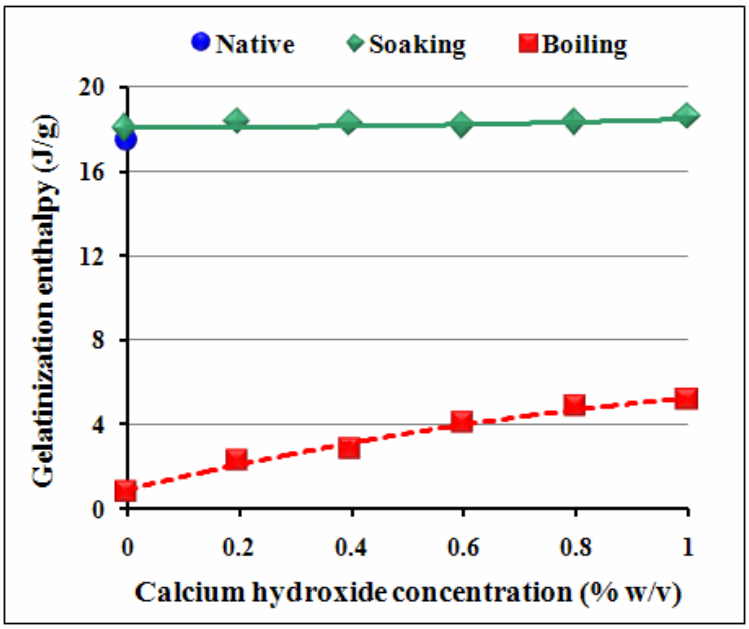


Figure 4.26 Effect of soaking or boiling pigeon pea grains in 0-1.0 % w/v calcium hydroxide solution on gelatinization enthalpy.

For starches from soaked grains, gelatinization temperatures slightly increased with [$Ca(OH)_2$] as depositing calcium on granule surface delayed migration of water into granules. Annealing of starch inside the granules occurred during boiling grains. Protein, amylose, calcium and phosphorus contents in modified flours were higher than those of native starch and starches from soaked grains, because starch-protein, protein-protein, protein-lipid, amylose-lipid, calcium-amylose and calcium-protein interactions occurred during boiling grains. Moreover, interactions of phytic acid with calcium, amylose and protein also occurred during boiling grains (Reddy *et al.*, 1982; Cheryan, 1980; Rojas-Molina *et al.*, 2009). These interactions resulted in rigid swollen pregelatinized granules of modified flours. These interactions might resist these granules to further gelatinize resulting in higher gelatinization temperatures. Boiling grains disrupt some of the double helix in crystalline and non-crystalline regions of granules. Therefore, fewer double helixes

left to break during gelatinization in DSC causing lower ΔH_{Gel} of modified flours. Mendez-Montealvo (2006) reported that nixtamalized maize flour had higher gelatinization temperature than its raw counterpart, because the Ca^{2+} ions stabilize starch structure of nixtamalized flour. Percentage relative crystallinity increased, and thus gelatinization temperatures and ΔH_{Gel} of modified flours increased as [$Ca(OH)_2$] used for boiling grains increased.

4.2.6 Effects of soaking or boiling grains in 0-1.0 % w/v $Ca(OH)_2$ solution on water and oil absorption capacities, swelling power, solubility, and amylose leaching

Soaking grains in water did not significantly affect water absorption capacity and oil absorption capacities at 25 and 70°C (Figure 4.27). At both temperatures, boiling grains gave higher water and oil absorption capacities than soaking grains. For each sample, water absorption capacity at 25°C was about half of that at 70°C, while oil absorption capacity at 25°C was about 0.7-0.8 times of that at 70°C. For each sample, water absorption capacity was about 2 and 3 times of oil absorption capacity at 25 and 70°C, respectively. At 25°C, water absorption capacity of starches from soaked grains increased with [$Ca(OH)_2$], while that of modified flours decreased as [$Ca(OH)_2$] increased up to 0.6 % w/v and then it increased with [$Ca(OH)_2$]. At 70°C, [$Ca(OH)_2$] dependency of water absorption capacity was opposite for starches from soaked grains and modified flours. At 25 and 70°C, oil absorption capacity slightly increased with [$Ca(OH)_2$] up to 0.6 % w/v and then level off for starches from soaked grains and modified flours.

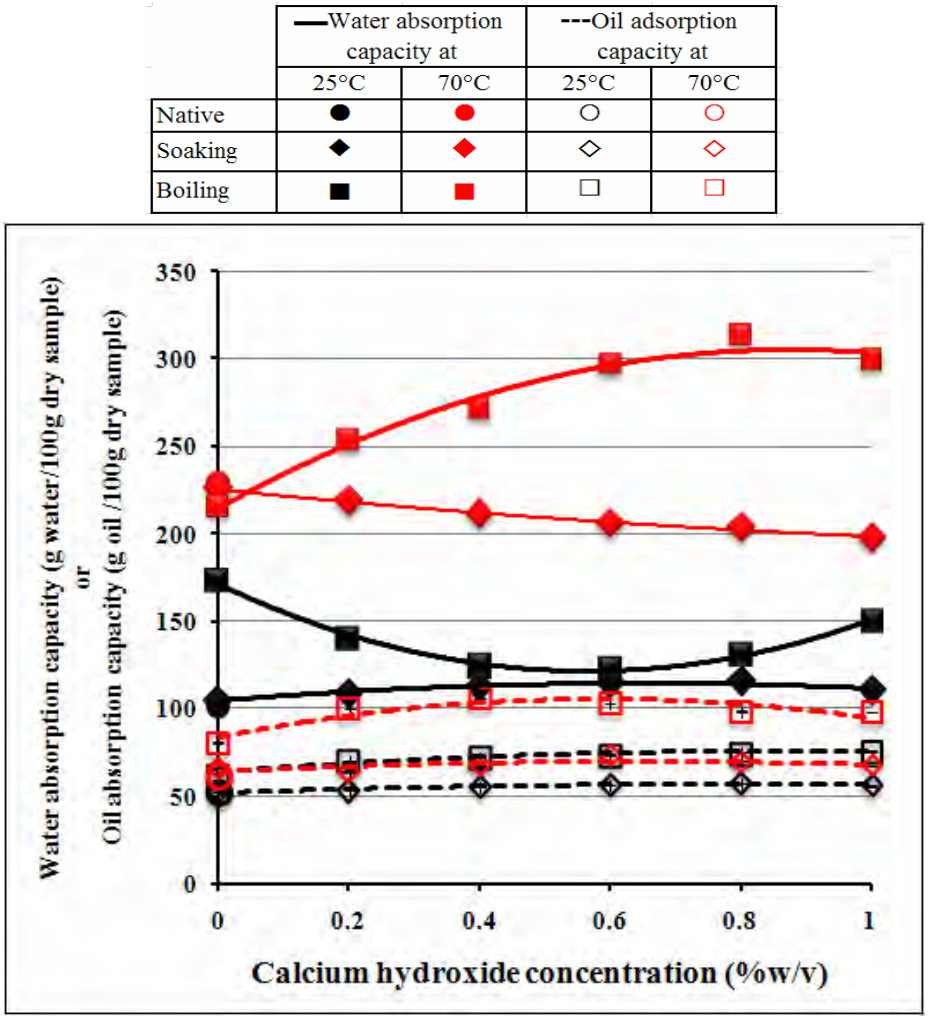


Figure 4.27 Effect of soaking or boiling pigeon pea grains in 0-1.0 % w/v calcium hydroxide solution on water absorption capacity and oil absorption capacity.

Since granules in modified flours were partial gelatinized and had wider channels as evidence in SEM images (Figure 4.19), therefore, they were more readily to absorb water or oil than native starch and starches from soaked grains (Sefa-Dedeh *et al*., 2004). At 25 and 70ºC, changing in water absorption capacity with [$Ca(OH)_2$] for starches from soaked grains and modified flours from boiled grains agreed with that reported by Sefa-Dedeh *et al*. (2004). At 70ºC, an increase in water absorption capacity with [$Ca(OH)_2$] for modified flours might be due to increases in calcium and

phosphorus contents. All samples had higher water absorption capacity than oil absorption capacity due to hydrophilic nature of starch and flour.

At each temperature, soaking grains in water did not affect swelling power, solubility and percentage amylose leaching (Figure 4.28, 4.29 and 4.30, respectively). At temperature below 70ºC, swelling power, solubility and percentage amylose leaching of modified flours were higher than those of native starch and starches from soaked grains (Figures 4.28, 4.29, and 4.30). At temperature over 70ºC, these three properties of modified flours were lower than those of native starch and starches from soaked grains. For each sample and temperature, swelling power, solubility and percentage amylose leaching of starches from soaked grains slightly decreased as [$Ca(OH)_2$] increased (Figures 4.31 A and B, 4.32 A and 4.33 A). At 50ºC, amylose did not leach out from any samples (Figures 4.28 C and 4.30 A). Like native starch, all starches from soaked grains and modified flours had the Arrhenius-type temperature dependence with $R^2 \sim 0.9$ (Figures 4.31 B, 4.32 B and 4.33 B). Slopes of the Arrhenius relationships for these three properties of native starch and starches from soaked grains were higher than those of modified flours. Slopes of the Arrhenius relationships for amylose leaching were higher than those of swelling power and solubility. These suggested that these three properties of native starch and starches from soaked grains depended on temperature stronger than modified starch, and amylose leaching depended on temperature more than swelling power and solubility. However, there were less than 10 % changes in these slopes with [$Ca(OH)_2$] used for soaking or boiling.

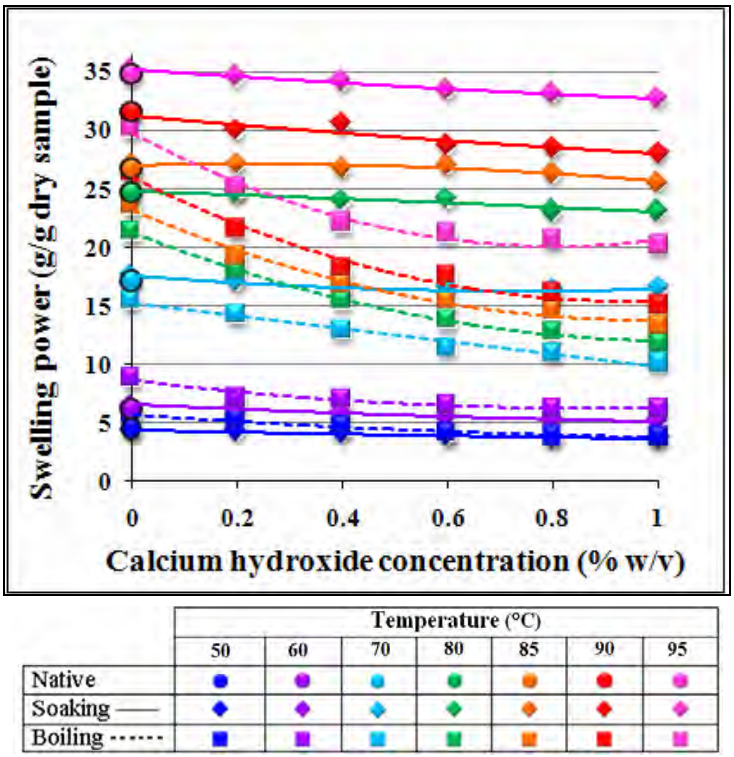


Figure 4.28 Effect of soaking or boiling pigeon pea grains in 0-1.0 % w/v calcium hydroxide solution on swelling power.

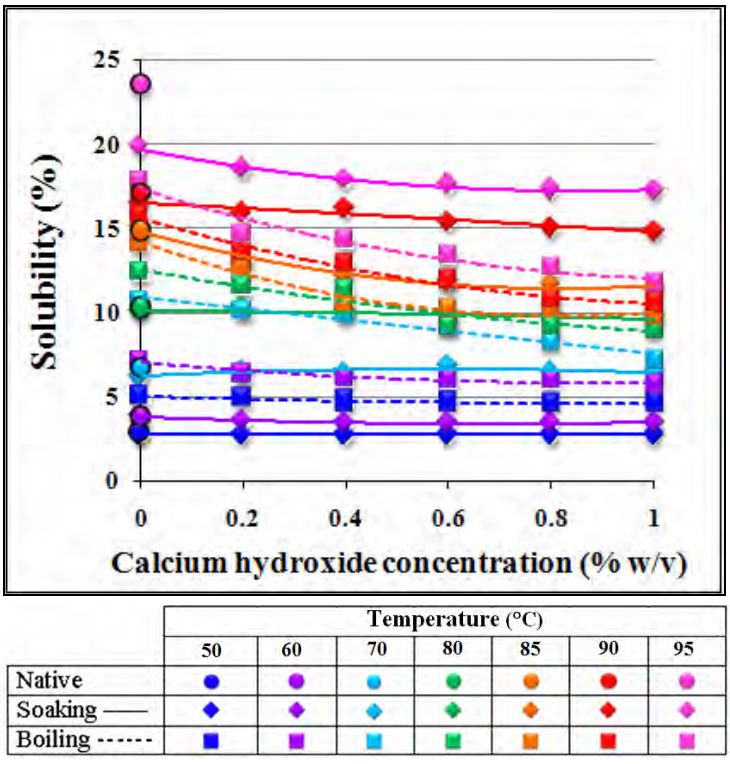


Figure 4.29 Effect of soaking or boiling pigeon pea grains in 0-1.0 % w/v calcium hydroxide solution on solubility.

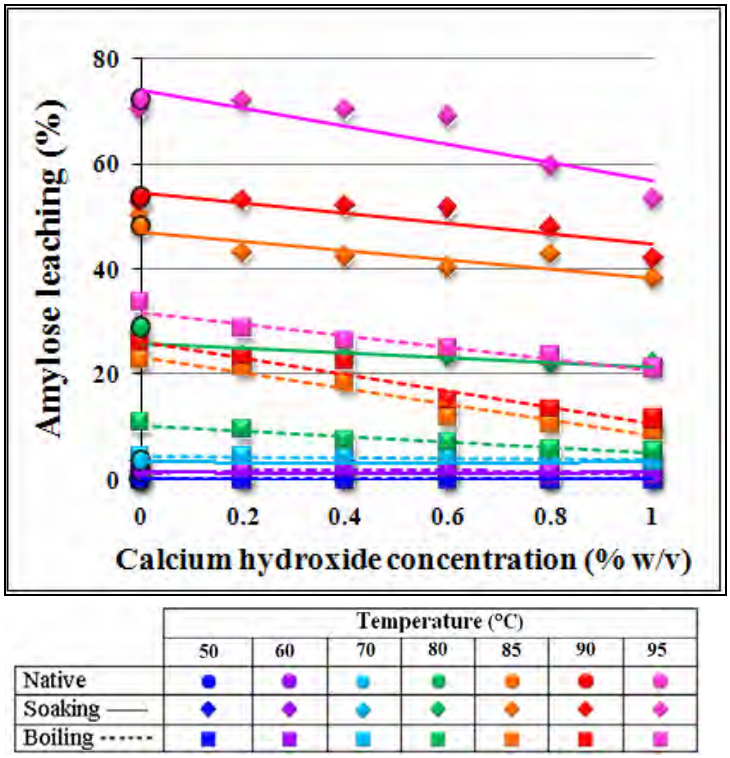
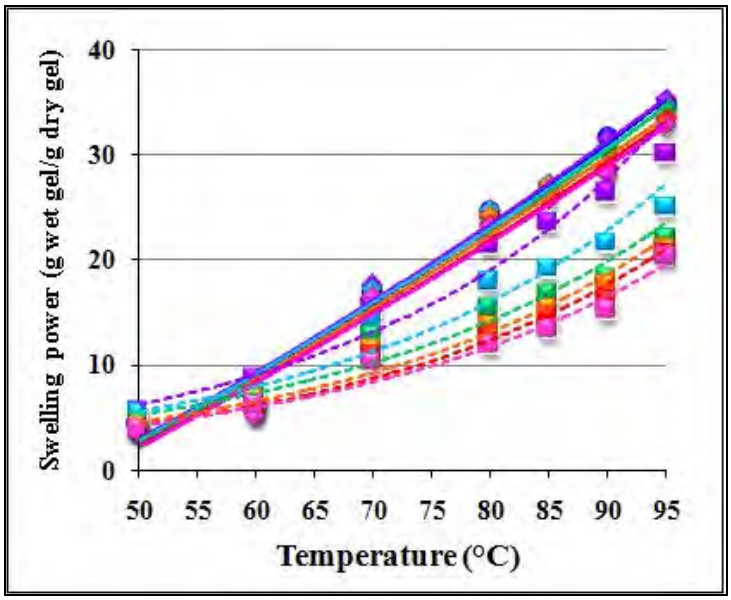
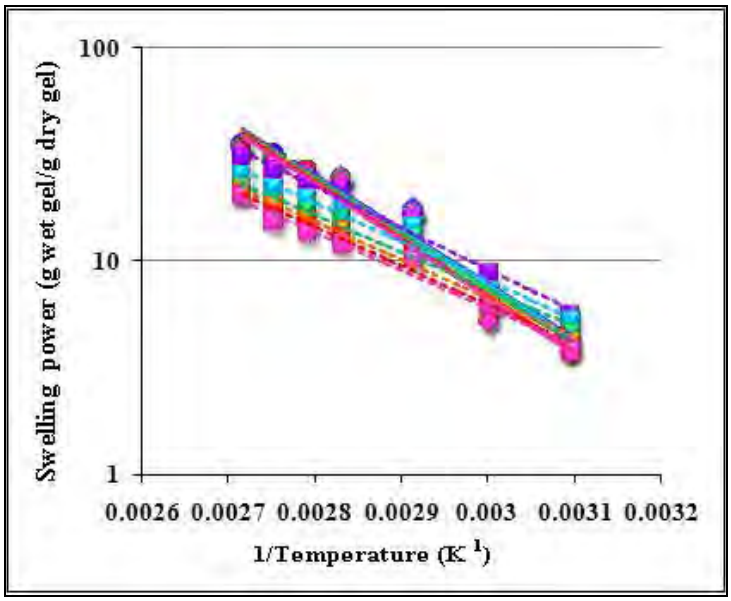


Figure 4.30 Effect of soaking or boiling pigeon pea grains in 0-1.0 % w/v calcium hydroxide solution on percentage amylose leaching.



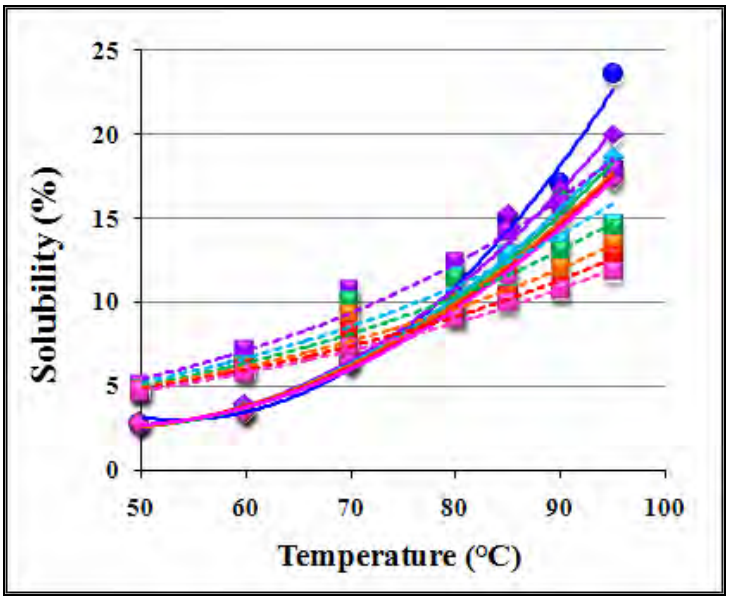
A



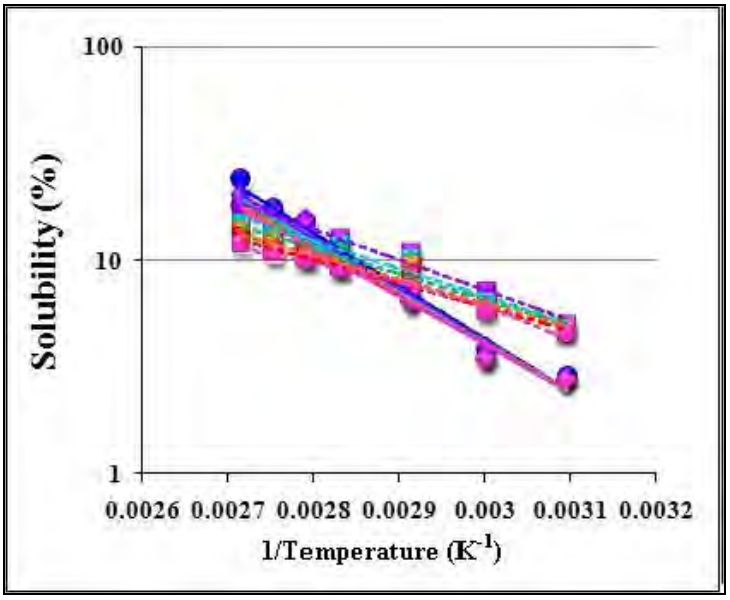
B

	[Ca(OH)$_2$] (% w/v)					
	0.0	0.2	0.4	0.6	0.8	1.0
Native	●					
Soaking ——	◆	◆	◆	◆	◆	◆
Boiling -----	■	■	■	■	■	■

Figure 4.31 Swelling power of pigeon pea starches (native and from soaked grains) and modified pigeon pea flours as a function of temperature (A) or 1/temperature (B).



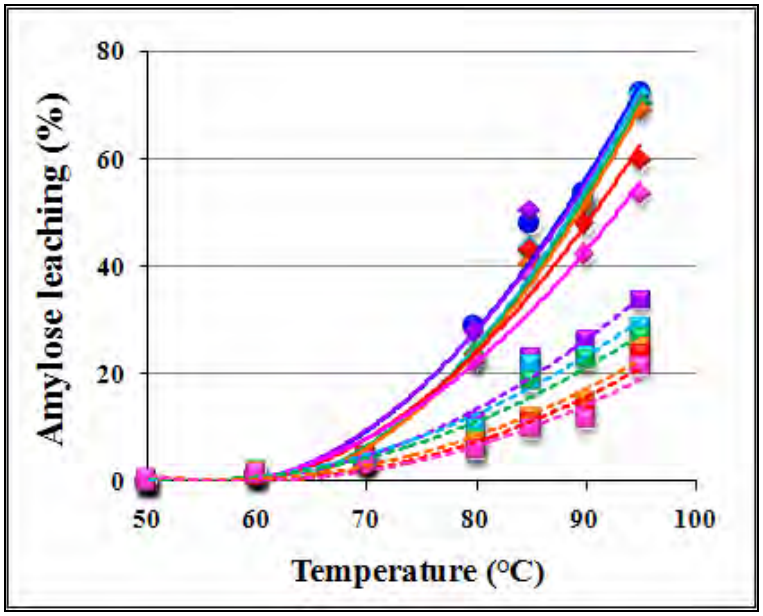
A



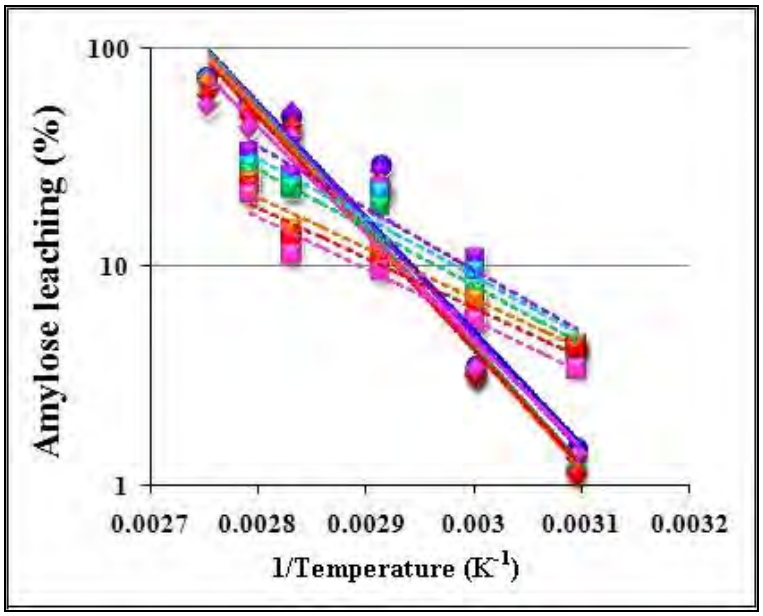
B

	[Ca(OH)$_2$] (% w/v)					
	0.0	0.2	0.4	0.6	0.8	1.0
Native	●					
Soaking ——	◆	◆	◆	◆	◆	◆
Boiling -----	■	■	■	■	■	■

Figure 4.32 Solubility of pigeon pea starches (native and from soaked grains) and modified pigeon pea flours as a function of temperature (A) or 1/temperature (B).



A



B

	[Ca(OH)$_2$] (% w/v)					
	0.0	0.2	0.4	0.6	0.8	1.0
Native	●					
Soaking ——	◆	◆	◆	◆	◆	◆
Boiling -----	■	■	■	■	■	■

Figure 4.33 Amylose leaching of pigeon pea starches (native and from soaked grains) and modified pigeon pea flours as a function of temperature (A) or 1/temperature (B).

Swelling power, solubility and percentage amylose leaching of modified flours were higher than those native starch and starches from soaked grains at temperature lower than T_o of native starch and starches from soaked grains. At temperature < 70ºC which was lower than T_o of native starch and starches from soaked grains, granules from boiled grains had wider channels, therefore it was easier for water to diffuse in and water soluble solids to diffuse out. In addition, depositing calcium on granule surface of starches from soaked grains might inhibit diffusion of components into and out of granules. This resulted in higher swelling power, solubility and amylose leaching of modified flours. At temperature higher than T_o of native starch and starches from soaked grains, granules of native starch and starches from soaked grains gelatinized causing higher values of these three properties than modified flours. Gelatinization of starch is thermal activated process. As gelatinization of granules extends with temperature, swelling power, solubility and percentage amylose leaching increase. At temperature ≥ 70ºC, depositing calcium on granule surface might diffuse into granule during gelatinization and heating might induce Ca-amylose interactions within granule, preventing transferring of components into and out of granules. Therefore, decrease in these three properties as $[Ca(OH)_2]$ increased might be due to an increase in Ca-amylose interactions. For modified flours, lipid content increased with $[Ca(OH)_2]$ might indicate an increase in amylose-lipid complexes resulting in decrease in these three properties.

4.2.7 Effects of soaking or boiling grains in 0-1.0 % w/v $Ca(OH)_2$ solution on pasting properties.

Pasting temperature of native starch and starches from soaking grains was about the same, ~84ºC (Figure 4.34). Soaking grains in water gave the temperature-viscosity profile of resulting starch paste almost overlapping to that of paste from native starch, with differences in peak viscosity, trough viscosity, final viscosity, breakdown and setback of resulting starch less than 5 %. Peak viscosity, trough viscosity, final viscosity and setback of native starch were lowest. Soaking grains in 0.2 % w/v $Ca(OH)_2$ solution resulted in starch paste having the highest peak viscosity, trough viscosity, final viscosity, breakdown and setback. Soaking grains in 0.8 and 1.0 % w/v $Ca(OH)_2$ gave almost overlapping of their temperature-viscosity profiles. Figure 4.35 shows that peak viscosity, trough viscosity, final viscosity, breakdown and setback of starch pastes from soaked grains decrease as [$Ca(OH)_2$] used for soaking increased above 0.2 % w/v. All modified starches obtained from boiled grains had viscosity lower than 200 mPa·s, and their peak and trough viscosities as well as breakdown and setback could not be determined (Figure 4.34). To evaluate effect of [$Ca(OH)_2$] used for boiling on the temperature-viscosity profile of their resulting pastes, starch suspensions were heated to 95ºC, holded at 95ºC for 30 min, cooled down to 50ºC then holded at 50ºC for 2 min, and their temperature-viscosity profiles were shown in Figure 4.35. The viscosity at the end of 95ºC and final viscosity were used to evaluate effect of [$Ca(OH)_2$] used for boiling as shown in Figure 4.36. These viscosities were decreased as [$Ca(OH)_2$] used for boiling increased (Figure 4.37).

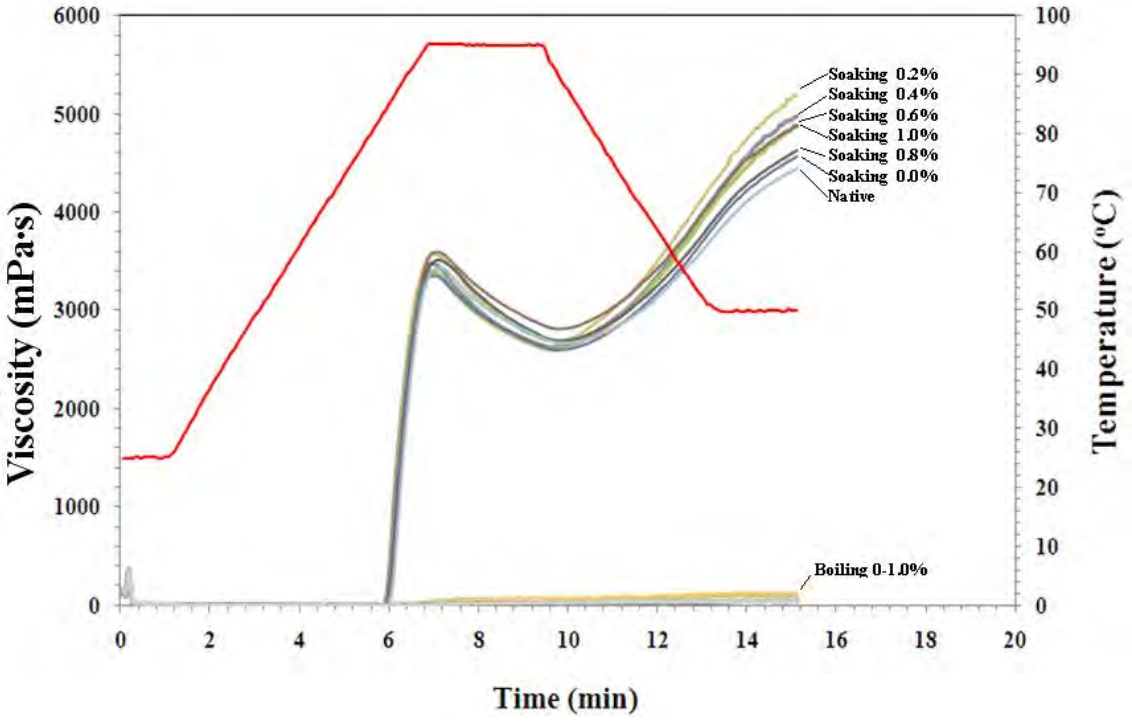


Figure 4.34 Effect of soaking and boiling pigeon pea grains in 0-1.0 % w/v calcium hydroxide solution on pasting properties of native starch, starches from soaked grains and modified flours.

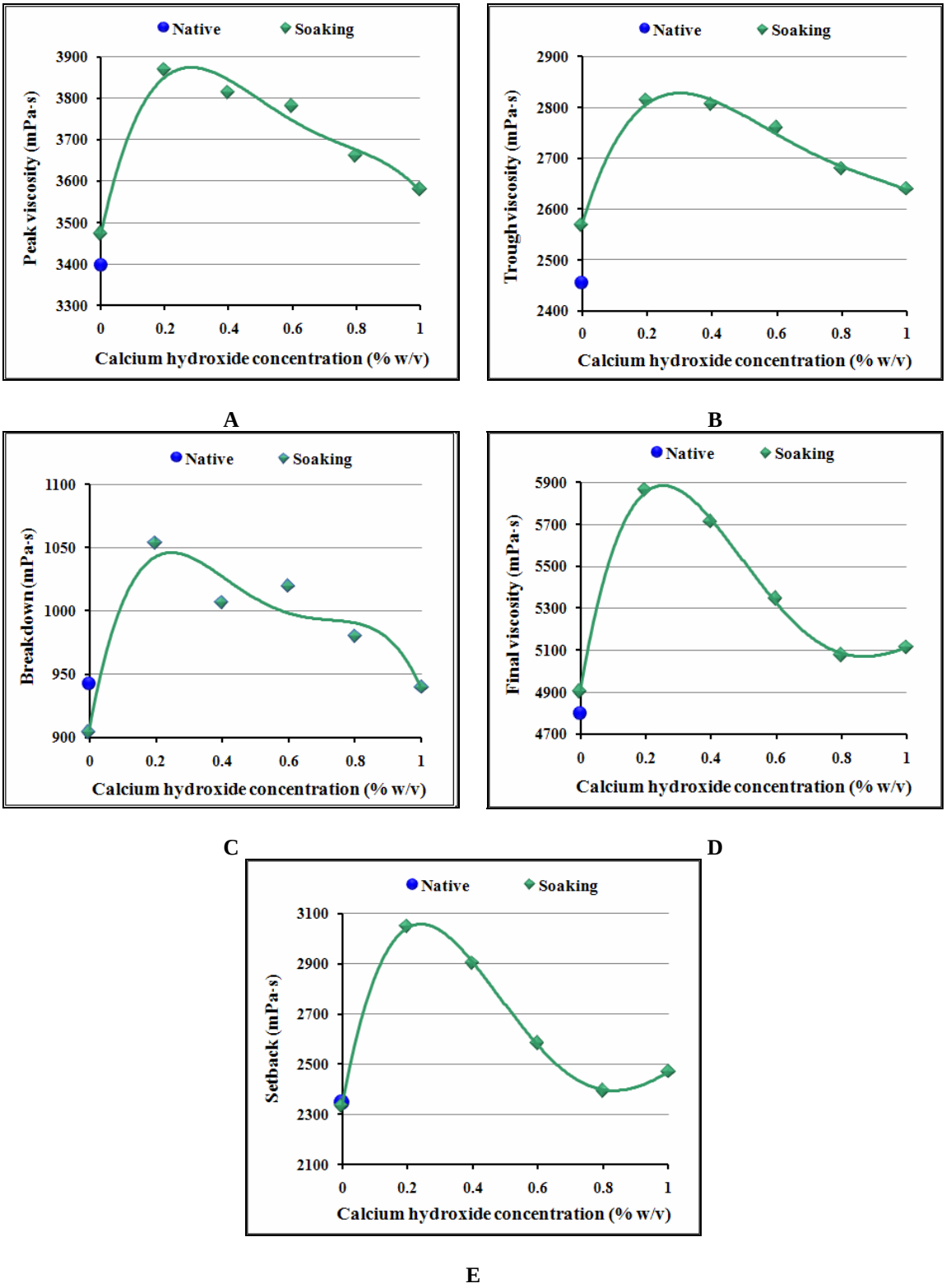


Figure 4.35 Effect of soaking pigeon pea grains in 0-1.0 % w/v calcium hydroxide solution on peak viscosity (A), trough viscosity (B), breakdown (C), final viscosity (D) and setback (E) of native starch and starches from soaked grains.

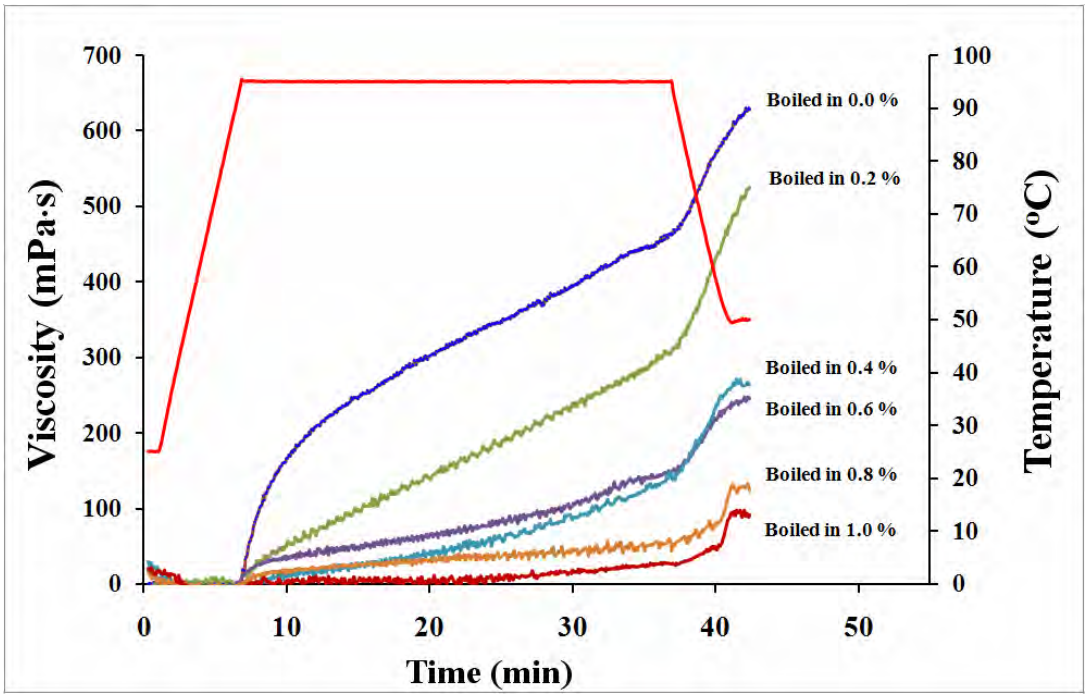
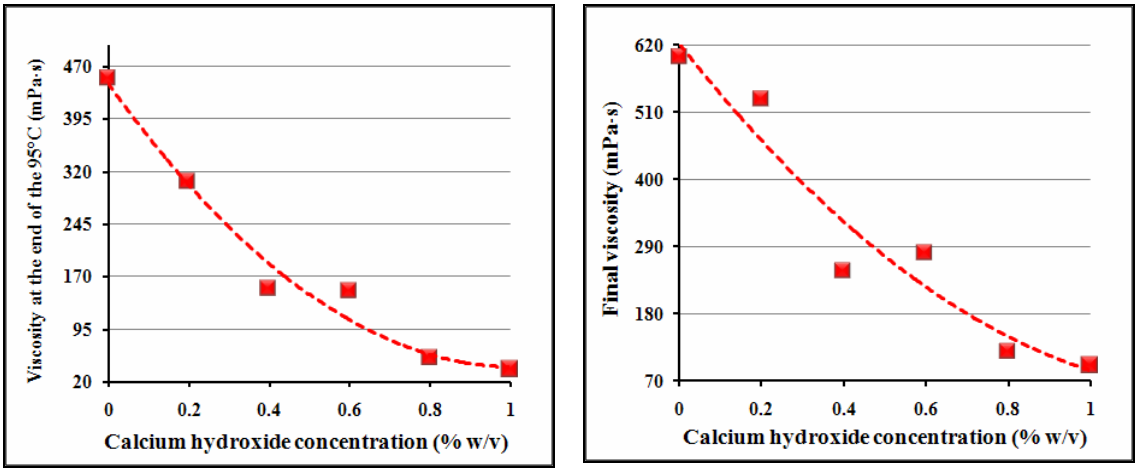


Figure 4.36 Effect of boiling pigeon pea grains in 0-1.0 % w/v calcium hydroxide solution on pasting properties of modified pigeon pea flours.



A B

Figure 4.37 Viscosity at the end of the 95°C (A) and final viscosity (B) of modified pigeon pea flours.

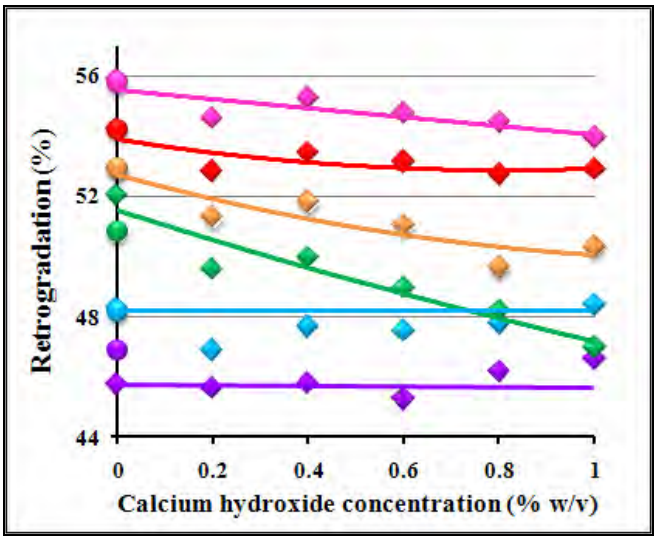
In this study, amylose concentration in melt phase at 95ºC was determined for analysis of amylose leaching. Amylose concentration in melt phase for native starch was 20.35 µg/ml. Amylose concentrations in melt phase were 20.44,

21.86, 18.36, 17.98, 16.59 and 16.34 µg/ml for starches obtained from grains soaked in 0, 0.2, 0.4, 0.6, 0.8 and 1.0 % w/v $Ca(OH)_2$ solutions, respectively. The highest amylose concentration in melt phase of starch obtained from grains soaked in 0.2 % w/v $Ca(OH)_2$ might be main reason for the highest viscosities and setback. Changing in amylose concentration in melt phase with [$Ca(OH)_2$] used for soaking also agreed well with changing in viscosities and setback with [$Ca(OH)_2$]. A higher amylose concentration in melt phase gave higher viscosity of melt phase and amylose-amylose interactions in melt phase. Besides the aforestated explanations, the presence of hydroxyl groups, OH^-, from $Ca(OH)_2$ enhanced granule swelling due to OH^- accelerating hydration resulting in an increase in viscosity. Anyhow, when [$Ca(OH)_2$] was higher than 0.2 % w/v, depositing calcium on granule surface increased to the amount that hydration and swelling of granule were prevented and thus viscosity decreased as [$Ca(OH)_2$] increased. The abovementioned reasons were responsible for the highest paste viscosities and setback of starch from grains soaked in 0.2 % w/v $Ca(OH)_2$ and decrease in these properties as [$Ca(OH)_2$] increased. For modified flours, their granules were partial gelatinized resulting in losing their capacity to form compact paste (Méndez-Montealvo *et al.*, 2007). Their amylose concentrations in melt phase were found to be ~13 µg/ml. Therefore, their viscosities were low.

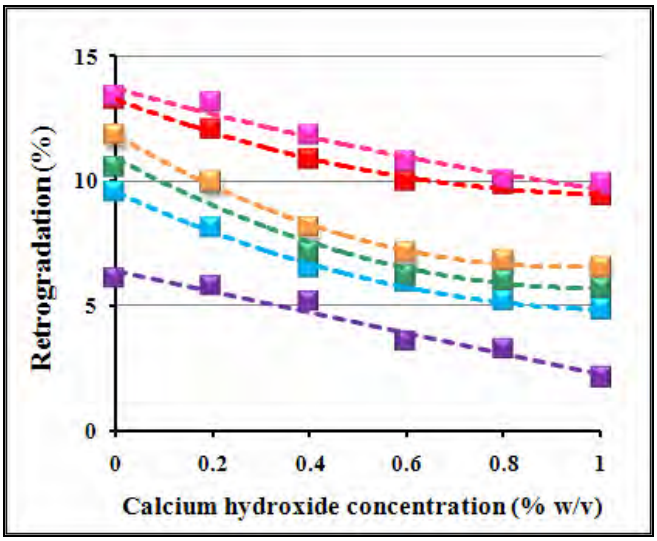
4.2.8 Effects of soaking or boiling grains in 0-1.0 % w/v $Ca(OH)_2$ solution on retrogradation and freeze-thaw stability.

At each storage duration, soaking grains in water did not affect percentage retrogradation expect for 1 and 4 day storage (Figure 4.38 A). For each starch, percentage retrogradation increased with storage duration. At 1 and 2 day

storage, [$Ca(OH)_2$] used for soaking did not significantly affect percentage retrogradation. Effect of [$Ca(OH)_2$] used for soaking on percentage retrogradation was found when pastes were stored for 3 days and longer, which an increase in [$Ca(OH)_2$] decreased percentage retrogradation. Slope of relationship between percentage retrogradation and [$Ca(HO)_2$] for 3 day storage was highest and this slope tended to decrease as storage duration increased. This suggested that effect of [$Ca(OH)_2$] on percentage retrogradation was strongest at 3 day storage. At each storage duration, percentage retrogradation of native starch was more than 4-fold higher than that of modified flours (Figure 4.38 A and B). For each modified flour, percentage retrogradation also increased with storage duration. At each storage duration, percentage retrogradation decreased as [$Ca(OH)_2$] used for boiling increased.



A

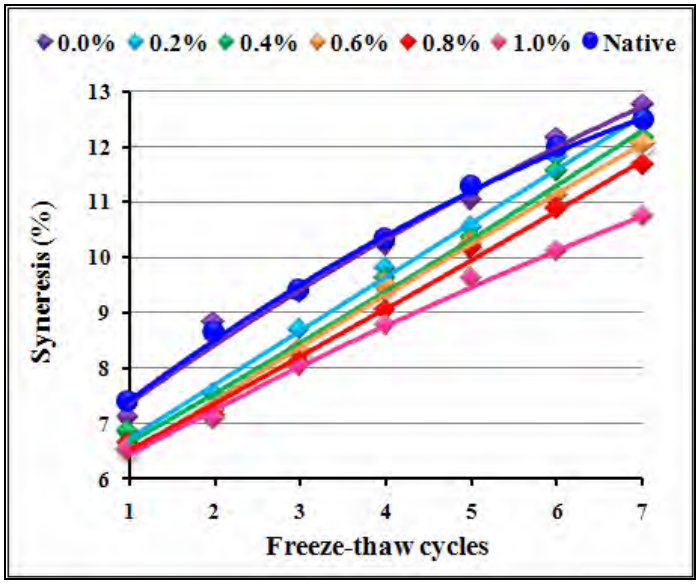


B

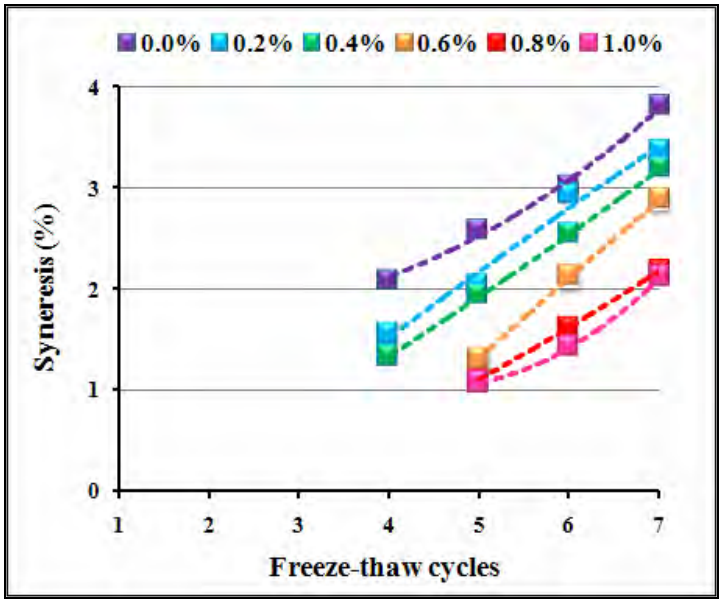
Figure 4.38 Effect of soaking (A) or boiling (B) pigeon pea grains in 0-1.0 % w/v calcium hydroxide solution on percentage retrogradation.

Comparing to native starch, soaking grains in water did not significantly affect percentage syneresis (Figure 4.39 A). For each freeze-thaw cycle, percentage syneresis decreased as [$Ca(OH)_2$] used for soaking increased. For

each starch, percentage syneresis increased with freeze-thaw cycle. Boiling grains decreased percentage syneresis (Figure 4.39 A and B). An increase in [$Ca(OH)_2$] used for soaking decreased percentage syneresis (Figure 4.39 B). For modified flours obtained from grains boiled in 0 - 0.4 % w/v $Ca(OH)_2$, no syneresis detected for the first three cycles. For modified flours obtained from grains boiled in > 0.4 % w/v $Ca(OH)_2$, no syneresis detected for the first four cycles. For each modified flour, percentage syneresis also increased with freeze-thaw cycle.



A



B

Figure 4.39 Effect of soaking (A) or boiling (B) pigeon pea grains in 0-1.0 % w/v calcium hydroxide solution on freeze-thaw stability.

Results of percentage retrogradation and syneresis indicated modified flours retrograded less than native starch and starches from soaked grains. Reasons for this were modified starches had amylose content and concentration of amylose in

melt phase lower than native starch and starches from soaked grains. Higher [$Ca(OH)_2$] used for boiling gave lower retrogradation. This might be due to starch-protein, protein-protein, protein-lipid, amylose-lipid, calcium-starch and calcium-protein interactions in granules formed during boiling grains retarded mobility of starch chains then resisted re-association of starch chains.

4.2.9 Effects of soaking or boiling grains in 0-1.0 % w/v $Ca(OH)_2$ solution on rheological properties

Steady shear behavior was determined at starch/flour concentration of 8 % w/w db, temperature of 75ºC and shear rate of 50-1000 s^{-1}. Soaking grains in water did not affect flow behavior of pigeon pea starch (Figure 4.40 and 4.41). Pastes of native starch and starches from soaked grains behaved as pseudoplastic with flow behavior index (n) ranging between 0.43-0.50 and consistency index about 20 $Pa{\cdot}s^n$. An increase in [$Ca(OH)_2$] used for soaking caused a slightly increase in flow behavior index. As [$Ca(OH)_2$] increased from 0 to 0.2 % w/v, consistency index slightly increased. Pastes of starches obtained from grains soaked in 0.4 - 0.6 % w/v $Ca(OH)_2$ had same consistency index. Consistency index tended to decrease as [$Ca(OH)_2$] increased from 0.6 to 1.0 % w/v. Comparing to native starch, consistency index of modified flours obtained from grains boiled in 0 - 0.2 % w/v $Ca(OH)_2$ were about 20-time lower. An increase in [$Ca(OH)_2$] used for boiling above 0.2 % w/v resulted in one magnitude order lower of consistency index. All pastes of modified flours were pseudoplastic. An increase in [$Ca(OH)_2$] used for boiling increased flow behavior index.

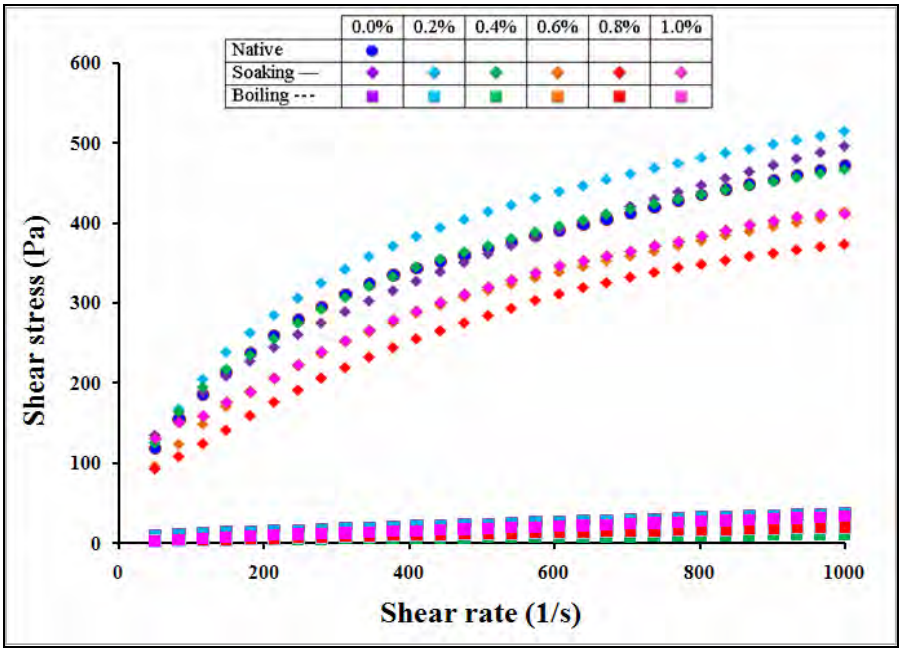
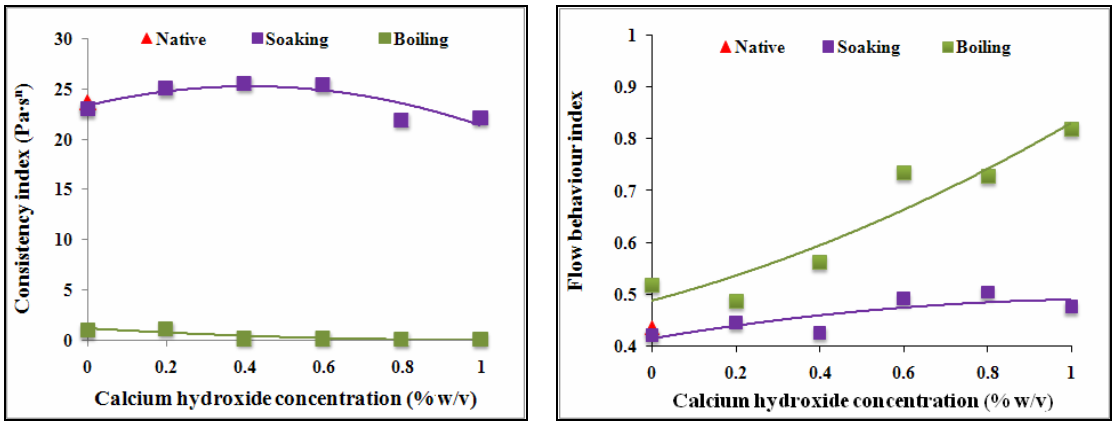


Figure 4.40 Effect of soaking or boiling pigeon pea grains in 0-1.0 % w/v calcium hydroxide solution on flow behavior measured at 8 % w/w db, 75°C and 50-1000 s^{-1}.



A **B**

Figure 4.41 Effect of soaking or boiling pigeon pea grains in 0-1.0 % w/v calcium hydroxide solution on consistency index (A) and flow behavior index (B) measured at 8 % w/w db, 75°C and 50-1000 s^{-1}.

Lower in value of flow behavior index indicate less shear stability of pastes due to granule breakdown (Elder and Schoch, 1959). For starches from soaked

grains, slightly increase in amylose content with [$Ca(OH)_2$] resulted in higher granule rigidity. Therefore, flow behavior index increased with [$Ca(OH)_2$]. For modified flours from boiled grains, starch-protein, protein-protein, protein-lipid, amylose-lipid, calcium-starch and calcium-protein interactions in granules, which formed during boiling grains in water or $Ca(OH)_2$ solutions, resulted in higher granule rigidity. Therefore, their flow behavior index was higher than that of native starch and starches from soaked grains. Furthermore, protein, amylose, calcium and phosphorus contents tended to increase with [$Ca(OH)_2$] used for boiling indicating an increase in these interactions with [$Ca(OH)_2$]. An increase in these interactions and percentage relative crystallinity with [$Ca(OH)_2$] were reasons for an increase in flow behavior index with [$Ca(OH)_2$]. This result agrees well with the result reported by Méndez-Montealvo *et al*. (2007), which stated that nixtamalized flour had a flow behavior index with higher tendency to Newtonian behavior. The reasons, explained for effects of soaking or boiling grains in water or $Ca(OH)_2$ solutions on viscosities and setback in pasting property section, can also explain these effects on consistency index.

Since modified flours did not form gel at flour concentration of 15 % w/w db. Therefore, linear viscoelastic properties (G´, G" and tan δ) were determined only for native starch and starches from soaked grains at starch concentration of 15 %w/w db, temperature of 25ºC, strain of 2 % and frequency of 0.1-100 rad/s. For each starch, G´ was almost constant over frequency of 0.1 – 100 rad/s and higher than G" (Figure 4.42). These suggested that this region was rubbery region for all starches and there was network formed from amylose-amylose interactions in melt phase. Each sample exhibited solid-like behavior as tan δ was lower than 0.2. Soaking grains in water did not affect viscoelastic properties. Difference in G´ among these

samples was lower than 10 %, however, G´ tended to increase with [$Ca(OH)_2$] used for soaking. Tan δ increased with [$Ca(OH)_2$] used for soaking.

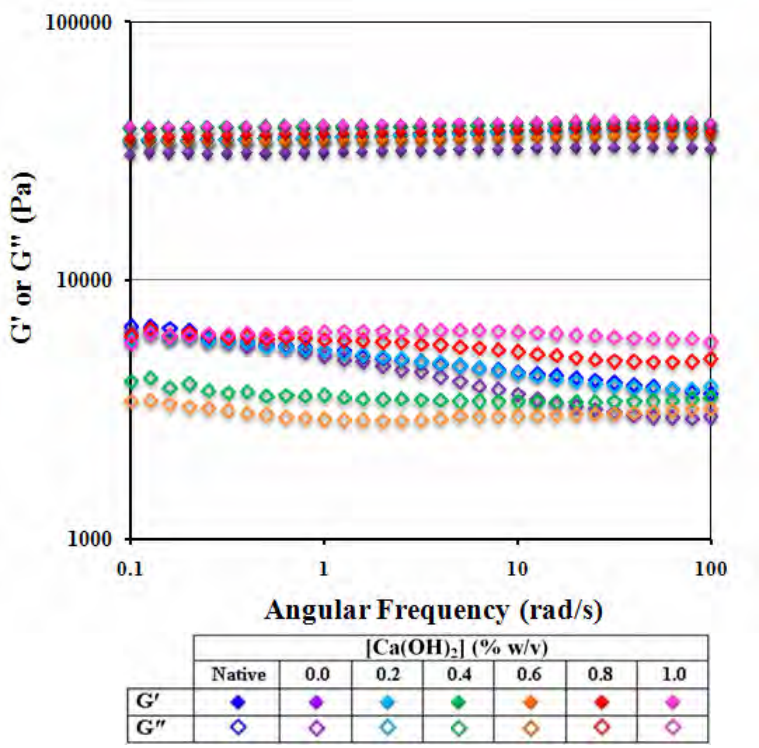


Figure 4.42 Effect of soaking pigeon pea grains in 0-1.0 % w/v calcium hydroxide solution on storage (G′) and loss (G′′) modulus measured in linear viscoelastic region at 15 % w/w db, 25°C, 2 % strain and 0.1-100 rad/s.

Modified flours could not form gel since their amylose contents and amylose were low and amylose leached out into melt phase was also low. Therefore, amylose-amylose interactions in melt phase were not enough to form network. For native starch and starches from soaked grains, networks were formed by amylose-amylose interactions and in the case of soaking grains in $Ca(OH)_2$ also by amylose-Ca-amylose interactions (Mondragón *et al*., 2006). The calcium inorganic cations form cross-links between two starch chains of the granule matrix by coordination of two ionized hydroxyl groups produced by the alkaline conditions; while at higher lime

concentrations the Ca ions are more likely to anchor on the surface of the starch granules (Bryant and Hamaker, 1997; Rodríguez *et al*., 1996). An increase [$Ca(OH)_2$] might increased amylose-Ca-amylose interactions resulting in more rigidity of the granule, which could be observed by an decrease in G´ (Bryant and Hamaker, 1997; Oosten, 1982).

CHAPTER V

CONCLUSIONS

For native starch, chemical compositions varied with starch granule size. Amylose and fiber contents were found to be direct function of granule size, while lipid and phosphorus contents were in opposite direction. Percentage relative crystallinity was inverse function of granule size. These resulted in differences in thermal and pasting properties, water absorption capacity, swelling power, solubility, amylose leaching, freeze-thaw stability and percentage hydrolysis for different granule size fraction. Furthermore, water absorption capacity depended strongly on granule surface area to volume ratio and peak viscosity depended on size and shape of starch granules.

Chemical compositions and structures of starch granules were modified by nixtamalization process. Formation of calcium-amylose, amylose-lipid, protein-protein, phytate-amylose and phytate-protein complexs, and calcium phytate, as well as starch gelatinization and solubilization, which occurred during boiling grains in 0-1.0 % w/v $Ca(OH)_2$ solutions, were responsible for the starch granule structural changes. These affected the physico-chemical properties of resulting flours.

Soaking grains in water at 6±1°C for 16 hr did not alter chemical compositions and structures of starch granules. Therefore, its properties did not change. Deposition of calcium on starch granule surface during soaking grains in $Ca(OH)_2$ solutions were responsible for calcium-amylose interactions in melt phase occurring when starch gelatinized, which in turn affected the physico-chemical properties, especially pasting and rheological properties.

Recommendation for future research

The further studies could be:

- effects of temperature, duration of heating and weight ratio of calcium hydroxide solution to grain used for boiling grains on structures and properties, and

- modification of starch using annealing and/or heat-moist processes in the presence of calcium ions.

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APPENDICES

APPENDIX A

Procedures for preparing amyloglucosidase solution, GOD-POD-ABTS reagent and glucose standard curve for starch content determination

Amyloglucosidase solution preparation

- Prepare 1.0 M acetate buffer
    - Dry sodium acetate (Ajax Finechem, NZ) at 85 °C for 4 hr
    - Mix 136.1 g dried sodium acetate with 800 ml distilled water
    - Adjust pH to pH 5 with glacial acetic acid (Ajax Finechem, NZ)
    - Add distilled water to obtain total volume of 1000 ml in volumetric flask
- Prepare 0.4 M acetate buffer
    - Dilute 1 M acetate buffer (40 ml) with distilled water in volumetric flask (100 ml)
- Prepare amyloglucosidase solution
    - Mix 0.67 mg amyloglucosidase (75UI/mg from *Aspergillus niger,* A3042, Sigma-Aldrich Pty, Ltd., Australia) with 10 ml 0.4 M acetate buffer

GOD-POD-ABTS reagent preparation

- Prepare tris-phosphate buffer (pH 7)
    - Mix 18.15 g tri-hydroxy-methyl-amino-methane (Ajax Finechem, NZ) with 25 g $NaH_2PO_4 \cdot 10H_2O$ (Ajax Finechem, NZ) and 450 ml distilled water
    - Adjust pH to pH 7 with orthophosphoric acid (Ajax Finechem, NZ)
    - Add distilled water to obtain total volume of 500 ml in volumetric flask

- Prepare GOD-POD-ABTS reagent
    - Mix 50 mg glucose oxidase (GOD) from *Aspergillus niger* type II (Sigma Chemical Co., St. Louis, MO, USA) with 1.5 mg peroxidase (POD) from Horseradish type I (Sigma Chemical Co., St. Louis, MO, USA) and 25 mg 2,2′ Azino-bis ethylbenzthiazolin sulfonate (ABTS) ammonium salt (Sigma Chemical Co., St. Louis, MO, USA) in 50 ml volumetric flask
    - Add tris-phosphate buffer (pH 7) to obtain total volume of 50 ml in volumetric flask

Glucose standard curve preparation

- Prepare 1 mg/ml glucose stock solution
    - Accurately weigh 200 mg pure glucose from potato (Fluka Chemie GmbH, Buchs, Switzerland) in 200 ml volumetric flask
    - Add deionized water to obtain total volume of 200 ml
- Prepare 10, 20, 30, 40, 50 and 60 μg/ml glucose standard solutions
    - Pipette 10, 20, 30, 40, 50 or 60 ml of 1 mg/ml glucose stock solution into 100 ml volumetric flask
    - Add deionized water to obtain total volume of 100 ml
- Prepare standard glucose concentration versus OD curve
    - Mix 800 μl glucose standard solution (10, 20, 30, 40, 50 or 60 μg/ml) or deionized water (for blank) with 200 μl amyloglucosidase solution
    - Incubate at 55°C for 1 hr 30 min in a controlled shaking water bath
    - Add 2 ml GOD-POD-ABTS reagent
    - Keep solution in dark room for 30 min
    - Measure OD of each standard glucose solution with spectrophotometer at 560 nm
    - Plot glucose concentration (μg/ml) versus OD (Figure A)

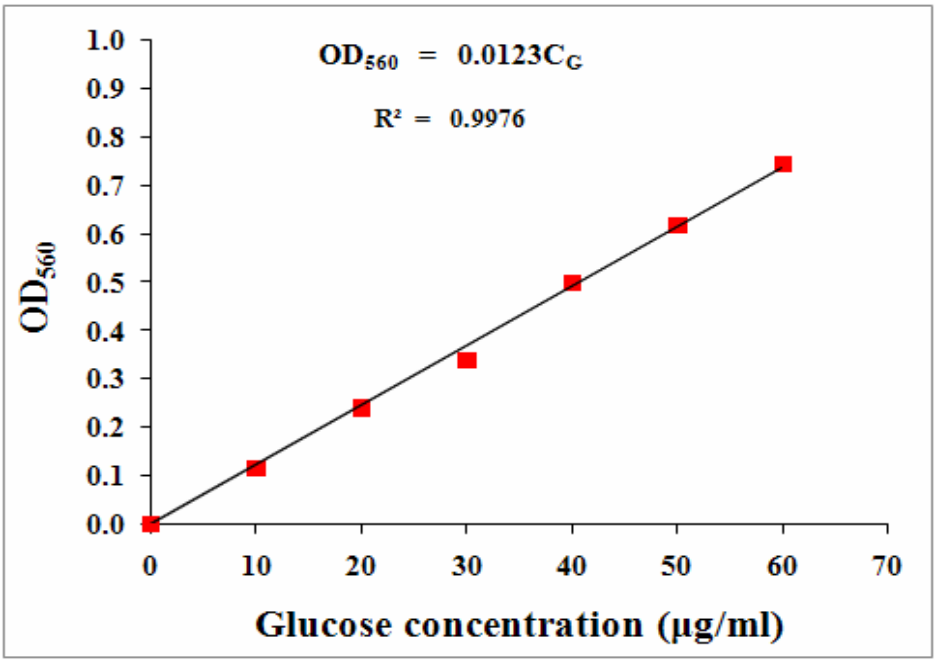

Figure A Glucose standard curve for starch content determination

$$C_G = (OD_{560})/0.0123 \quad [A]$$

where

C_G = glucose concentration (µg/ml)

OD_{560} = OD at 560 nm

APPENDIX B

Procedure for preparing phosphorus standard curve for phosphorus content determination (Heyns, 1959)

Standard phosphorus solution preparation

- Prepare 1 mg P/ml phosphorus stock solution
    - Accurately weigh 0.4387 g potassium dihydrogen phosphate standard (CAS No. 7778-77-0, Fisher Scientific, UK) in 1000 ml volumetric flask
    - Add deionized water to obtain total volume of 1000 ml
- Prepare 20 ppm phosphorus standard solutions
    - Pipette 20 ml of 1 mg P/ml phosphorus stock solution into 100 ml volumetric flask
    - Add deionized water to obtain total volume of 100 ml

Phosphorus standard curve preparation

- Prepare 1.66, 3.33, 6.66, 9.99 and 13.33 ppm phosphorus standard solutions
    - Pipette 5, 10, 20, 30 or 40 ml of 20 ppm phosphorus standard solutions into 100 ml volumetric flask to obtain 1.66, 3.33, 6.66, 9.99 or 13.33 ppm phosphorus standard solutions, respectively
    - Add deionized water to obtain total volume of 100 ml
- Prepare standard phosphorus concentration versus OD curve
    - Mix 10 ml standard phosphorus solution (1.66, 3.33, 6.66, 9.99 or 13.33 ppm) or deionized water (for blank) with 2 ml Vanadate-Molybdate reagent (Ajax Finechem, NZ)
    - Measure OD of each standard phosphorus solution with spectrophotometer at 435 nm
    - Plot phosphorus concentration (μg/ml) versus OD (Figure B)

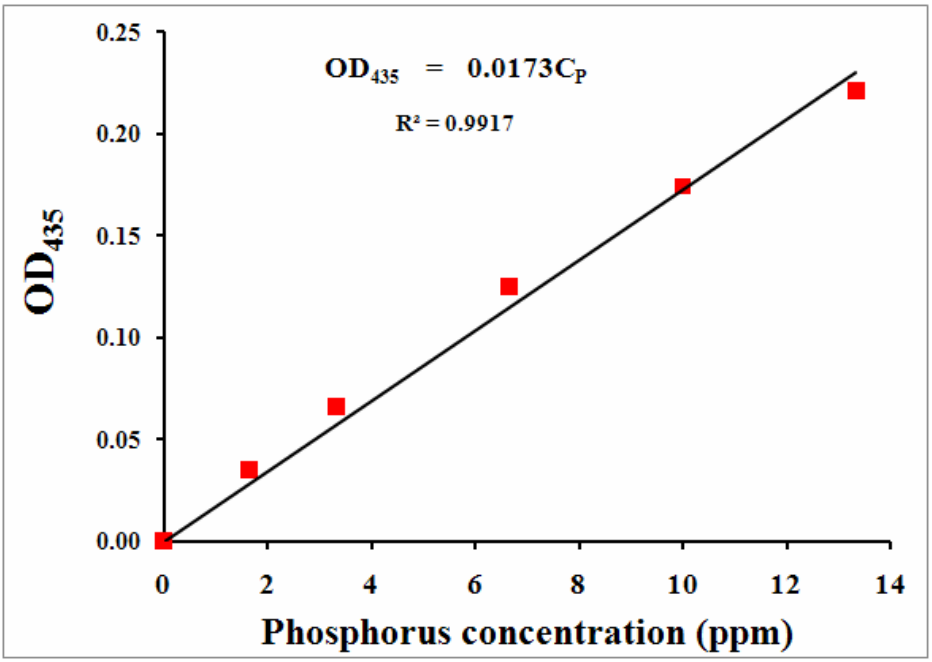

Figure B Phosphorus standard curve for phosphorus content determination

$$C_P = (OD_{435})/0.0173 \quad [B]$$

where

C_P = phosphorus concentration (μg/ml)

OD_{435} = OD at 435 nm

APPENDIX C

Procedure for preparing amylose standard curve for amylose leaching determination

Iodine solution (2 %w/v) preparation

- Prepare 2 %w/v iodine solution
    - Accurately weigh 0.2 g iodine (Ajax Finechem, NZ) and 2.0 g potassium iodide (Ajax Finechem, NZ) in 100 ml volumetric flask
    - Add deionized water to obtain total volume of 100 ml
    - Stir the solution for 4 hr

Standard amylose solution preparation

- Prepare 1 mg/ml amylose stock solution
    - Accurately weigh 50.4 mg pure amylose standard from potato (Fluka Chemie GmbH, Buchs, Switzerland) in 50 ml volumetric flask
    - Add 0.5 ml 95 %v/v ethanol (Ajax Finechem, NZ) and 4.5 ml 1 M NaOH (Ajax Finechem, NZ)
    - Heat at 100 °C for 10 min in a controlled shaking water bath.
    - Cool the amylose standard solution in an ice bath
    - Add deionized water to obtain total volume of 50 ml
- Prepare 4.48, 8.96, 13.44 and 26.88 μg/ml amylose standard solutions
    - Pipette 0.5, 1.0, 1.5 or 2.0 ml of 1 mg/ml amylose stock solution into 100 ml volumetric flask to obtain 4.48, 8.96, 13.44 or 26.88 μg/ml amylose standard solutions, respectively
    - Add 1 ml 1 M acetic acid and 2 ml 2 %w/v iodine solution
    - Add deionized water to obtain total volume of 100 ml
    - Keep the solution in dark room for 20 and 25 min
    - Measure OD of each amylose solution with spectrophotometer at 620 and 545 nm for the solution kept in dark room for 20 and 25 min, respectively

Standard amylopectin solution preparation

- Prepare 1 mg/ml amylopectin stock solution
    - Accurately weigh 50.5 mg pure amylopectin standard from potato (Fluka Chemie GmbH, Buchs, Switzerland) in 50 ml volumetric flask
    - Add 0.5 ml 95 %v/v ethanol (Ajax Finechem, NZ) and 4.5 ml 1 M NaOH (Ajax Finechem, NZ)
    - Heat at 100 °C for 10 min in a controlled shaking water bath.
    - Cool the amylopectin standard solution in an ice bath
    - Add deionized water to obtain total volume of 50 ml
- Prepare 22.30, 44.59, 62.43 and 89.18 μg/ml amylopectin standard solutions
    - Pipette 2.5, 5.0, 7.0 or 10.0 ml of 1 mg/ml amylopectin stock solution into 100 ml volumetric flask to obtain 22.30, 44.59, 62.43 or 89.18 μg/ml amylopectin standard solutions, respectively
    - Add 1 ml 1 M acetic acid and 2 ml 2 %w/v iodine solution
    - Add deionized water to obtain total volume of 100 ml
    - Keep the solution in dark room for 20 and 25 min
    - Measure OD of each amylopectin solution with spectrophotometer at 620 and 545 nm for the solution kept in dark room for 20 and 25 min, respectively

Amylose standard curve preparation

- Plot amylose concentration (µg/ml) versus OD

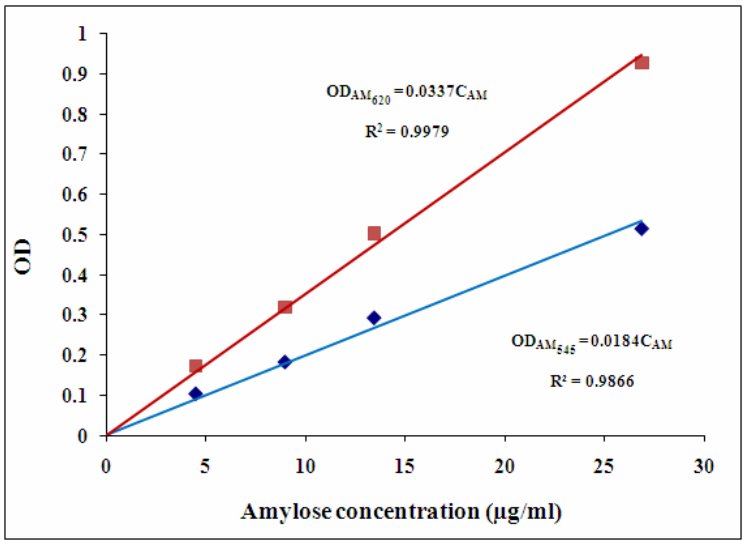

Figure C Amylose standard curve for determination of amylose concentration in the supernatant obtained in section 3.4.3

Amylopectine standard curve preparation

- Plot amylopectin concentration (µg/ml) versus OD

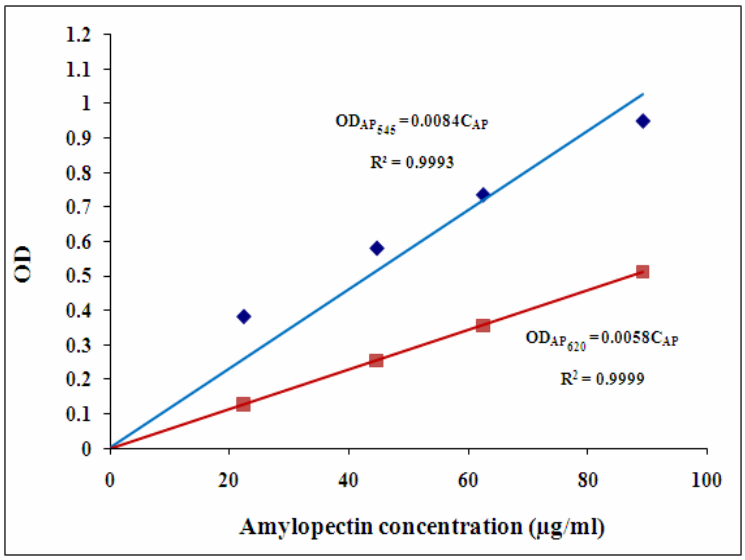

Figure D Amylopectin standard curve for determination of amylose concentration in the supernatant obtained in section 3.4.3

As

$$OD_{545} = C_{AM}.S_{AM545} + C_{AP}.S_{AP545}$$

$$OD_{620} = C_{AM}.S_{AM620} + C_{AP}.S_{AP620}$$

Therefore,

$$C_{AM} = (S_{AP620}.OD_{545} - S_{AP545}.OD_{620}) / (S_{AM545}.S_{AP620} - S_{AM620}.S_{AP545}) \quad [C]$$

Where:

C_{AM} = Amylose content (µg/ml)

C_{AP} = Amylopectin content (µg/ml)

OD_{545} = Optical density of the sample at 545nm

OD_{620} = Optical density of the sample at 620nm

S_{AP545} = Slope of the pure amylopectin standard curve measured at 545nm

S_{AP620} = Slope of the pure amylopectin standard curve measured at 620nm

S_{AM545} = Slope of the pure amylose standard curve measured at 545nm

S_{AM620} = Slope of the pure amylose standard curve measured at 620nm

VITA

Mrs. Piyanuch Roskhrua was born on October 23, 1973 in Lampang, Thailand. She obtained her B.Sc. degree in Food Science and Technology form Rajamongala Institute of Technology in 1996 and M.Sc. in Agro-Industry from Naresaun University in 2002. Since 2002, she has worked for the Department of Food Technology, Faculty of Agro-Industry Agro-Industry, Rajamangala University of Technology Lanna (Nan Campus). In 2007, she received a scholarship from the Royal Thai Government to study in the Agro-Industry Consortium Ph.D. program at the Department of Food Technology, Chulalongkorn University.