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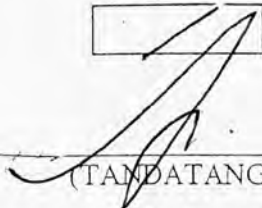
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**A PRELIMINARY STUDY ON THE FORMATION OF
CITRATE RESISTANT SAGO STARCH**

CHOI YIK HUEY

PERPUSTAKAAN
UNIVERSITI MALAYSIA SABAH

**THESIS SUBMITTED IN PARTIAL FULFILLMENT
FOR DEGREE OF BACHELOR OF FOOD SCIENCE
WITH HONOURS IN
FOOD TECHNOLOGY AND BIOPROCESS**

**SCHOOL OF FOOD SCIENCE AND NUTRITION
UNIVERSITI MALAYSIA SABAH
2009**

DECLARATION

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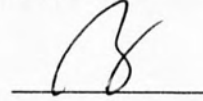


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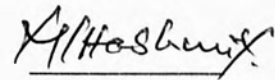
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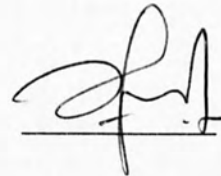
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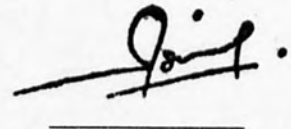
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ABSTRAK

KAJIAN ASAS DALAM PEMBENTUKAN KANJI SAGU RINTANG SITRAT

Kesan keadaan tindakbalas terhadap pembentukan kanji rintang (RS) dan kestabilan pendidihannya dikaji dengan menggunakan Rekabentuk Komposit Tengah (CCD) yang merangkumi tiga-faktor dan tiga-peringkat. RS telah disediakan melalui modifikasi asid sitrik pada suhu tindakbalas (100, 125 and 150°C), kandungan kanji (1.0, 1.5 and 2.0 g/ml asid sitrik, berat kering asas kanji) dan suhu tindakbalas (3, 6 and 9 jam). Sifat fiziko-kimia kanji sitrat diukur secara kualitatif dan kuantitatif. Dalam kajian ini, suhu tindakbalas menunjukkan impak kuat ke atas pembentukan RS sebelum dan selepas pendidihan. Kandungan RS tertinggi (38.1%) dicapai di bawah keadaan 2.0 g kanji sagu/ml asid sitrik, pada 150°C selepas 9 jam. Secara amnya, RS terbentuk adalah tidak stabil terhadap pendidihan akibat kekurangan paut-silang. Peningkatan penghadaman in vitro yang seiring dengan RS menunjukkan tanda hidrolisis separa. Kejadian esterifikasi di antara asid sitrik dan kanji sagu disahkan oleh kehadiran puncak karbonil pada 1730 cm⁻¹ dalam spektra FT-IR. Namun, keputusan dari darjah penggantian dan keterlarutan dalam dimetilsulfoksida gagal untuk mencirikan paut-silang yang terbentuk di antara paras RS yang berbeza. Kanji sitrat dengan RS pertengahan menunjukkan sifat-sifat pempesan dan terma yang lebih rendah berbanding sampel dengan RS rendah. Manakala, tiada profil pempesan, endoterma dan pembentukan ion kompleks-iodin yang jelas diperhatikan bagi kanji sitrat dengan RS tinggi. Memandangkan pembentukan RS dalam kajian ini agak rendah, kajian lanjut untuk meningkatkan kandungan RS adalah diperlukan.

ABSTRACT

The effect of reaction conditions on the formation of resistant starch (RS) and its boiling stability was investigated using three-factor, three-level Central Composite Design (CCD). RS was prepared by citric acid modification at reaction temperature (100, 125 and 150°C), starch content (1.0, 1.5 and 2.0 g/ml citric acid, dry starch basis) and reaction time (3, 6 and 9 hr). The physicochemical properties of citrate starches were measured qualitative and quantitatively. In this study, reaction temperature displayed pronounced impact on formation of RS before and after boiling. Highest RS content (38.1%) was attained under conditions of 2.0 g sago starch/ml citric acid, at 150°C for 9 hr. Generally, RS formed was not boiling-stable due to insufficient cross-linking. Increase in *in vitro* digestibility with RS showed signs of partial hydrolysis. Occurrence of esterification between citric acid and sago starch was confirmed by the presence of carbonyl peak at 1730 cm⁻¹ in the FT-IR spectra. However, results from degree of substitution and solubility in dimethylsulfoxide were unsuccessful in characterizing cross-linking formed among different RS levels. Compared to low RS, citrate starch with intermediate RS exhibited lower pasting and thermal properties. Meanwhile, no pasting profile, endotherm peak and formation of iodine-complex ion were observed for citrate starches with high RS. Since RS formation found in this study was considerably low, further research to increase RS content is needed.

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LIST OF ABBREVIATIONS

Cu_2SO_4	-	Copper (II) sulphate
CL	-	Cross-linking
DP	-	Degree of Polymerization
DMSO	-	Dimethylsulfoxide
DNS	-	3,5-dinitrosalicylic acid
DS	-	Degree of substitution
GLP-1	-	Glucagons-like peptide
GPC	-	Gel permeation chromatography
LCDA	-	Land Custody and Development Authority
LCL	-	Lower cross-linking
LS	-	Lower substitution
PYY	-	Pancreatic peptide tyrosine tyrosine
HCl	-	Hydrochloric acid
HCL	-	Higher cross-linking
HP	-	Hydroxypropylation
HS	-	Higher substitution
KH_2PO_4	-	Monopotassium phosphate
KI	-	Potassium iodine
MCL	-	Middle cross-linking
Na_2HPO_4	-	Dibasic sodium orthophosphate
NaOH	-	Sodium hydroxide
OSA	-	Octenyl succinic anhydride
POCl_3	-	Phosphoryl chloride
RSM	-	Response Surface Methodology

RS	-	Resistant Starch
RS1	-	RS type 1
RS2	-	RS type 2
RS3	-	RS type 3
RS4	-	RS type 4
sb	-	Starch basis
SCFA	-	Short fatty acid chain
STMP	-	Sodium trimetaphosphate
STPP	-	Sodium tripolyphosphate
T _m	-	Melting temperature
T _g	-	Glass transition temperature
T _o	-	Onset temperature
T _p	-	Peak temperature
T _c	-	Complete temperature
v/v	-	Volume per volume
w/w	-	Weight to weight
wwb	-	Wet weight basis

LIST OF SYMBOLS

\pm	-	Plus minus
$^{\circ}\text{C}$	-	Degree Celcius
%	-	Percentage
>	-	More than
α	-	Alpha
cm	-	Centimeter
G	-	Gram
J/g	-	Joule per gram
ΔH	-	Enthalpy
hr	-	Hour
mg	-	Milligram
min	-	Minute
mg/ml	-	Milligram per milliliter
mm	-	Millimeter
ml	-	Milliliter
M	-	Molarity
N	-	Normality
nm	-	Nanometer
rpm	-	Rate per minute
sb	-	Starch basis
U/g	-	Units per gram
U/ml	-	Units per milliliter
μm	-	Micrometer

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CHAPTER 1

INTRODUCTION

1.1 Introduction

According to Englyst & Hudson (1996), starch is classified nutritionally as rapid digestible (RDS), slow digestible (SDS) and resistant starch (RS). As compared to the other two dietary classes, RS is assumed to resist digestion and enter the colon. Thus, RS has been referred to as the sum of starch and starch degradation products not absorbed in the small intestine of healthy individuals (Englyst *et al.*, 1996a; Englyst & Hudson, 1996b). In account to its digestibility, RS had been highly linked to many physiological implications such as colonic fermentation (Heningsson *et al.*, 2003) and postprandial glycemia (Mitra *et al.*, 2007). Consequently, RS contained foods may perhaps be the potential candidate for reducing prevalence of various diet-related diseases, like diabetes and obesity (Chun-Kuang *et al.*, 2007), with the highest rate in Asia region, where estimated population of diabetic individuals reaching 380 million by 2025 (Gill, 2007).

Based on the starch's nature and its environment in food, RS is further characterized into four major types i.e. RS type 1 (RS1); physical inaccessible starch, RS type 2 (RS2); native resistant starch, RS type 3 (RS3); retrograded starch (Asp, 1996) and RS type 4 (RS4); chemical modified starch derivatives like acetylated and phosphorylated starches. Since RS1 and RS2 are native starches, their potential RS content will be lost subsequent to gelatinization, which is a disruption of granular structure by heating with excess of water during food processing. RS3, arising after hydrothermal treatment is thermally stable when heated above 100°C as retrograded starch melts at 155°C (Keren *et al.*, 2003). On the other hand, RS4, produced by cross-linking, is also resistance towards enzymatic digestion (Eerlingen & Delcour, 1995) and low pH (Sae-Hun & Malshick, 2006) through supplementary action of hydrogen bonds

with chemical covalent bonds that act as bridges between starch molecules (Tharanathan, 2005).

In mild acid hydrolysis with common acids like hydrochloric acid below gelatinization temperature, amorphous regions are hydrolysed prior to the crystalline ones (Jakakody & Hoover, 2002) to yield short linear chains, which in turn participate in the formation of enzyme resistant crystalline structure (Eerlingen & Delcour, 1995). Yet in reality, correlation between resistant starch and lintnerisation (where starch is reacted with 2.2 M hydrochloric acid at a temperature of 30 to 40°C) can hardly be deduced from earlier researches. This is because though extensive lintnerisation can increase amylose leaching and solubility, which is widely believed to reflect higher interaction between amylose–amylose chains (Jakakody & Hoover, 2002), relatively short amylose chain length with degree of polymerization of less than 22-65 glucose residues might not have dimensions to take part in crystallization process and resultant in poorer RS yield (Eerlingen & Delcour, 1995). The fallout of lintner starches from the aforesaid was further associated by the fact that prolonged hydrolysis up to 20 days will reduce the average DP chains to 10 ± 18 from 22 ± 30 (Jacobs *et al.*, 1998).

Hence, citric acid hydrolysis is deemed to have prospective outlook in production of cheap and yet tailored made digestion profiles. Apart from being rated nutritional harmless in contrast to hydrochloric acid, an inorganic acid, its wide usage in food processing, offers a new alternative to advancement of resistant starch (Wepner *et al.*, 1999).

While being a weak acid, citric acid can incites resistant starch formation by means of continuous duo-functionalities i.e. acid hydrolysis and cross-linking (Tharanathan, 2005). Via cross-linking, substitution of citric groups will reduce crystallites strength besides sterically hinder granule swelling, retrogradation, and enzyme-substrate complex configuration (Shin *et al.*, 2007). Thus, there is a wide disparity in the pathway and method for resistant starch formation between citric acid and conventional acids used. Moreover, limited information regarding resistant starch content and citric acid hydrolysis could also contribute to need of further clarification on

impact of different reaction time and temperatures on the hydrolysis and substitution rate.

Up till now, RS formation is mainly emphasized on the wheat, corn and potato starches and very few researches had been carried out on RS formation from local tropical starches like sago though sago is commercially established in South East Asia. In Malaysia itself, sago starch's current large-scale commercial plantation, developed by Sarawak's Land Custody and Development Authority (LCDA), is estimated to be over 28,000 hectares and its world-wide annual exportation to Singapore, Japan and other countries has reached about 25,000 to 40,000 (Sarawak State Government, 2007). Consequently, the overall intention of this study was to uncover the prospect of RS formation in sago using unconventional method i.e. citric acid modification. Therefore, the effects of starch amount, reaction temperature and time on formation of citrate resistant starch were examined by Response Surface Methodology (RSM). Further investigation was carried out to characterize the citrate starch.

1.2 Objectives

The main objectives of this research were to investigate:

1. The influence of reaction parameters (sago starch content, reaction temperature and time) and their interaction on the formation of citrate resistant sago starch.
2. The physicochemical characteristics of citrate sago starch produced.

CHAPTER 2

LITERATURE REVIEW

2.1 Resistant Starch

Up till now, relationship between resistant starch (RS) and dietary fiber had been clearly undermined within the food industry itself. The widespread of erroneous prevalence is due to the fact that resistant starch is classically defined as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals, similar to those of dietary fiber (Brouns *et al.*, 2002; Champ *et al.*, 2003). Further categorization of resistant starch into four main types (as shown in Table 2.1) adds to the complication.

Table 2.1: Classification of types of resistant starch (RS), food sources, and factors affecting their resistance to digestion in the colon

Types of RS	Description	Food sources	Resistance minimized by
RS1	Physically protected	Whole- or partly milled grains and seeds, legumes	Milling, chewing
RS2	Ungelatinized resistant granules with type B crystallinity, slowly hydrolyzed by α -amylase	Raw potatoes, green bananas, some legumes, high amylose corn	Food processing and cooking
RS3	Retrograded starch	Cooked and cooled potatoes, bread, cornflakes, food products with repeated moist heat treatment	Processing conditions
RS4	Chemical modified starches due to cross-linking with chemical reagents	Foods in which modified starches have been used (e.g. breads and cakes)	Less susceptible to digestibility in vitro

Source: Nugent, 2005

Such imposed diversities are caused by breadth and complexity associated starch structures, which form the resistant starch. As such, analytical quantification using both total dietary fiber (enzymic gravimetric) and Englyst method had led to dispute in accuracy of RS measurement and its characterization (Champ, 1996; Champ *et al.*, 2003; Haralampu, 2000).

2.1.1 Health and Non-Health Potential

Generally, RS appears to confer significant advantage to human digestive health. Research by Munster *et al.* (1994) imparted that reduction in risk of colon cancer had been linked to starch's resistance in colon. The rise in short fatty acid chain (SCFA) especially butyrate during colonic fermentation will in turn impede conversion of primary to secondary bile acids through decrease in cecal pH. Consequently, colonic carcinogenesis could be reduced with decrease in colonic mucosal proliferation (Scheppach *et al.*, 1991; Van Munster *et al.*, 1994). Chapman *et al.* (1994)'s study on the impact of butyrate, acetate and propionate on gut metabolism of 15 ulcerative colitis patients showed that sufficient and sustained level of SCFA may be essential for the maintenance of healthy gut. These patients found to have low levels of butyrate in gut and thus, have a low rate of butyrate oxidation by the mucosa (Brouns *et al.*, 2002).

The potential of RS as a prebiotic is supported by Queiroz-Monic *et al.* (2005) when animals fed with leguminous-containing diets indicated lower counts of Enterobacter and Bacteroides as compared to Bifidobacterium. Thus, RS is thought to decrease infectious intestinal diseases by suppressing putrefactive and pathogenic bacteria (Fooks *et al.*, 1999) yet increasing bifidogenic effects. Furthermore, RS can prevent and manage obesity by affecting energy density of diet. It is illustrated that rats fed with RS, have raised colon fermentation, intestinal expression of gut hormones, pancreatic peptide tyrosine tyrosine (PYY) and glucagons-like peptide (GLP-1) and lowered abdominal fat (Keenan *et al.*, 2006). Based on Behall *et al.* (2006)'s clinical study, consumption of high amylose corn starch lowered postprandial glucose and glycemic response in normal and obese adult women. However, positive impact of insulin response can only be detected with a minimum intake of 5 to 6 g of RS. Meanwhile, diets high in RS are reported to result in serum cholesterol and total lipids reduction when rats were adapted to potato resistant

starch. Other animal studies also depicted depression of lipo-protein fraction triacylglycerol and total cholesterol (Bobboi *et al.*, 2004).

Nevertheless, to be acknowledged by the food industry and consumer as a functional ingredient, RS also contributes to high quality of end products, apart from providing valuable physiological impacts. Physically, RS has particularly lower water holding capacity than traditional fiber. This is supported by Wepner *et al.* (1999) when moisture content of pasta dough was lowered as citrate resistant starch, which acted as a substitute for durum wheat, swollen to a lesser extent. As such, RS has the ability to form end product with improved texture, appearance and mouthfeel via better extrusion.

Moreover, RS can increase coating crispiness apart from reducing oil uptake. Sanz *et al.* (2008) replaced wheat flour with a commercial RS3 (Novelose 330) at 10, 20 and 30% in frying batter. The battered food was analyzed by 50 consumers and no significant difference was reported in "appearance", "flavour" and "overall acceptance". Nevertheless, the "crispiness", "fat" and "moisture" attributes were significantly different. Achievement of crispier fried batter has been linked to high gelatinization temperature and resistance towards granular disruption, attributed from high amylose content of RS. At the same time, RS is able to reinforce moisture content barrier against fat absorption and moisture loss, through thermogelling mechanism. Owing to its white colour, bland taste and fine particle structure, RS can simply be integrated into food formulations without texture and appearance changes (Murphy, 2001; Nugent, 2005).

2.1.2 Applications in Food Industry

The spearhead for resistant starch production are based on high amylose corn (Novelose 330, Actistar, CrystaLean). Currently, the commercial manufactured resistant starches can easily prevail over native sources of RS like banana, legumes and potatoes as the former are tolerant towards processing and storage conditions (Nugent, 2005). Whereas, the latter's low gelatinization temperature affects its RS stability. Example, marketed RS3 like Novelose 330, which contains 30% total dietary fiber, has a melting temperature of 121.5°C (Sajilata *et al.*, 2006).

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