รายงานวิจัยฉบับสมบูรณ์

โครงการการศึกษาการผลิตกากมะพร้าวดัดแปรเพื่อใช้เป็นสารเสริมใยอาหารในผลิตภัณฑ์อาหาร

Studies on modifications of copra meal for utilization as fiber supplements in food products

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สนับสนุนโดยสำนักงานคณะกรรมการการอุดมศึกษาและ สำนักงานกองทุนสนับสนุนการวิจัย

สารบัญ

บทคัดย่อ

กากมะพร้าวที่สกัดน้ำมันและคั้นน้ำกะทิออกแล้ว เป็นส่วนเหลือทิ้งจากอุตสาหกรรมกะทิและอุตสาหกรรมน้ำมันมะพร้าว กากมะพร้าวเป็นแหล่งของใยอาหารที่ดี ประกอบด้วยปริมาณใยอาหารทั้งหมดถึง 60.9% ข้อดีสำคัญที่ทำให้กากมะพร้าวเหมาะสมกับการผลิตใยอาหารมากกว่าแหล่งอื่นๆคือ กากมะพร้าวไม่มีลิกนินและมีสีขาวดังนั้นในการเตรียมใยอาหารจากกากมะพร้าวจึงไม่จำเป็นต้องมีขั้นตอนการกำจัดลิกนินและการฟอกสี แม้ว่ากากมะพร้าวเป็นแหล่งของใยอาหารที่ดี การใช้กากมะพร้าวเป็นสารเสริมใยอาหาร โดยการเติมลงในอาหารโดยตรงในปริมาณมากนั้นเป็นไปได้ยาก เนื่องจากการเติมกากมะพร้าวจะเปลี่ยนสมบัติทางกายภาพและสมบัติทางประสาทสัมผัสของอาหาร เนื่องจากค่าสมบัติทางน้ำ (Hydration properties) ได้แก่ค่าการอุ้มน้ำ WHC (water holding capacity) และ WRC (water retention capacity) และค่าการพองตัว SC (swelling capacity) มีค่าสูงมากเมื่อเปรียบเทียบกับใยอาหารจากแหล่งอื่น เนื่องจากค่า WHC ซึ่งมีค่าสูง การเติมกากมะพร้าวลงในผลิตภัณฑ์อาหารจะทำให้เนื้อสัมผัสแย่ลงเนื่องจากกากมะพร้าวจะดูดความชื้นจากรอบๆตัวมัน ดังนั้นถ้าสามารถเปลี่ยน สมบัติทางน้ำของกากมะพร้าวให้มีค่าลดลงได้ เราก็จะสามารถใช้กากมะพร้าวเติมลงไปในอาหารเพื่อเป็นแหล่งใยอาหาร หรือทดแทนการใช้แป้งในการผลิตอาหารที่มีปริมาณคาร์โบไฮเดรตและแคลอรี่ลดลง

งานวิจัยนี้ศึกษาการไฮโดรไลซ์หรือย่อยกากมะพร้าวด้วยสารละลายกรดไฮโดรคลอริกและเอนไซม์แมนนาเนสเพื่อลดค่าการอุ้มน้ำหรือ WRC และค่าการพองตัวหรือ SC ของกากมะพร้าว เพื่อปรับปรุงสมบัติของกากมะพร้าวให้เหมาะสมในการเติมลงไปในผลิตภัณฑ์อาหาร การใช้กรดไฮโดรคลอริกที่ความเข้มข้น 0.5% ลดค่า SC และ WRC ในขณะที่ค่า bulk density (BD) และปริมาณใยอาหารที่ละลายน้ำได้มีค่าเพิ่มขึ้นอย่างมีนัยสำคัญ จากการวิเคราะห์รูปแบบน้ำตาลโมโนแซคคาไรด์ gel penetration profile และ FT-IR spectra บ่งบอกถึงการเปลี่ยนแปลงโครงสร้างของกากมะพร้าว มีการทำลายโครงสร้างภายในเนื่องจากการใช้กรดทำให้โครงสร้างของกากมะพร้าวจับตัวกันแน่นขึ้นในขณะที่ความสามารถในการอุ้มน้ำลดลง การใช้กากมะพร้าวดัดแปรเติมในส่วนผสมของผลิตภัณฑ์ขนมอบได้แก่ ขนมปัง และคุกกี้ เพื่อทดแทนแป้งสาลีหรือเพื่อเพิ่มใยอาหาร พบว่าคุณภาพของผลิตภัณฑ์ขนมปังและคุกกี้ที่ได้มีคุณภาพดีกว่าผลิตภัณฑ์ที่ใช้กากมะพร้าวปกติ แสดงว่ากากมะพร้าวที่ผ่านการไฮโดรไลซ์ไม่แสดงลักษณะการอุ้มน้ำ และแย่งจับน้ำจากส่วนผสมอื่นทำให้มีลักษณะปกติระหว่างผสมส่วนผสมตรงกันข้ามกับการใช้กากมะพร้าวปกติ ซึ่งแย่งจับน้ำและพองตัวจนทำแห้งส่วนผสมแห้งและผสมเข้ากันยาก Response surface methodology (RSM) แสดงให้เห็นว่าความเข้มข้นของกรด อุณหภูมิและเวลาในการไฮโดรไลซ์มีผลต่อสมบัติของกากมะพร้าวดัดแปร โดยมีการสร้างสมการทำนายความสัมพันธ์ระหว่างปัจจัยเหล่านี้กับสมบัติของกากมะพร้าวดัดแปร

ค่า SC และ WRC ลดลงอย่างมีนัยสำคัญเมื่อไฮโดรไลซ์กากมะพร้าวด้วยเอนไซม์ทางการค้าที่มีแอกติวิตี้ของแมนนาเนส เอนไซม์ทางการค้า Acti SF-R (SF-R) ไฮโดรไลซ์กากมะพร้าวด้วยอัตราเร็วสูงสุดตามด้วย E mannanase GMP (E-man) เอนไซม์ทั้งสองลดค่า SC และ WRC ของกากมะพร้าวอย่างมีนัยสำคัญ ศึกษาอิทธิพลของปริมาณเอนไซม์ อุณหภูมิและ pH ในการไฮโดรไลซ์ต่อค่า hydrolysis yield, SC, WRC และ BD ของกากมะพร้าวโดยใช้ RSM พบว่าที่สภาวะในการไฮโดรไลซ์

กากมะพร้าวด้วยเอนไซม์ทั้งสองชนิดที่ให้ค่า SC, WRC และ BD ใกล้เคียงกัน การใช้ SF-R ให้อัตราการไฮโดรไลซิสสูงกว่าทำให้ค่า hydrolysis yield ต่ำกว่าการใช้ E-man แสดงให้เห็นว่าแอคติวิตี้ของ endo-β-mannanase ในเอนไซม์ทางการค้าสำคัญที่สุดต่อการเปลี่ยนแปลง (ลด) สมบัติการอุ้มน้ำของกากมะพร้าว ในขณะที่แอคติวิตี้ของ β-mannosidase มีผลต่อการลดลงของ hydrolysis yield ในขณะที่มีผลต่อการเปลี่ยนแปลงสมบัติการอุ้มน้ำน้อยมาก แอคติวิตี้ของ endoglucanase ส่งเสริมอัตราการการไฮโดรไลซิสของกากมะพร้าวแต่ไม่มีผลต่อการเปลี่ยนแปลงสมบัติการอุ้มน้ำของกากมะพร้าว การใช้กากมะพร้าวดัดแปรด้วยเอนไซม์ในผลิตภัณฑ์ขนมปังทำให้ให้เนื้อสัมผัสและคุณภาพของขนมปังดีกว่าการใช้กากมะพร้าวปกติอย่างมีนัยสำคัญ

คำสำคัญ: กากมะพร้าว ไฮโดรไลซิส กรด เอนไซม์ แมนนาเนส สมบัติกากอุ้มน้ำ ใยอาหาร

Abstract

Coconut residue or copra meal (CM), by-product taken after pressing cream and oil out of the coconut meat, is a good source of dietary fiber but high water holding capacity of CM limits the quantity of fiber incorporated into food products. This study focused on the modification of CM physiochemical properties using acid and enzymatic hydrolysis in order to improve its potential utilization as source of food fibers or low-calories bulk ingredients in food applications. Acid hydrolysis using 0.5% HCl significantly reduced swelling capacity (SC) and water retention capacity (WRC) whereas bulk density (BD) and soluble dietary fiber content (SDF) of the modified CM significantly increased. Monosaccharide composition profile, gel penetration profile and FT-IR spectra indicated the destruction of CM matrix structure. This destruction increased compactness of the structure and lessened the ability of CM to hold water. Substituting the modified CM for wheat flour in bread and cookies significantly improved bread and cookies qualities compared to the use of the untreated CM. Response surface methodology (RSM) showed that HCl concentration, hydrolysis temperature and time influenced properties of the modified CM. The models predicting their relationships were also generated.

SC and WRC were reduced significantly using enzymatic hydrolysis by commercial enzymes containing β-mannanase. Actipro SF-R (SF-R) hydrolyzed CM at the highest rate followed by E mannanase GMP (E-man) and significant reduction in SC and WRC was observed. Influences of enzyme content, hydrolysis temperature and pH on hydrolysis yield, SC, WRC and BD of CM were studied using RSM. The generated quadratic models predicted that SF-R hydrolyzed CM in higher rate but produced lower hydrolysis yield than E-man under the condition giving similar SC, WRC and BD. The activity of endo-β-mannanase in the commercial enzymes was the most important for modifying CM hydration properties whereas the presence of β-mannosidase caused major loss in hydrolysis yield without significant changes in CM hydration properties. The activity of endoglucanase enhanced the hydrolysis of CM but showed no effect on the modification of CM properties. Substituting the modified CM for wheat flour significantly improved bread qualities.

Keywords: Copra meal; acid; enzymes; mannanase; hydrolysis; hydration properties; dietary fiber

Executive Summary

Dietary fiber has been shown to have important health implications in the prevention for risks of chronic diseases such as cancer, cardiovascular diseases and diabetes mellitus. A study on effectiveness of the dietary fiber component of coconut flour, copra meal after coconut oil was separated by pressing in an expeller, as a functional food showed that the glycemic index of coconut flour supplemented foods decreased with increasing levels of dietary fiber from coconut flour. Moreover, fifteen and 25% dietary fiber from coconut fiber reduced serum total cholesterol, LDL cholesterol and triglycerides of humans with moderately raised serum cholesterol levels.

Thailand is one of the largest exporters of coconut products. Each year abundant amounts of by-product taken after extraction of coconut milk and oil, so called coconut residue or copra meal, are produced. Copra meal is a good source of dietary fiber**.** Total dietary fiber of 60.9%, 56.8% insoluble and 3.8% soluble, was reported in copra meal after coconut oil was separated. Despite, beneficial effects of dietary fiber from copra meal and other sources, changes in physical properties and sensory qualities produced by the fibers limit the quantity of fiber incorporation into food products. Since high water holding capacity (WHC) associated with the fiber, this is deleterious to product texture, as the fiber sorbs moisture from its surrounding. Our preliminary study indicated that acid and enzyme hydrolysis was successfully used to modify hydration properties of copra meal and improved qualities of bread and cookies supplemented with the modified copra meal significantly. The improvement of functionality and potential applications of copra meal fiber would increase its utilization, and subsequently profit the coconut industry, and also the development of fiber-rich foods.

The objective of this study is to investigate factors affecting the modifications of hydration properties of copra meal by acid and enzymatic hydrolysis using hydrochloric acid and commercial enzymes containing mannanase activity. Response surface methodology (RSM) will be used to obtain the most optimum and practical conditions for the process. The formations of monosaccharide, disaccharide during acid and enzymatic hydrolysis were also monitored. Monosaccharide compositions analysis, gel penetration technique and FT-IR were used to investigate the structural changes of modified CM. Baking products prepared by substitution of wheat flour for modified copra meal were also studied for their overall qualities.

Acid hydrolysis using 0.5% HCl significantly reduced swelling capacity (SC) and water retention capacity (WRC) whereas bulk density (BD) and soluble dietary fiber content (SDF) of the modified CM significantly increased. Monosaccharide composition profile, gel penetration profile and FT-IR spectra indicated the destruction of CM matrix structure. This destruction

increased compactness of the structure and lessened the ability of CM to hold water. Substituting the modified CM for wheat flour in bread and cookies significantly improved bread and cookies qualities compared to the use of the untreated CM. Response surface methodology (RSM) showed that HCl concentration, hydrolysis temperature and time influenced properties of the modified CM. The models predicting their relationships were also generated.

SC and WRC were reduced significantly using enzymatic hydrolysis by commercial enzymes containing β-mannanase. Actipro SF-R (SF-R) hydrolyzed CM at the highest rate followed by E mannanase GMP (E-man) and significant reduction in SC and WRC was observed. Influences of enzyme content, hydrolysis temperature and pH on hydrolysis yield, SC, WRC and BD of CM were studied using RSM. The generated quadratic models predicted that SF-R hydrolyzed CM in higher rate but produced lower hydrolysis yield than E-man under the condition giving similar SC, WRC and BD. The activity of endo-β-mannanase in the commercial enzymes was the most important for modifying CM hydration properties whereas the presence of β-mannosidase caused major loss in hydrolysis yield without significant changes in CM hydration properties. The activity of endoglucanase enhanced the hydrolysis of CM but showed no effect on the modification of CM properties. Substituting the modified CM for wheat flour significantly improved bread qualities.

Chapter 1 Introduction

Thailand is one of the largest exporters of coconut products. Each year abundant amounts of by product taken after pressing cream and oil out of the coconut meat, so called coconut residue or copra meal (CM), are produced. CM is a good source of dietary fiber. CM after coconut oil separation contains total dietary fiber of 60.9% with 56.8% insoluble and 3.8% soluble (Trinidad et al. 2006). Trinidad et al. (2006) studied effectiveness of the dietary fiber component of coconut flour, copra meal after coconut oil was separated by pressing in an expeller, as a functional food. This reported 15 and 25% dietary fiber from coconut flakes could reduce serum total cholesterol, LDL cholesterol and triglycerides of humans. Despite, beneficial effects of dietary fiber from CM and other fiber sources, changes in physical properties and sensory qualities produced by the fibers limit the quantity of fiber incorporation into food products. High water holding capacity (WHC) associated with some fibers is deleterious to product texture, as the fiber sorbs moisture from its surrounding (Onwulata and Elchediak, 2000).

To ease of formulation when dietary fiber (DF) is used to replace wheat flour, WHC need to be low close to that of wheat flour. Several treatments could change WHC of fibers or non-carbohydrate polysaccharides. Particle size reduction by method such as micronization of increased WHC of carrot insoluble fiber (Chau et al., 2007), oat bran and microcrystalline cellulose (Cadden, 1987) but the effect varied on fiber sources. The decrease in WHC was found for wheat bran after particle size reduction (Cadden, 1987). The treatments that promote a breakdown of the cell wall matrix will cause the reduction in WHC. Drying cauliflower fiber at high temperature (75°C) was found to decrease WHC (Femenia et al., 1997). A 35% reduction in WHC of observed by pressure treatment (Onwulata and Elchediak, 2000).

Coconut fiber contained more than twice as much WHC, water retention capacity (WRC) and swelling capacity (SC) as apple, pea, wheat and carrot fiber (Raghavendra et al. 2006) indicating its limitation as fiber supplement for food products. Studies on methods for improving CM fiber functionality and potential applications would increase its utilization, and also the development of fiber-enhanced foods with reduced carbohydrate and caloric content, and subsequently profit the coconut industry. Acid hydrolysis is a possible method to disrupt coconut fiber matrix structure and subsequencely reduce its WHC. The first objective of this study was to investigate modification of CM properties by acid hydrolysis. Gel penetration and FT-IR were used to investigate the structural changes of modified CM. Modified CM was also substituted to wheat flour in bread and cookies and their properties were studied.

Since consumers increased demands on food products with low chemical treatment, in this recent research we studied the hydrolysis of CM using commercial enzymes containing mannanase activity. β-Mannanases (mannan-endo-1,4-β-mannosidase, E.C. 3.2.1.78) are hydrolytic enzymes which catalyze the random cleavage of β-1,4 mannosidic linkages within the backbones of mannan, galactomannan, glucomannan and galactoglucomannan (Titapoka et al. 2008). β-Mannanase is a major enzyme for CM hydrolysis since the main chain of the major polysaccharide in copra meal is made up of (1⟶4) – linked β-D-mannosyl residues and a few α-1,6 galactose units attached to the O-6 position of some of the mannose building blocks (Banta, 1998).

The destruction of the CM complex structure is likely to decrease its hydration properties and reduce its ability to trap water which helps improving physical properties of fiber from CM. The second objective of the study was to investigate the modification of CM hydration propertied by enzymatic hydrolysis using commercial enzymes containing β-mannanase activity. The modification of CM could improve its functional properties and promote its utilization as a source of food fibers or low-calories bulk ingredients for food applications.

Chapter 2 Materials and methods

2.1 Raw material

Dried CM, by product taken after extraction of coconut milk, was obtained in ground dried form from a coconut milk factory located in Nakonphatom, Thailand. Upon arrival to the laboratory the dried CM was package in polypropylene bags, sealed and stored at 4°C until used. Crude fat content of dried CM determined by extracting the sample with petroleum ether in a Soxhlet apparatus was 29.38±0.10%.

2.2 Preparation of raw material

To reduce fat content, dried CM (100 g) was mixed with hexane (1 L) for 18 h. After separating hexane, CM was kept in a hot air oven (ShelLab, 1375FX, Cornelis, Oregon) at 45 $^{\circ}$C for 3 h to remove hexane (Raghavendra et al., 2004). Fat content of the defatted CM was 8.04±0.34%. The defatted CM was ground using a blender and sieved through a 300 µm pore size.

2.3 Acid hydrolysis

Hydrolysis of CM was performed in a 250 ml Erlenmeyer flask. Five grams of dried defatted CM was mixed with 165 ml of HCl solution at the required concentration. The mixture was incubated in a circulating water bath (Polyscience, 8205, Pleasant Prairie, Wisconsin) at required temperature for required time. The reactions were terminated by the removal of HCl solution from the treated CM. The treated CM was washed thoroughly by distilled water until the draining water was neutral. The treated CM was dried in a hot air oven at 60°C for 18 h. The dried modified CM was weighed and the hydrolysis yield (%) was calculated by dividing the dried CM weight after hydrolysis by the initial dried CW weight and multiplying the results by 100.

2.4 Determination of physicochemical properties of modified CM

Before determination of physicochemical properties, all modified CM samples were dried, ground in a blender to a specific particle size, which was between 250 and 400 µm and used for determination of hydration properties.

Swelling capacity (SC) of the modified CM in water was measured in terms of the bed volume technique and calculated in ml hydrated sample per g dry matter as described by Robertson et al. (2000).

Water retention capacity (WRC) was the quantity of water that remains bound to the hydrated fiber following the application of centrifugation. Results were expressed as g water per g dry matter of original substrate as described by Robertson et al. (2000) with slight modification. One gram of modified CM was hydrated in 30 ml distilled water in a centrifuge tube. After equilibration (18 h), samples were centrifuged (3,000xg for 20 min) (Sorvall, Biofuge Stratus, Germany). The pellet was weighed freshly and after drying in a hot air oven at 105 °C for 8h, the residue dry weight was recorded.

Loose bulk density of powders is a measured density after a powder is freely poured into a container. The bulk density (BD) is expressed in grams per milliliter (g/ml) (Chau et al., 2006)

2.5 Separation and analysis of dietary fiber (DF) content of modified CM

Dried CM (1 g) was suspended in phosphate buffer pH 6.0. After pH was adjusted to 6.0, starch was hydrolyzed by adding 0.1 ml of heat stable α-amylase (heat-stable solution, A3306, Sigma) into the solution and placed in boiling water bath 15 min. After cooling to room temperature, the pH of the reaction mixture was adjusted to 7.5 and proteins were digested with 0.1 ml of protease (Protease from *Bacillus licheniformis*, P4860, Sigma) at 60°C for 30 min. After cooling, pH was adjusted to 4.5 and the digestion was continued by adding 0.1 ml amyloglucosidase (Amyloglucosidase solution from *Aspergillus niger,* A9913*,* Sigma*)* at 60°C for 30 min. The suspension was filtrated with a sintered funnel. The filtrate was added with 4 volumes of 95% ethanol, kept overnight to precipitate the soluble dietary fiber (SDF). The SDF suspension was filtrated with a sintered funnel. The sintered funnels with the SDF precipitate were dried at 105°C and weighed. This method was used to obtain SDF. To obtain total dietary fiber (TDF), the suspension after enzymatic treatment was added with 4 volumes of 95% ethanol and kept overnight before filtration with a sintered funnel and washed with 95% ethanol and acetone. The sintered funnels with the precipitate were dried at 105°C and weighed. This method was use to obtain the TDF without correction for undigested protein and ash.

2.6 Molecular weight chromatography of modified CM SDF

SDF obtained from the method in the section 2.5 (after alcohol precipitation) was re-dissolved with distilled water (5 ml) and centrifuged (3,000xg for 10 min). A half milliliter of the supernatant was loaded onto a Sepharose CL-6B column (1.0 cm i.d. x 30 cm; Amersham Pharmacia Biotech, Uppsala, Sweden). The column was equilibrated and eluted

with degassed distilled water at a flow rate of 1 ml/ min. Fractions (0.5 ml each) were collected throughout the elution. A sample of each elution volume was subjected to the phenol-sulfuric acid method to detect the presence of polysaccharides. The column void volume (V_o) was determined by blue dextran (MW 2,000,000) using distilled water as a mobile phase. Elution volume (V_e) of standard dextrans of 40,000 Da and 500,000 Da (*Amersham biosciences*) were determined and used to conduct a standard curve. The standard curve of log MW and Ve/Vo was used to evaluate molecular range of modified CM SDF.

2.7 Monosaccharide compositions of CM

TDF obtained from the method in the section 2.5 (after alcohol precipitation) was weighed into a glass beaker after which 3 ml of 72% H_2SO_4 was added. The contents in the beaker was stirred as incubated in 30°C water bath for 1 h. After that 87 ml of distilled water was added and the mixture was autoclaved at 120ºC for 1 h. The solution was then neutralized by adding $Ba(OH)_2$. The filtered solution (0.45μm) were analyzed for monosaccharide compositions by the ion chromatography system (ICS 3000).

2.8 FTIR

The FT-IR spectra of modified CM were recorded on Fourier transform infrared spectrometer (Spectrum GX, Perkin Elmer) equipped with DTGS Mid -IR (KBr) detector. The spectra range was 4000-400 cm^{-1}, resolution 4 cm^{-1} and 16 scans were collected for each sample.

2.9 Effect of SC and WRC of CM in bread and cookie properties

A baking test was carried out in which untreated or treated CM with the same particle size was added to the bread and cookies for substituting wheat flour. The standard ingredients for a single loaf bread formulation contained 200 g wheat flour, 11 g water, 30 g sucrose, 33.33 g butter, 17 g egg, 2 g salt and 2 g active dry yeast extract. Loaf volume (g), loaf volume (ml), specific volume (ml/g) were determined. Moisture content was determined by standard method. Bread slices (2.5 cm thickness) without their crusts were used to determine bread firmness. Firmness were measured using a texture analyzer (Brookfield Model QTS25, Middleboro, MA) equipped with 38.1 mm ϕ Perspex cylinder probe. The center of the bread slice was subjected to 40% deformation (from 25 mm to 10 mm) at the test speed of 2 mm/sec and the maximal force needed to break through the

bread slices was used to quantify the bread firmness. A total of 5 replicates were used for each treatment and the results averaged.

The standard ingredients for cookies contained 75 g wheat flour, 30 g butter, 30 g sucrose, 25 g egg, ¼ tea spoon baking powder and 0.25 g vanilla. Width/thickness ratio was determined. Firmness was measured using a texture analyzer as previous described equipped with 2mm ϕ stainless steel cylinder probe. The probe was placed onto the center of the cookie piece and the distance of 3mm and the maximal force was used to quantify the cookie firmness. A total of 10 replicates were used for each treatment and the results averaged.

2.10 Effect of acid concentration, temperature and time on acid hydrolysis of CM

Response Surface Methodology was applied to predict the relationship between more than one variable (HCl concentration (X_1), hydrolysis temperature (X_2) and time (X_3)) with the obtained responses (the hydrolysis yield, BD, SW and WRC). The analysis develops a model by fitting the experimental data in a generalized smooth curve, from which a specific predicted response could be calculated.

A central composite design (CCD) with three factors was applied to investigate influences of HCl concentration (0.5-1.5%), hydrolysis temperature (40-90ºC) and time (6-24 h). The optimal exact two factors designs (Borkowski, 2003) was applied to investigate influences of HCl content (X_1) and hydrolysis time (X_3) at constant temperature of 77°C on physical properties of modified CM. Each acid hydrolysis experiment was done according to the procedure described in the section 2.3.

2.12 The enzymes

Three commercial enzymes containing β-mannanases used in this study included; E Mannanase GMP or E-man (An enzyme preparation that contains mannanase as main activity and β-glucanase, xylanase and cellulase as side activities. The enzyme is produced by a selected strain of *Trichoderma reseei*, Actipro SF-R or SF-R (Enzyme preparation for food use containing pectinase and hemicellulase derived from a selected stain of *Aspergillus niger* with declared activity of $\geq$28,500 AVJP/g) and Actipro SF-J94 Max or SF-J94 (Enzyme preparation for food use containing pectinase derived from a selected stain of *Aspergillus niger* with declared activity of $\geq$132,000 AVJP/g). All the enzymes were products of DSM food specialties.

2.13 Enzyme activity determination

β-Mannanase was determined using 0.5% locust bean gum as substrate. The substrate was suspended by homogenizing and heating to 80°C for 10 min in 0.1 M sodium phosphate buffer, pH 6.0 or other pH studied. Enzyme sample (0.05 ml) was incubated with the substrate solution (0.95 ml) at 60°C or other temperature studied and the reducing sugars formed were determined by the method of Somogyi and Nelson (Ratto and Poutanen, 1988). One unit of β-mannanase activity was defined as the amount of enzyme that releases 1 μmol of reducing sugar equivalent to D-mannose per minute under the experimental conditions.

β-mannosidase was determined by incubating a mixture of 50 μl of 1.25 mM *p*-nitrophenyl-β-D-mannopyranoside 175 μl of 20 mM phosphate buffer (pH 7.0) and 25 μl of suitably diluted enzyme solution at 50°C for 15 min. The reaction was stopped by adding 350 μl of 0.2 M Na_2CO_3 and the liberated *p*-nitrophenol was measured at 450 nm. One unit of β-mannosidase was defined as the amount of enzyme that liberates 1 μmol of *p*-nitrophenol per minute under the experimental conditions.

Endoglucanase was determined using 1.0 % hydroxyethycellulose in 0.05 M citrate buffer, pH 4.8 as a substrate according to Ghose (1987). One unit of Endoglucanase was defined as the amount of enzyme that liberates μmol/ml of glucose equivalent released in 1 min per ml enzyme under the experimental conditions.

Glucoamylase was determined using *p*-nitrophenyl-β-D-glucopyranoside (PNPG) as a substrate according to AOAC Official Method 994.09. One unit of activity is the amount of glucoamylase that frees 0.1 μmole of *p*-nitrophenol/min from PNPG substrate under the conditions of the assay.

2.14 pH and temperature optima of β-mannanase

pH optima of β- mannanase of each commercial enzyme was determined by measuring β-mannanase activity at pH values ranging from 3-9 (acetate buffer for pH 3-6 and phosphate buffer for pH 7-9). Optimal temperature of β-mannanase for each enzyme was determined by assaying at different temperatures (25-70°C). All samples were analyzed in duplicates.

2.15 Enzymatic hydrolysis of CM

Hydrolysis of CM was performed in a 250 ml Erlenmeyer flask. Five grams of dried defatted CM was mixed with 165 ml of buffer at the required pH. Then enzymes at required amount was added, the mixture was incubated in a circulating water bath (Polyscience, 8205, Pleasant Prairie, Wisconsin) at required temperature for 6 h. The reactions were terminated by heating the mixture at 85°C for 10 min. The hydrolyzed CM was washed thoroughly by distilled water and dried in a hot air oven at 60°C for 18 h. The dried modified CM was weighed to calculate the hydrolysis yield (%) by dividing the dried CM weight after hydrolysis by the initial dried CW weight and multiplying the results by 100.

2.16 Determination of sugar content in the hydrolysis solution

Mannose content in the hydrolysis solution was measured by using HPLC system (Shimadzu, Kyoto, Japan). The solution (2 ml) was filtered through a 0.45 μm filter and a portion (10 μL) filtrate was injected into the HPLC column (Inertsil® NH_2 5 μm, dimension 4.6 × 250 mm). The mobile phase was CH_3CN/H_2O:80/20 at flow rate 1.0 ml/min, at 30 °C. The HPLC was performed using a refractive index detector with detection limit of 0.01 mmol/L.

Mannobiose and mannotriose content in the hydrolysis solution was measured by using the same HPLC system and HPLC column described previously. The mobile phase was $C_2H_6O/C_4H_8O_2/CH_3CN/H_2O$:30/30/23/17 at flow rate 1.0 ml/min, at 26 °C.

D-mannose (Merck, Germany), 1,4-beta-D-*mannobiose* and 1,4-beta-D-*mannotriose* (Megazyme, Australia) were use as standards.

2.17 Effect of the enzyme content, hydrolysis temperature and pH on properties of the hydrolyzed CM

RSM was applied to predict the optimal hydrolysis conditions of CM by SF-R and E-man which could produce the modified CM with the highest hydrolysis yield and BD with the lowest SW and WRC. Two sets of the optimal exact three factor designs (Borkowski, 2003) were employed in this study, requiring 10 experiments as. The selected variables were: enzyme concentration, temperature and pH. Each acid hydrolysis experiment was done according to the procedure described in the method section 2.15 and the experimental range and levels of independent variables are shown in **Table 8**. The experimental data obtained were fitted by the following quadratic/(second-degree) polynomial equation as shown below:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (1)$$

where Y is the predicted response, b_0 the constant, b_1, b_2, and b_3 the linear coefficients, b_{12}, b_{13}, and b_{23} the cross-product coefficients, and b_{11}, b_{22}, and b_{33} are the quadratic coefficients.

2.18 Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) . When ANOVA indicated significant F values, multiple sample comparison was also performed by Duncan's new multiple range test ($p < 0.05$) in order to detect significant differences using SPSS statistics 17.0 program.

Chapter 3 Results and discussion

3.1 Effect of acid hydrolysis on hydration properties

Treatment of 0.5%HCl at 90°C resulted in the significant reduction in SC and WRC values of CM ($p<0.05$) (**Table 1**). WRC and SC of modified CM significantly decreased with increased hydrolysis time ($p<0.05$). SC and WRC decreased 2.6 and 2.3 times and BD increased 2.4 times as the hydrolysis time increased to 24 h. The ability to bind water is generally affected by the number and nature of water-biding sites was well as the fiber structure (Chau et al. 2006). The decrease in SC and WRC was likely to cause by the destruction cell structure limiting the ability of the cells to hold water. Femenia (1997) found that heat drying promoted a breakdown of the cell wall matrix but freeze drying conserve matrix structure as a result a decrease in water holding capacity (WHC) of cauliflower fiber was observed after drying at high temperature (75°C) whereas freeze drying increased the WHC. Dynamic pulsed pressure treatment which caused the increased extractable hemicelluloses and subsequence disruption of cells resulted in a reduction in fiber WHC (Onwulata and Elchediak, 2000). BD of modified CM significantly increased with increased hydrolysis time ($p<0.05$). The increased in BD of modified CM indicated the decrease in particle internal porosity and surface area of the CM as a result of the structural disruption of CM by acid treatment. The increase in BD made CM to be less bulky and its physical property became closely to that of flour.

3.2 Effect of acid hydrolysis on DF content, monosaccharide compositions and molecular weight distribution of modified CM SDF

To study effect of acid hydrolysis on DF content and molecular weight distribution of modified CM, CM was treated with 0.5% HCl at 90°C for 1, 6 and 24 hr. There was no changed in TDF content for both treated and untreated CM whereas a significant increase in SDF content was found for the modified CM (**Table 2A**). The increase in the SDF content was also reported for corn bran fiber treated with xylanase (Hu et al. 2008). Marconi et al. (2000) demonstrated that cooking, both conventional (boiling) and microwave (in sealed containers), resulted in increases in the soluble fiber fraction and decreases in the insoluble fraction of chickpeas and common beans. These changes were attributed to a partial solubilization and depolymerization of hemicellulose and insoluble pectic substances.

Major monosaccharide in TDF of CM are mannose followed by glucose and some galactose and other sugars. Balasubramaniam (1976) which found that the polysaccharides of fresh mature coconut kernels were galactomannans (61%), mannans (26%), and cellulose

(13%). The mannose content might be lower than expected because only TDF of CM was analyzed for the monosaccharide profiles. Significant decrease in monosaccharide contents was observed in TDF of the modified CM indicated alteration of CM structure (**Table 2B**).

Figure 1 shows gel-permeation profiles of SDF of untreated and modified CM. The profile indicated two groups of SDF molecules with different molecular size at about 40,000 Da for the first eluting peak and another peak at very low MW (fraction no. 40-50). A larger low MW peak and the smaller high MW peak were found for SDF of the modified CM. The result showed that high MW polysaccharides were converted to low MW polysaccharides. This indicated structural modification of SDF of the modified CM.

3.3 FT-IR analysis of the modified CM

The FT-IR spectra of copra meal treated with 0.5%HCL at 90^{O}C for 1, 6 and 24 h are shown in **Figure 2**. The spectra shows similar characteristic of polysaccharide structure. Hydrogen bonded OH stretching at around 4000-3000 cm^{-1} and CH_2-stretching at 2800-3000 cm^{-1} is observed for all the spectra. As hydrolysis time increased, OH stretching band became boarder and the maximum absorbance of hydrogen bonded OH stretching is shifted to lower wavenumber (from 3377-3370 cm^{-1}). According to Oh et al. (2005) who studied cellulose treated with NaOH and CO_2, these changes indicated changed in intramolecular hydrogen bonding. There was a decrease in the absorbance of the band at 1647 cm^{-1} of treated CM. The bands in this region show bending vibrations of water molecules (Virkki et al., 2005) or OH bending of bound water (Oh et al. 2005). There were some differences between the samples in the region 800-1200 cm^{-1}. Although we cannot indicate exactly the structure changes of the modified CM, yet FT-IR spectra indicated changes in the structure of the modified CM. CM is hydrolyzed to smaller molecules or segments by HCl resulting in the disrupted CM matrix structure. Decreased WRC and SC and FT-IR spectra actually indicated the destruction of CM matrix structure. Lin et al. (2009) reported that HCl treatment had effect on molecular degradation (a decrease in molecular size distribution) and surface structure of cotton cellulose.

3.4 Effect of SC and WRC of CM in bread and cookie properties

Table 3 shows properties of bread prepared by substituting 10% wheat flour for untreated and modified CM. Specific volume of bread loaf decreased and crumb firmness increased as CM was substituting for wheat flour. As SC and WRC of the modified CM decreased, the changes in specific volume and firmness were significantly decreased and less decrease in product quality was found. Specific volume decreased 52% for loaf prepared from

the untreated CM and only 35% for loafs prepared by the modified CM (24 h). Lower loaf volume and firmer texture is commonly observed in fiber-supplemented breads (Filipovic et al. 2007; Hu et al. 2009; Chen 1988; Sangnark and Noomhorm, 2003). The volume of bread depends significantly on the quantity of gluten (Hu et al., 2009) and interaction between gluten and fiber material (Chen, 1988). The reduction of gluten content also changes crumb structure which will impaired carbon dioxide retention (Hu et al., 2009). Crumb firmness increased 5 times for bread prepared from untreated CM and only 2 times for bread prepared from the modified CM (24 h). High hydration properties of fiber had negative effects on product qualities especially during dough development because of the competition for water between fiber and dough components that were activated by water during dough development, such as yeast action and gluten structure (Chen, 1988). Addition of only 3% rice bran insoluble fiber into bread formulation caused 3.7 times increase in firmness (Hu et al, 2009) and 5 times for the addition of 10% defatted rice bran (Abdul-Hamid and Luan , 2000) whereas only 2 times increase in firmness when using modified CM at 10% substitution. The untreated CM completed with other ingredients for water resulted in the adverse effects on the bread qualities as the SW and WRC were reduces this effect was less. Therefore, the result indicated that the modified CM has improved functional properties of CM for substituting wheat flour in bread without changing the bread formulation. As the development of the bread formulations, bread quality could be improved and the modified CM could be added into bread formulation as source of food fibers or low-calories bulk ingredients at equivalent quantity as the traditional breads. Addition of fiber into bread formulation instead of replacing the flour and adjusting bread formulation by adding more water was commonly used to improve loaf volume in fiber bread or gluten free bread (Sangnark and Noomhorn, 2003) and this method could be used to improve the volume of the bread loafs prepared from the modified CM as well.

Table 4 shows properties of cookies prepared by substituting 10% and 20% wheat flour for untreated and modified CM. Width/thickness ratio of cookies decreased and firmness increased as CM was substituting for wheat flour. There was a further decrease in width /thickness ratio and increase in firmness as higher amount of CM was substituted to the formulations (from 10% to 20%). With the decrease in SC and WRC for CM treated for 1 to 24h, the decrease in width/thickness ratio and the increase in firmness were less compared to those of untreated sample. As WRC and SC decreased, the less decrease in width/thickness ratio indicated that cookies spread better than those prepared from the untreated CM. The strong water-binding characteristics of CM fiber caused cookie dough to be drier in appearance

than control dough as a consequence, the dough could not spread well, and the cookies were small and thick similarly to study by Chen et al. (1988).

Moisture content increased for cookie containing modified CM. The softer texture of cookies containing modified CM might be partly due to this higher moisture content than the untreated DF samples. The low moisture content for untreated DF samples was because the effect of competition for water between DF and other ingredients was more significant in the modification of cookies texture. DF with high WRC and SC normally sorbs more water as a result the products exhibited high moisture content because commonly water was added to aid cookie dough formation for cookie that incorporated with DF (DeFouw et al., 1982) but this was not the case for our studies. At 20% substitution, cookies incorporated with modified CM after 24 h incubation maintained their qualities comparable to the control with only slight firmer texture. Compared to fiber from other source such as apple, wheat bran and oat bran, changes in cookies qualities were detected on addition at less than 10% concentration on a flour replacement basis (Chen et al., 1988). This observation suggested that modified CM is a promising source of food fibers or low-calories bulk ingredients in food applications.

3.5 Effect of acid concentration, temperature and time on acid hydrolysis of CM

In the previous sections we demonstrated that CM is hydrolyzed to smaller molecules or segments by HCl resulting in the disrupted CM matrix structure causing the decrease in SC and WRC of modified CM. The modified CM caused less quality changes in bread and cookie when used to replace wheat flour. To obtain CM with desired physiochemical properties, in this section we studied factors influenced modified CM properties including acid concentration, hydrolysis temperature and time and created models to predict their relationships.

A central composite design (CCD) with three factors was applied and the quadratic models with coded variables are shown in Eqs. 1 and 2, which represent the hydrolysis yield (Y_1) and bulk density (Y_2) as a function of temperature (X_2) and time (X_3).

$$Y_1 = 80.6991-18.4341x_2-3.8119x_3-5.3176x_2^2-2.1781x_2x_3+1.6952x_3^2 \quad (1)$$

$$Y_2 = 108.3400+56.6298x_2+4.8545x_3+34.5979x_2^2+9.5419x_2x_3+1.7641x_3^2 \quad (2)$$

For Y_1 representing hydrolysis yield, the model did not show lack of fit (p-value = 0.1100) and R^2 of 0.9539. For Y_2 representing bulk density, the model did not show lack of fit (p-value = 0.1305) and R^2 of 0.8997 indicated that the models fit well with the raw data. The model

indicates that as the process temperature (X_2) increased the hydrolysis yield decreased and BD increased. An increased in the hydrolysis time (X_3) results in a similar trend as the increase in process temperature (X_2). We analyzed the relationship between yield versus temperature and BD versus temperature when hydrolysis time was constant using equation (1) and (2). We found that when these plots were overlaid the temperature given the highest yield and BD was 77°C. Since the variable X_1 (HCl concentration) could not be fitted to the same quadratic model as the other two variables indicated that their relationships was not quadratic. We used the optimal exact two factors designs (Borkowski, 2003) to investigate influences of HCl content (X_1) and hydrolysis time (X_3) at constant temperature of 77°C on physical properties of modified CM.

The effects of HCl content and hydrolysis time on yield, BD, SC and WRC when temperature was set at 77°C was studied and results was shown on **Figure 3**. The data indicate that as the HCl content increased the hydrolysis yield, SC and WRC was decreased where as BD was increased. Similar trend was found for the effect of hydrolysis time. At process temperature of 77°C, the quadratic models are shown in Eqs. (3), (4), (5) and (6). The models did not showed lack of fit and R^2 values were high indicated that the models fit well with the raw data (**Table 5**).

The confirmation experiment was done at 0.75%HCl, temperature of 77°C and hydrolysis time of 18 h in duplicate and the result was 70.76±1.04% for yield, 218.99% ±4.75% for BD, 28.79±1.75% for SC and 28.96±0.02% for WRC. The result was in good agreement with the predicting values which are 70.69% for yield, 242.42% for BD, 29.52% for SC and 28.81% for WRC.

3.6 Screening of commercial food grade enzymes on hydrolysis of CM

The activity of β-mannanase measured at optimal pH and temperature of the enzyme at pH 5.0 and 60°C for three commercial enzymes used in this study were shown in **Table 6**. The enzyme containing the highest activity of β-mannanase was E-man. β-mannosidase, endoglucanase, glucoamylase were also detected at various extents. SF-R contained the highest activity of β-mannosidase, endoglucanase, glucoamylase. SF-J94 had the lowest activity of β-mannanase , β-mannosidase and endoglucanase compared to the other two enzymes. Balasubramaniam (1976) found that the polysaccharides of fresh mature coconut kernels were galactomannans (61%), mannans (26%), and cellulose (13%). Therefore, the

commercial enzymes used in this study contained important enzymes for breaking down CM complex structure.

Hydrolysis of CM using these three commercial enzymes was studied and the progress of CM hydrolysis was determined by measuring reducing sugars produced during hydrolysis (**Figure 4)**. The enzyme contents providing the same β-mannanase activity were used to hydrolyze CM. The result showed that SF-R hydrolyzed CM at the highest rate followed by E-man. The lowest hydrolysis rate was found for SF-J94. This was likely because SF-R contained the highest activity of β-mannosidase, endoglucanase, glucoamylase and SF-J94 had the lowest activities of these enzymes.

Table 7 shows changes in hydrolysis yield and hydration properties of CM after enzymatic hydrolysis. The hydrolysis yield of CM treated with E-Man and SF-R were lower than that of the control, whereas treating by Actipro SF-J94 resulted in similar hydrolysis yield as the control. Moreover, the hydrolysis of CM using E-man and SF-R reduced SC and WRC but not by using SF-J94.

SF-J94 had higher β-mannosidase activity and glucoamylase compared to E-man. These two major enzymes in SF-J94 broke down side chain residues of CM, therefore they had less effects on lowering yield and hydration properties of CM. The increase in hydrolysis time from 6 h to 24 h only slightly lowered the yield and WRC of the hydrolyzed CM (**Table 7**). The low activity of endo β-mannanase resulted in the inability of the enzyme to hydrolyze inside structure of CM as a consequence there was no change in hydration properties and only the loss of yield was observed for SF-J94.

Considering β-mannosidase activity, both SF-J94 and SF-R had β-mannosidase activity whereas E-man contained under detectable activity of β-mannosidase. E-man had higher endoglucanase compared to SF-J94. The result from **Table 7** suggested that endo β-mannanase and endoglucanase were likely important enzymes for CM hydrolysis to lower hydration properties of CM. E-man had only endo β-mannanase therefore the reduction in hydrolysis yield was less than that of SF-R which contained higher β-mannosidase, endoglucanase and glucoamylase. Since SF-J94 caused no change in hydration properties, we did not study further for this enzyme. SF-R and E-man contained high level of β-mannanase and endoglucanase and the increase in hydrolysis time from 6 to 24 h resulted in the modified CM with lower SW and WRC values (data not shown). Therefore, these two enzymes were used for the next experiments.

To investigate the influences of endoglucanase on the hydrolysis of CM, we hydrolyzed CM using purified cellulase or endoglucanase (from *Aspergillus niger* powder, ≥0.3 units/mg

solid 5 KU,SIGMA#C1184). The enzyme catalyzes the hydrolysis of endo-1,4-β-D-glycosidic linkages. The increase in reducing sugar content was observed during hydrolysis of CM using purified cellulase (data not shown). This result corresponded well with the reduction in the hydrolysis yield after 6 h (**Figure 5**) indicating that CM structure was destructed by enzymatic hydrolysis using cellulase. Cellulose is one of the non starch polysaccharide component found in mature coconut kernels. Balasubramaniam (1976) identified linkages between cellulose and mannan in CM and found that mannanase and cellulase were needed to breakdown copra mannan.

However, the hydrolysis of CM using cellulase did not lower hydration properties (SW and WRC) of the hydrolyzed CM. Besides, slight increase in the hydration properties was observed (**Figure 5**). For the hydrolysis of CM using E-man which contained lower amount of endoglucanase activity compared to the commercial endoglucanase, the hydration properties of modified CM was lowered than the control. The result suggested that endoglucanase activity affected hydrolysis of CM but had no influence on the modification of CM hydration properties compared to mannanase activity. Therefore it was likely that the higher endoglucanase activity in SF-R could enhance CM hydrolysis and caused loss in hydrolysis yield but showed less effect on the reduction of the CM hydration properties compared to the action of E-man.

3.7 Effect of the enzyme content, hydrolysis temperature and pH on properties of the hydrolyzed CM

We investigated influences of enzyme content (X_1), temperature (X_2) and pH (X_3) on some physical properties of modified CM using three factors designs for RSM. Two sets of experiments were performed for two kinds of commercial enzymes; E-man and SF-R. The experimental designs and results are shown in **Table 8** and the quadratic models generated from raw data are shown in Eqs. (7) to (11) for E-man and Eqs. (12) to (16) for SF-R.

To check model adequacy, residual analysis was performed. Normal probability plot of the residuals showed that the residuals were along a straight line indicated that the normal assumption is satisfied (**Figure 6A**). Plot of residuals versus predicted responses showed that the residuals scatter randomly which indicated that model is adequate (**Figure 6B**). Both normal probability plot of the residuals and plot of residuals versus predicted responses for all responses, Y1 to Y5, resulted in patterns which indicated the reliability of the predicted models. This suggested that the generated models could explain the data variation and represented the actual relationships between the parameters.

For E-man, the confirmation experiment was done at enzyme content of 1,200 units, temperature of 40°C at pH 5.0 in duplicate and the result were 54.44±0.34% for yield, 163.54% ±6.71% for BD, 74.82±1.31% for SC and 41.21±2.73% for WRC. The result was in good agreement with the predicting values which were 57.18% for yield, 169.91% for BD, 79.37% for SC and 39.72% for WRC.

For SF-R, the confirmation experiment was done at enzyme content of 579.5 units, temperature of 40°C at pH 5.0 in duplicate and the result were 58.57±0.87% for yield, 118.52% ±0.22% for BD, 88.33±0.08% for SC and 66.47±4.56% for WRC. The result was in good agreement with the predicting values which are 58.07% for yield, 124.54% for BD, 87.05% for SC and 67.14% for WRC.

Figure 7A shows effects of temperature and pH on hydrolysis yield and hydration properties of modified CM at constant level of E-man. As temperature increased SC, WRC and yield were increased where as BD was decreased. The pH optimum to obtain low SC and WRC and high BD was 4-4.4. Temperature optimum to obtain low SC and WRC and high BD was 40-44 °C. **Figure 7B** shows effects of E-man content and pH on hydrolysis yield and hydration properties of modified CM at constant temperature. SC, WRC and yield were decreased as the enzyme content increased whereas BD was increased.

Effects of temperature and pH on hydrolysis yield and hydration properties of modified CM at constant level of SF-R was in the same trend as those of E-man. The pH optimum to obtain low SC and WRC and high BD was 4. Temperature optimum to obtain low SC and WRC and high BD was 40-46°C. Similar to E-man, SC, WRC and yield were decreased as the enzyme SF-R content increased whereas BC was increased. Kotcharian et al. (2004) studied enzymatic degradation of carrot cell wall materials (CWM) and found that with increasing degree of cell wall degradation, yields of cell wall materials decreased. The treatment showed to reduced SC and WRC of CMW significantly and an increase in bulk density was also observed similarly to our results.

Considering values calculated from the models generated, at the same pH (5.0), temperature (40°C) and mannanase contents, 700 units, the hydrolysis rate measured as reducing sugar production rate (Y1) of E-man was much lower than that of SF-R (28.52% and 71.62%) whereas the hydrolysis yield of E-man was higher than that of SF-R (78.36% and 50.66%). At this condition SC was 95.35% for E- man and 84.28% for SF-R whereas WRC values was similar (66.79% and 65.94% for E-man and SF-R, respectively. BD was 136.06% and 126.46% for E-man and SF-R, respectively. The use of these two enzymes at the same mannanase activity (700 units) gave closed values of SC, WRC and BD however the hydrolysis

by SF-R caused significant reduction in hydrolysis yield due to high hydrolysis rate. **Figure 8** showed relationships between hydrolysis rate measured as reducing sugar production rate (Y1) which indicated the progress of CM hydrolysis reaction and mannose and mannobiose liberated in the supernatant of the reaction mixture from enzymatic hydrolysis of CM using E-Man and SF-R. Hydrolysis of CM using SF-R produced higher content of mannose than mannobiose whereas using E-Man released more mannobiose than mannose. For both enzymes the trend for mannotriose liberated was in lower rate than the released of mannose and mannobiose (data not showed). This result indicated that SF-R hydrolyzed CM into smaller molecules than E-Man did. It was noticed that E-man contained only endo-β- mannanase and under detectable level of β-mannosidase activity which breaks down the sugar residues at the side chain of the polymer. Therefore, this result suggested that the majority of polysaccharide chains of CM were broken down into monosaccharide when using SF-R as a result the yield loss was at a higher rate than that of E-man whereas the reduction in hydration properties was less than that of E-man. This result indicated that β-mannosidase activity in SF-R caused less significant in modification of CW hydration properties but caused loss in hydrolysis yield. Besides, studies showed that mannooligosaccharides such as mannobiose and mannotriose could function in the selective growth of human-beneficial intestinal microflora (Kurakake et al. 2006). Therefore, enzymatic hydrolysis of CM especially by using E-Man could be applied to produce these health beneficial products as well.

Major monosaccharide in total dietary fiber (TDF) of CM are mannose followed by glucose and some galactose and other sugars (**Table 10**). Hydrolysis of CM using HCl and enzymes caused significant reduction in monosaccharide content. The decreased in glucose contents were more enhanced by enzymatic hydrolysis due to the presence of endoglucanase and glucoamylase. The loss of mannose was higher by using SF-R than E-man due to its high β-mannosidase. The CM treated with E-Man had properties close to HCl treated CM. Their monosaccharide composition profiles were similar. The significant loss in the mannose content for CM treated with SF-R corresponded to its low hydrolysis yield.

3.8 Effect of modified CM on bread properties

Table 11 shows properties of bread prepared by replacing 5% of wheat flour for untreated and enzymatic modified CM. Specific volume of bread loafs decreased and crumb firmness increased significantly as untreated CM was substituting for wheat flour. Only slight decrease in the volume was observed for those with the enzymatic modified CM whereas the bread firmness was not significant different from the control. As SC and WRC of the modified CM decreased, the changes in specific volume and firmness were significantly lowered and less

decrease in product quality was found. Lower loaf volume and firmer texture is commonly observed in fiber-supplemented breads (Filipovic et al. 2007; Hu et al. 2009; Chen 1988; Sangnark and Noomhorm, 2003). The volume of bread depends significantly on the quantity of gluten (Hu et al., 2009) and interaction between gluten and fiber material (Chen, 1988). The reduction of gluten content also changes crumb structure which will impaired carbon dioxide retention (Hu et al., 2009). Crumb firmness increased 4 times for bread prepared from the untreated CM whereas no changes in firmness for the bread prepared from the enzymatic modified CM. High hydration properties of fiber had negative effects on product qualities especially during dough development because of the competition for water between fiber and dough components that were activated by water during dough development, such as yeast action and gluten structure (Chen, 1988). The untreated CM completed with other ingredients for water resulted in the adverse effects on the bread qualities as the SW and WRC were reduces this effect was less. After 2 day of storage, the bread firmness increased for all treatment as a result of moisture loss. The highest firmness was observed for the bread incorporated with the untreated CM. There was no significant difference in the firmness for the control bread and the bread prepared from enzymatic modified CM. Therefore, the result indicated that the enzymatic modified CM improved functional properties of CM for substituting wheat flour in bread without changing the bread formulation.

Chapter 4 Conclusions

Acid hydrolysis of CM was an effective method to decrease hydration properties of CM. The modified CM had lower SC and WRC and higher BD compared to the untreated one. Acid hydrolysis modified CM metric structure which increased compactness of the metric structure and decreased the sites of water to bind the modified CM. The use of modified CM in bread and cookies improved the product qualities significantly. With development of the food formulations, the qualities of foods incorporated with the modified CM could be further improved. RSM showed that HCl concentration, hydrolysis temperature and time influenced hydrolysis yield, SC, WRC and BD of the modified CM. Depending on the properties of fiber desired to be incorporated into food formulations, we can use the models generated from this method to predict properties of the modified CM. Acid hydrolysis is a simple method for lowering hydration properties of food fiber and may be used in other kinds of fibers possessed high water holding capacity similarly to CM to improve their properties and utilization as source of food fibers or low-calories bulk ingredients.

The decrease in SC and WRC by enzymatic hydrolysis using mannanase was likely to cause by the destruction of CM metric structure, resulting in the increase in cell compactness limiting the ability of CM to hold water (indicated by the decrease in WRC and SC) similarly to what happened by acid hydrolysis of CM. The increased in BD of modified CM indicated the decrease in particle internal porosity and surface area of the CM as a result of the structural disruption of CM by acid and enzymatic treatments. Enzymatic hydrolysis of CM can be used to produce modified CM with low hydration properties. Endo β-mannanase which breaks down the inside backbones of mannan, galactomannan, glucomannan and galactoglucomannan showed to be the most important enzymes to lower hydration properties of the CM. The presence of β-mannosidase activity (break down the sugar residues at the side polymer chain) caused loss in hydrolysis yield. The presence of endoglucanase activity showed less influence on modifying CM hydration properties however may enhance the hydrolysis of other hydrolase.

The use of modified CM in bread improved the product qualities significantly. In summary, enzymatic hydrolysis of CM is a promising method to produce modified CM of desired physiochemical properties exhibited potential source of food fibers or low-calories bulk ingredients in food application. Moreover, the concept could be applied in the process such as coconut oil extraction. Once the cell compartments of CM were disrupted by enzymatic hydrolysis, the oil could be easily extracted and this process would enhance the oil yield. At the same time, the CM after oil extraction will be modified and may be used as food ingredients. In

this study, mannose, mannobiose and mannotriose were detected in the supernatant of the reaction mixture from enzymatic hydrolysis of CM using E-Man and SF-R. Studies showed that mannooligosaccharides such as mannobiose and mannotriose could function in the selective growth of human-beneficial intestinal microflora (Kurakake et al. 2006). Therefore, enzymatic hydrolysis of CM especially by using E-Man could be applied to produce these health beneficial products as well. Further research on these approves have currently been conducted in our research group.

Table 1 Properties of modified copra meal treated with 0.5%HCl at 90°C for 1, 6 and 24 h

Time of hydrolysis (h)	Swelling capacity, SC (ml/g dried CM)	Water retention, WRC (g/g dried CM)	Bulk density, BD (g dried CM/ml)
0	14.15±0.54[d]	4.650.03[d]	0.136±0.004[a]
1	11.55±0.29[c]	3.20±0.03[c]	0.152±0.003[b]
6	7.17±0.24[b]	2.38±0.04[b]	0.232±0.007[c]
24	5.47±0.29[a]	2.00±0.04[a]	0.330±0.012[d]

Table 2 Dietary fiber content and monosaccharide compositions of untreated and modified CM (1% HCl at 90°C)

A.

Sample	TDF*		SDF*	
Untreated	77.15	$\pm 0.00^{ns}$	0.40	$\pm 0.04^{a}$
1h	77.37	$\pm 0.27^{ns}$	3.22	$\pm 0.24^{b}$
6h	77.15	$\pm 2.08^{ns}$	3.72	$\pm 1.59^{b}$
24h	77.05	$\pm 2.32^{ns}$	3.05	$\pm 0.21^{b}$

*TDF is total dietary fiber content in percentage; SDF is soluble dietary fiber content in percentage

B.

Sample	Arabinose	Galactose	Glucose	Xylose	Mannose
Untreated	10.46	6.97	288.99	6.21	381.46
1h	1.80	2.05	106.91	2.05	129.80
24h	1.40	0.66	74.82	0.71	168.93

*mg/g dried TDF

Table 3 Properties of bread prepared by substituting 10% wheat flour for untreated and modified CM *

Copra meal treated with 0.5%HCl at 90°C	Loaf weight (g)		Loaf volume (ml)		Specific Volume (ml/g)		Crumb firmness (g)		Moisture content (%)	
Without substitution	44.02	±0.29^{c}	112.67	±4.62^{b}	2.62	±0.09^{c}	498.67	±22.50^{a}	29.82	±0.98^{ns}
0hr	43.41	±0.11^{ab}	54.67	±4.16^{a}	1.26	±0.11^{a}	2657.67	±118.43^{d}	27.53	±0.51^{ns}
1hr	43.18	±0.17^{ab}	60.00	±2.00^{a}	1.39	±0.04^{a}	1959.67	±45.61^{c}	27.05	±0.03^{ns}
6hr	43.50	±0.32^{bc}	62.00	±3.46^{a}	1.43	±0.07^{a}	1167.50	±20.51^{b}	29.28	±0.19^{ns}
24hr	42.84	±0.03^{a}	72.00	±2.00^{b}	1.69	±0.03^{b}	994.33	±116.82^{b}	28.81	±0.48^{ns}

*CM treated with 0.5% HCl for 1, 6 and 24 h at 90°C. SC (ml/g) was 4.65 for untreated CM, 3.20 for 1h, 2.38 for 6h and 2.00 for 24h treated CM. WRC (g/g) was 14.15 for untreated CM, 11.55 for 1h, 7.17 for 6h and 5.47 for 24h treated CM.

Table 4 Properties of cookies prepared by substituting 10% and 20% wheat flour for untreated and modified CM

Samples	Width/thickness ratio				Firmness (g)				Moisture (%)			
	10%		20%		10%		20%		10%		20%	
No substitution	3.223	±0.091^{c}	3.227	±0.102^{b}	564.50	±102.90^{a}	439.88	±56.17^{a}	5.541	±0.057^{a}	5.541	±0.057^{a}
0h	2.850	±0.080^{a}	2.689	±0.069^{a}	2211.63	±374.95^{d}	2973.00	±494.44^{c}	5.460	±0.171^{a}	5.134	±0.243^{a}
1h	2.895	±0.063^{a}	2.677	±0.042^{a}	1356.13	±200.60^{c}	986.75	±134.39^{b}	7.082	±0.500^{c}	8.959	±0.200^{c}
6h	3.026	±0.059^{b}	2.736	±0.016^{a}	1307.63	±163.44^{c}	1012.00	±93.46^{b}	6.857	±0.303^{b}	7.733	±0.248^{b}
24h	3.406	±0.086^{d}	3.185	±0.044^{b}	705.00	±92.67^{c}	746.88	±105.55^{ab}	7.524	±0.052^{c}	7.236	±0.479^{b}

*CM treated with 0.5% HCl for 1, 6 and 24 h at 90°C

Table 5 Analysis of variances for fitted model for different responses. Equations beneath the table indicate relationships between each response and the variables.

Response	Source	Df	Sum of squares	Mean squares	F value	Pr > F
Hydrolysis yield (Y_1)	Lack of Fit	3	2.02	0.67	0.38	0.77
	Pure Error	9	15.75	1.75		
	Total Model	5	495.89	0.96	66.99	<.0001
	R-Square = 0.96					
Bulk density (Y_2)	Lack of Fit	3	2264.68	754.89	3.72	0.05
	Pure Error	9	1827.93	203.10		
	Total Model	5	71567	0.94	41.97	<.0001
	R-Square = 0.94					
Swelling capacity (Y_3)	Lack of Fit	3	91.27	30.42	1.81	0.21
	Pure Error	9	150.96	16.77		
	Total Model	5	2135.76	0.89	21.16	<.0001
	R-Square = 0.90					
Water retention capacity (Y_4)	Lack of Fit	3	2.18	0.73	0.24	0.87
	Pure Error	9	27.51	3.05		
	Total Model	5	1564.62	0.98	126.47	<.0001
	R-Square = 0.98					

$$Y_1 = 69.76-3.70X_1-4.99X_3+1.39X_1^2-0.86X_1X_3+2.16X_3^2 \quad (3)$$
$$Y_2 = 249.46+39.74X_1+61.45X_3-5.76X_1^2+10.20X_1X_3-39.81X_3^2 \quad (4)$$
$$Y_3 = 26.76-7.51X_1-9.59X_3+6.55X_1^2+1.66X_1X_3+6.57X_3^2 \quad (5)$$
$$Y_4 = 26.93-7.18X_1-8.03X_3+2.90X_1^2+2.08X_1X_3+5.08X_3^2 \quad (6)$$

Table 6. Activity of commercial enzymes on substrates (cellulose and hemicellulose), pH 5.0 and temperature at 60 °C for β-mannanase activity

Enzyme	Activity (unit)			
	β-mannanase	β-mannosidase	Endoglucanase	Glucoamylase
E-man (25 mg/ml)	57.24	nd	726.67	12.37
SF-R	3.60	79.45	3,053.33	781.70
SF-J94	1.03	12.18	67.67	25.45

nd : not detected
β-mannanase: 1 unit = μmol of mannose equivalent released in 1 min per ml enzyme
β-mannosidase: 1 unit = nmol nitrophenol released in 1 min per ml enzyme
Endoglucanase: 1 unit = μmol/ml of glucose equivalent released in 1 min per 0.1 ml enzyme
Glucoamylase: 1 unit = nmol nitrophenol released in 1 min per ml enzyme

Table 7. Effect of enzymatic hydrolysis at optimum temperature and pH for 6 h and 24 h on dried CM weight after hydrolysis or hydrolysis yield, swelling capacity (SC) and water retention capacity (WRC) of the hydrolyzed CM

Hydrolysis time / CM properties	6 h			24 h		
	Yield (g/3g CM)	SC (ml/g)	WRC (g/g)	Yield (g/3g CM)	SC (ml/g)	WRC (g/g)
Untreated	2.77±0.028	20.00±0.00	14.31±0.75	2.70±0.030	20.00±0.00	14.31±0.29
E-Man	1.84±0.007	18.75±1.77	13.18±0.77	1.24±0.007	13.00±0.71	7.67±0.00
SF-R	0.86±0.028	12.50±0.00	5.18±0.00	nd	nd	nd
SF-J94	2.48±0.007	20.00±0.00	15.25±0.14	2.26±0.028	20.00±0.00	14.31±0.51

nd : not determination

Table 8. Experimental range and levels of independent variables for hydrolysis of CM by E Mannanase GMP (25mg/ml) and Actipro SF-R

Independent variables	Symbol	Range and levels for E-man*			Range and levels for SF-R*		
		-1	0	1	-1	0	1
Enz (ml)	X_1	0.13	1.38	2.64	0.1	0.05	2
temp (°C)	X_2	40	60	80	40	60	80
pH	X_3	4	6	8	4	6	8

* The ranges of the enzyme used were 60-1200 units for E-man and 38-760 units for SF-R
One unit of β-mannanase activity was defined as changes in absorbance at 540 nm due to the reducing groups formed per minute (0.001 ΔA540/min) under the assay conditions.

Table 9. Experimental design and results obtained by hydrolysis of CM by E Mannanase GMP and by Actipro SF-R; Equations beneath the table indicate relationships between each response and the variables.

Runs	Variables			Responses for E-man					Responses for SF-R				
	X_1	X_2	X_3	Y_1	Y_2*	Y_3*	Y_4*	Y_5*	Y_1	Y_2*	Y_3*	Y_4*	Y_5*
1	0.2912	-1	-1	27.42	77.37	52.39	91.67	59.23	57.13	58.19	133.77	78.33	54.84
2	-1	0.2912	-1	7.48	93.38	50.43	110.00	98.10	9.06	90.34	101.58	100.00	94.95
3	-1	-1	0.2912	4.12	95.81	49.97	121.67	94.22	5.53	96.39	96.68	113.33	100.02
4	-0.1925	1	-0.1925	10.90	97.24	54.07	113.33	107.98	17.38	94.52	91.44	109.58	101.90
5	-0.1925	-0.1925	1	11.66	93.05	52.35	118.33	80.31	4.68	91.69	98.52	108.33	99.26
6	1	-0.1925	-0.1925	72.06	61.15	66.60	81.67	46.54	56.27	56.98	116.14	85.00	82.03
7	-1	1	1	2.68	98.23	50.46	116.67	93.43	10.66	94.28	90.58	116.67	107.68
8	1	1	-1	21.21	95.25	58.23	116.67	101.03	0.62	99.38	95.25	100.00	109.52
9	1	-1	1	15.65	83.44	49.54	111.67	83.24	17.52	85.27	96.06	117.50	103.38
10	1	1	1	5.40	100.77	53.08	115.00	110.21	11.28	89.24	92.94	113.33	109.43

Sugar content (mg/ml) in liquid fraction of the hydrolysis mixture after 6 h

Y_1 is reducing sugar production rate (mg mannose eq./g dry copra meal •min)

*The values are in percentage of those of untreated samples: 4.53 g for dried CM weight after hydrolysis (Y_2), 0.17 g/ml for bulk density (Y_3), 15 ml/g for swelling capacity (Y_4) and 11.95 g/g for water retention capacity (Y_5)

The quadratic models generated for E-man hydrolysis:

$$Y_1=41.3446+17.7907x_1-4.7488x_2-6.4197x_3+9.0989x_1^2-7.3048x_1x_2-23.1779x_2^2-9.2739x_1x_3+6.7606x_2x_3-19.0858x_3^2 \quad (7)$$

$$Y_2=78.3669-8.7903x_1+6.9692x_2+4.3775x_3-5.3546x_1^2+7.4618x_1x_2+11.2373x_2^2+3.7585x_1x_3-4.3860x_2x_3+9.6697x_3^2 \quad (8)$$

$$Y_3=127.2882+14.3882x_1-12.5964x_2-9.2342x_3+7.9576x_1^2-12.5580x_1x_2-13.5931x_2^2-5.4252x_1x_3+11.0794x_2x_3-16.5964x_3^2 \quad (9)$$

$$Y_4=97.0960-6.6532x_1+4.2504x_2+7.3345x_3-4.0480x_1^2+7.3686x_1x_2+8.7379x_2^2-0.7154x_1x_3-6.6190x_2x_3+9.9182x_3^2 \quad (10)$$

$$Y_5=68.9473-11.1579x_1+14.7332x_2+3.6797x_3-4.2091x_1^2+9.2422x_1x_2+20.9639x_2^2+11.4557x_1x_3-9.9104x_2x_3+6.7153x_3^2 \quad (11)$$

The quadratic models generated for SF-R hydrolysis:

$$Y_1=36.8441+14.1557x_1-10.1116x_2-10.3125x_3+1.0948x_1^2-11.1846x_1x_2-7.7104x_2^2-2.1187x_1x_3+17.5812x_2x_3-17.3479x_3^2 \quad (12)$$

$$Y_2=74.1339-10.1939x_1+9.0382x_2+7.0233x_3-2.3494x_1^2+7.2670x_1x_2+10.7142x_2^2+3.2369x_1x_3-12.2552x_2x_3+8.1050x_3^2 \quad (13)$$

$$Y_3=108.5021+5.6336x_1-9.3609x_2-8.6864x_3-2.2616x_1^2-1.9871x_1x_2-6.8109x_2^2-2.2715x_1x_3+10.7029x_2x_3-0.0603x_3^2 \quad (14)$$

$$Y_4=96.0172-6.1843x_1+4.5360x_2+11.1224x_3+0.6202x_1^2+1.3829x_1x_2+9.0164x_2^2+3.1314x_1x_3-7.5889x_2x_3+1.2767x_3^2 \quad (15)$$

$$Y_5=86.3736-5.4486x_1+9.7615x_2+11.5914x_3+5.2335x_1^2+4.8392x_1x_2+6.4579x_2^2+0.5794x_1x_3-12.3708x_2x_3+0.1129x_3^2 \quad (16)$$

Table 10. Monosaccharide compositions of untreated and modified CM

Sample	Arabinose	Galactose	Glucose	Xylose	Mannose
Untreated	10.46	6.97	288.99	6.21	381.46
HCl **	1.40	0.66	74.82	0.71	168.93
SF-R	1.84	1.17	7.32	0.77	23.19
E-Man	1.10	2.31	6.00	0.73	161.88

*mg/g dried TDF

** CM was treated with 0.5% HCl for 24h at 90ºC

Table 11. Properties of bread prepared by 5% replacement of wheat flour for untreated and modified CM

Sample	Specific Volume (ml/g)	Crumb firmness (g)	
		Day 0	Day 2
Control	3.48 ± 0.01^{c}	278.33 ± 9.45^{a}	1152.67 ± 179.17^{a}
Untreated	2.76 ± 0.16^{a}	819.67 ± 16.56^{b}	3152.67 ± 198.43^{b}
E-man*	3.14 ± 0.02^{b}	303.50 ± 9.19^{a}	1141.00 ± 2.83^{a}

*hydrolysis of CM was done using E-man (1200 units/5 g dried CM) at pH 4.0 and 40ºC for 6 h

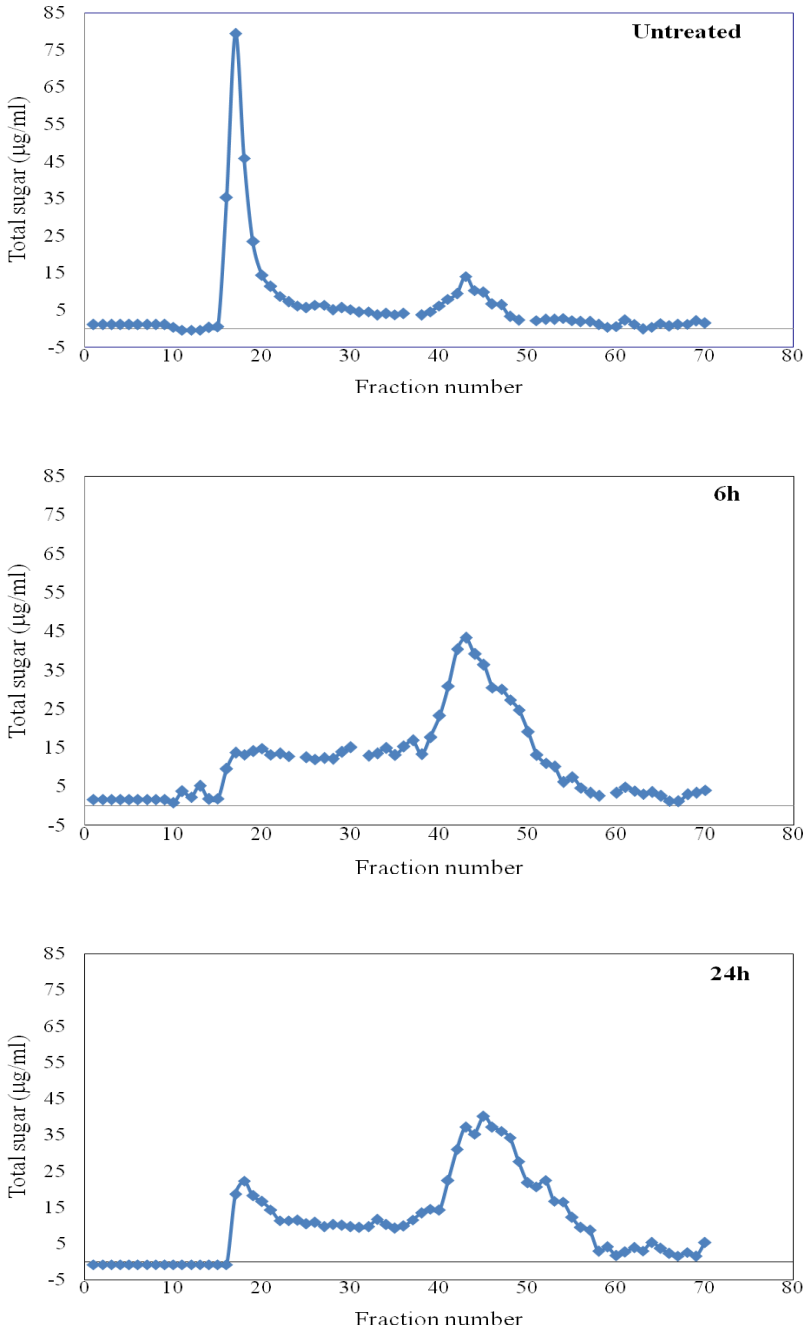


Figure 1. Gel-permeation profiles of SDF of untreated and modified CM (CM treated with 1% HCl for 6 and 24 h at 90°C) on Sepharose CL-6B column (1.0 cm i.d. x 30 cm); Fraction volume, 0.5 ml

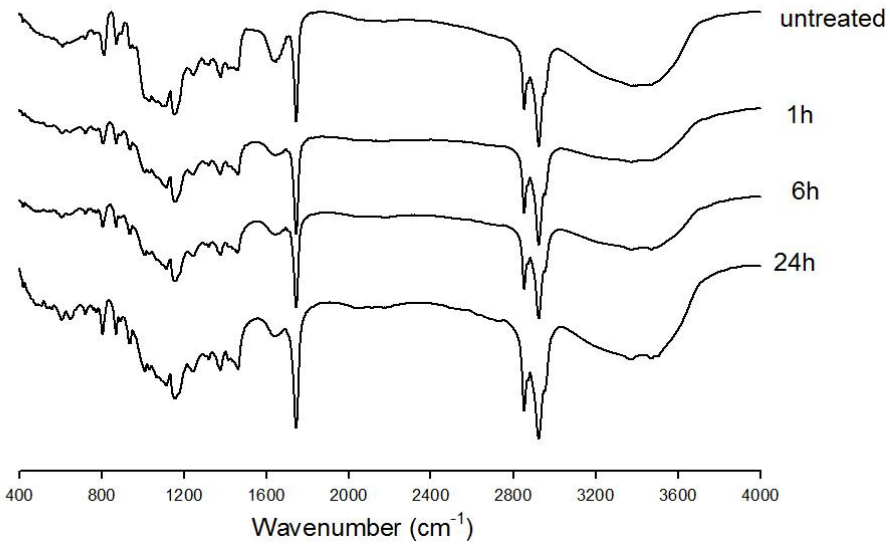


Figure 2. FT-IR spectra of copra meal treated with 0.5%HCL at 90^{O}C for 1, 6 and 24 h

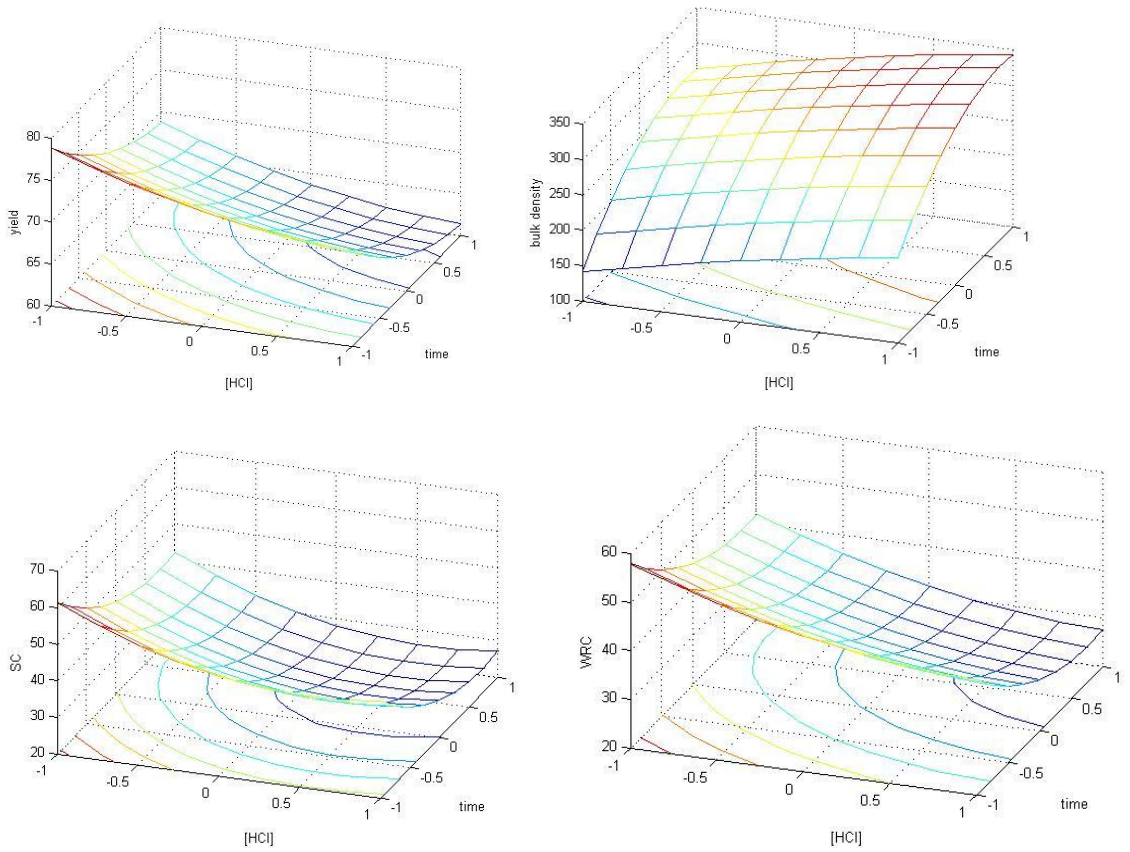


Figure 3. Effect of HCl content and hydrolysis time at constant hydrolysis temperature of 77^{o}C on yield, BD, SC and WRC of modified CM

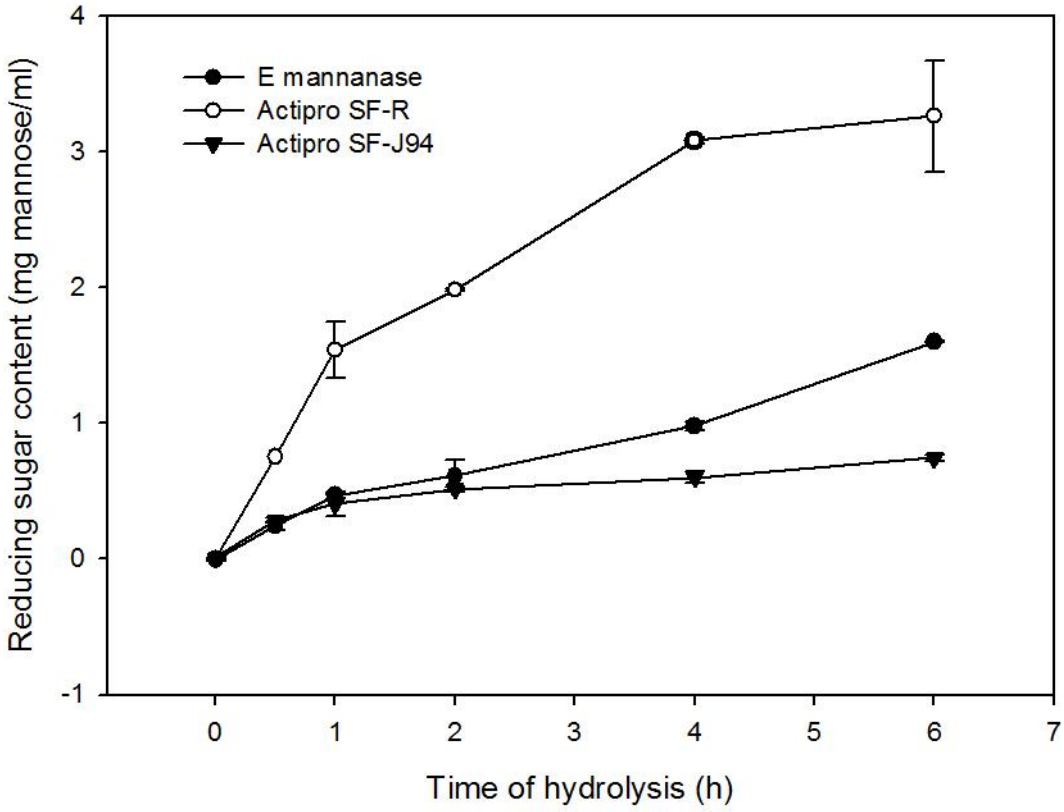


Figure 4. Hydrolysis curves of β-mannanases under the condition of S= 3% (w/v), E/S=64.97U/g at 60 °C and pH 5.0. The values were average of two replicates.

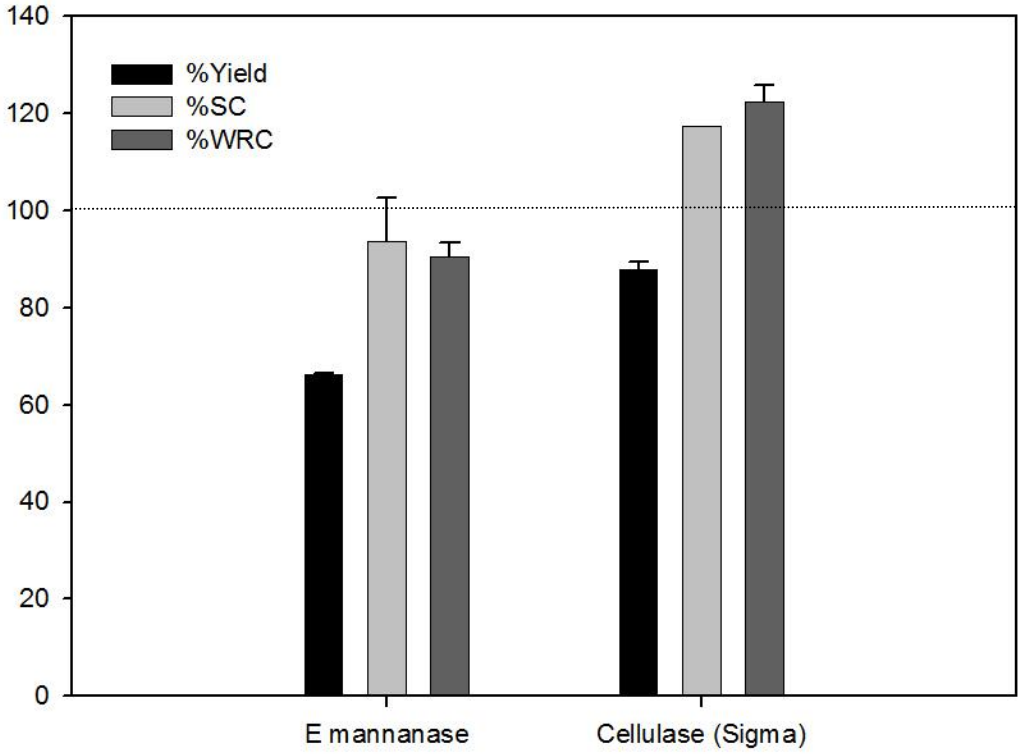
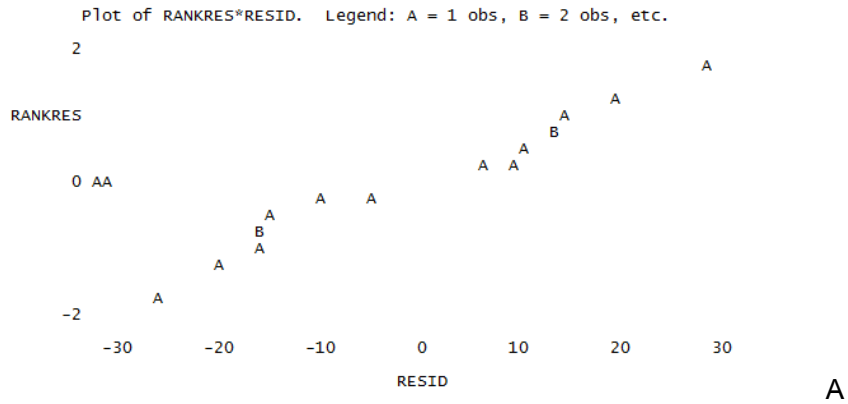
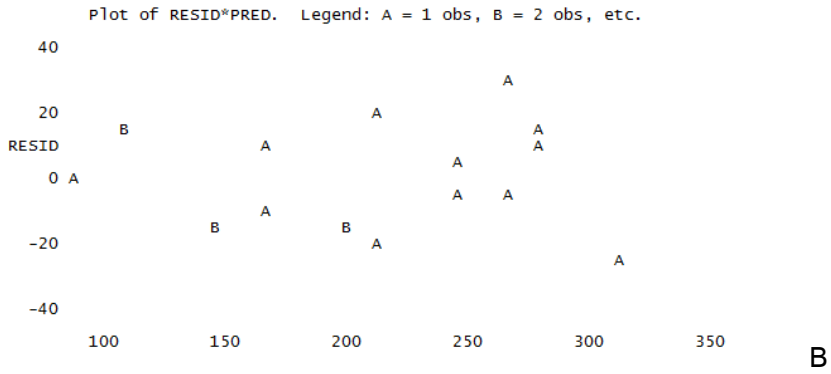


Figure 5. Changes in yield, SC and WRC of the treated CM (50ºC and pH 4.8 for 6 h) using E mannanase and cellulase compared to the non-hydrolyzed CM (100%). Endoglucanase activities in the reaction mixture were 479,084.23 unit for cellulase and 114,499.87 unit for E mannanase. The values were average of two replicates. The (‥‥) on the figure indicated the values of the non-hydrolyzed CM.



A



B

Figure 6. Examples of residual analysis

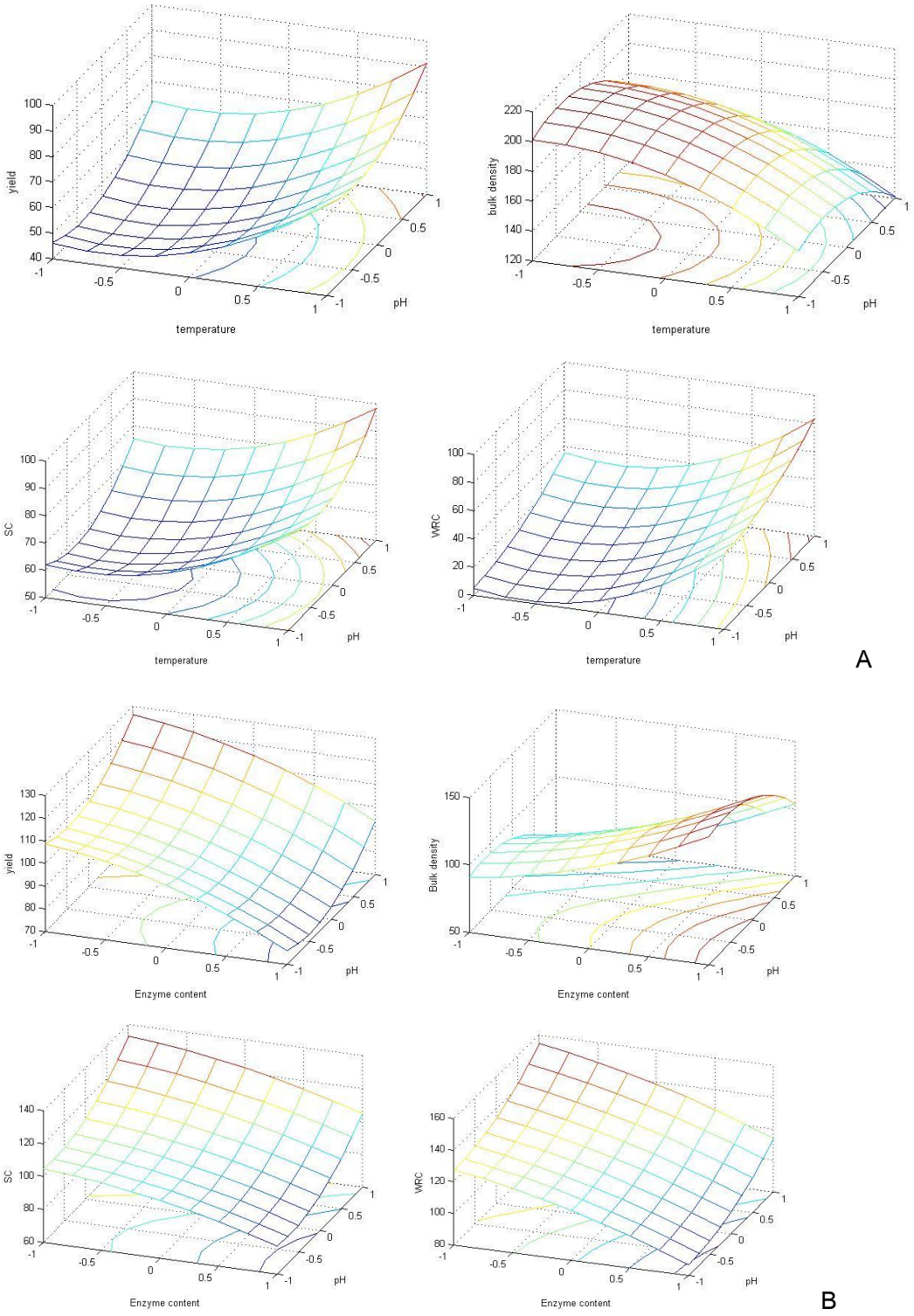
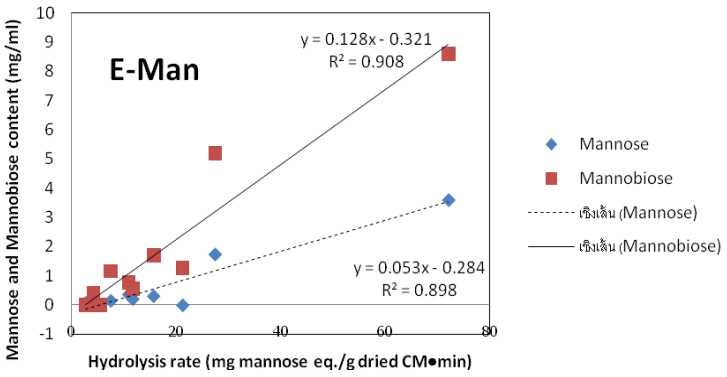
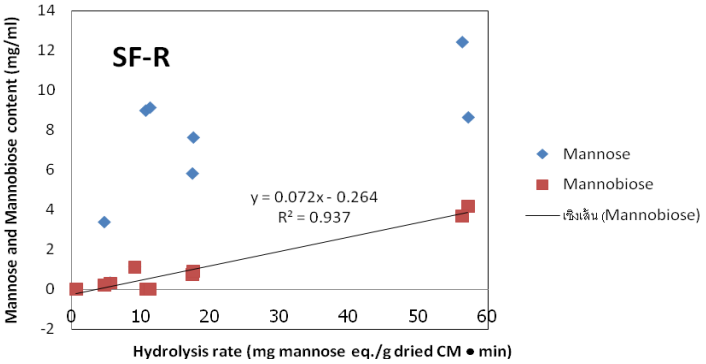


Figure 7. Effects of temperature and pH on hydrolysis yield and hydration properties of modified CM at constant level of E mannanase (A) and effects of E mannanase content and pH on hydrolysis yield and hydration properties of modified CM at constant temperature (B)



A



B

Figure 8. Relationships between hydrolysis rate and mannose and mannobiose liberated during hydrolysis of CM using E-Man and SF-R

Chapter 5 References

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Output of the project

1. Tipawan Thongsook and Sirilux Chaijamrus. Modification of physiochemical properties of copra meal by dilute acid hydrolysis. Submitted to International Journal of Food Science and Technology.
2. Tipawan Thongsook and Sirilux Chaijamrus. Enhancing the functionality of food fibers from copra meal by enzymatic modification. Submitted to Food and Bioproducts Processing.