EFFECT OF KAPPA-SEMI-REFINED CARRAGEENAN ON THE GELATINISATION AND RETROGRADATION OF CORN STARCH AND WHEAT FLOUR

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THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF BACHELOR OF FOOD SCIENCE WITH HONOURS (FOOD SCIENCE AND NUTRITION)

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

2012

PUMS 99:1

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS

JUDUL: EFFECT OF KAPPA-SEMI-REFINED CARRAGEENAN ON THE GELATINISATION AND RETROGRADATION OF CORN STARCH AND WHEAT FLOUR

IJAZAH: BACHELOR OF FOOD SCIENCE AND NUTRITION WITH HONOURS (FOOD SCIENCE AND NUTRITION

SESI PENGAJIAN: 2012

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ACKNOWLEDGEMENT

First and foremost, I would like to express my deepest gratitude and appreciation to my supervisor, Dr. Lee Jau-Shya, who has been very dedicated in guiding me throughout this past one year from the thesis proposal, in conducting laboratory work, and to the completion of this thesis.

Not forgetting, to my proposal and thesis examiners, Dr. Siew Chee Kiong, Dr. Afsaneh Farhadian, and Mr. Mohd Nazri bin Abdul Rahman, who have given me valuable opinions and suggestions in improving my thesis.

Grateful thanks are also forwarded to the laboratory assistants of the School of Food Science and Nutrition, particularly Mr. Wilter Kisin, Mr. Duasin Liman, Madam Doreen, and Madam Marni who have been very helpful in contributing to the completion of this thesis. Special thanks to Kok Heung, who have also been helpful in giving constructive advices on laboratory experiments.

I would also like to thank my friends and coursemates who have never hesitate to lend me a helping hand in many occassions when conducting my final year project, and also for their encouragements and moral support.

Last but not least, my thanks to my beloved family members who have continuously showered me with their love and support to the completion of this thesis.

Seah Jia Ling
20 May 2012

ABSTRACT

The effect of kappa-semi-refined carrageenan (SRC) with different concentration (0.1%, 0.5%, 1.0%, and 1.5%) on the gelatinisation and retrogradation of corn starches with different amylose content (waxy, normal, high amylose) and wheat flours with different protein content (low protein, medium protein, high protein) was determined. The analysis conducted included pasting properties, thermal behaviour, freeze-thaw stability and syneresis tests. Addition of SRC to corn starches of different amylose content affected the pasting properties of the corn starches differently. Addition of SRC significantly increased the pasting temperature of waxy corn starch but decreased the pasting temperature of normal corn starch ($p<0.05$). However, pasting temperature of high amylose corn starch was not altered with addition of SRC ($p>0.05$). Breakdown viscosities of waxy and normal corn starch were reduced with SRC addition ($p<0.05$), suggesting additional heat and shear resistance of these starches provided in the presence of SRC. For high amylose corn starch, addition of SRC decreased the peak and final viscosity ($p<0.05$). Only the setback viscosity of high amylose corn starch was found to decrease with increasing SRC concentration ($p<0.05$), while the setback viscosities of waxy and normal corn starch were not affected ($p>0.05$). Pasting temperature of wheat flours were not modified by SRC addition ($p>0.05$). However, addition of SRC increased the peak viscosity and reduced the setback viscosity of all wheat flours ($p<0.05$). Addition of SRC increased the gelatinisation end temperature and temperature range of waxy and normal corn starch, while significantly decrease the gelatinisation enthalpy of high amylose corn starch ($p<0.05$), indicating reduced water availability for starch gelatinisation in the presence of SRC. Addition of 1.5% and 0.1% SRC significantly increased the retrogradation of waxy and high amylose corn starch respectively ($p<0.05$). The gelatinisation properties of wheat flours were not affected by SRC ($p>0.05$). The effect of SRC on retrogradation of wheat flours measured by Differential Scanning Calorimeter displayed inconsistent results. However, addition of 0.1% SRC promoted the retrogradation of low protein wheat flour ($p<0.05$). Addition of SRC successfully decreased syneresis in freeze-thaw cycle and refrigeration ($4^{\circ}C$) for one week for both corn starches and wheat flours ($p<0.05$). Overall, the effect of SRC on gelatinisation and retrogradation of starch was found to be affected by the amylose content of corn starch. Nonetheless, these properties were less affected by the protein content of commercial wheat flour.

ABSTRAK

KESAN PENAMBAHAN KARAGINAN SEPARA TULEN KAPPA KE ATAS PENGELATINAN DAN RETROGRADASI KANJI JAGUNG DAN TEPUNG GANDUM

Kesan karaginan separa tulen kappa (KST) pada kepekatan berlainan (0.1%, 0.5%, 1.0%, dan 1.5%) terhadap pengelatinan dan retrogradasi kanji jagung dengan kandungan amilosa yang berbeza (waxy, biasa, amilosa tinggi) dan tepung gandum dengan kandungan protein yang berbeza (berprotein rendah, berprotein sederhana, berprotein tinggi) telah dikaji. Analisis yang dijalankan termasuk sifat pempesan, sifat terma, ujian kestabilan pembeku-cairan, dan ujian sineresis. Penambahan KST ke atas kanji jagung dengan kandungan amilosa yang berbeza memberi kesan yang berbeza terhadap sifat pempesan kanji jagung. Penambahan KST meningkatkan suhu pempesan kanji jagung waxy tetapi menurunkan suhu pempesan kanji jagung biasa ($p<0.05$). Namun, suhu pempesan kanji jagung amilosa tinggi tidak dipengaruhi oleh penambahan KST ($p>0.05$). Kelikatan breakdown kanji jagung waxy dan kanji jagung biasa didapati menurun apabila ditambah dengan KST ($p<0.05$), mencadangkan peningkatan rintangan kanji jagung waxy dan kanji jagung biasa terhadap haba dan ricihan dengan penambahan KST. Bagi kanji jagung amilosa tinggi, penambahan KST menurunkan kelikatan puncak dan kelikatan akhir ($p<0.05$). Hanya kelikatan setback kanji jagung amilosa tinggi berkurang dengan peningkatan kandungan KST ($p<0.05$), manakala kelikatan setback kanji jagung waxy dan kanji jagung biasa tidak dipengaruhi oleh KST ($p>0.05$). Suhu pempesan tepung gandum tidak diubah dengan penambahan KST ($p>0.05$). Namun, penambahan KST meningkatkan kelikatan puncak dan menurunkan kelikatan setback ke atas semua tepung gandum ($p<0.05$). Penambahan KST meningkatkan suhu pengelatinan akhir dan julat suhu pengelatinan kanji jagung waxy dan kanji jagung biasa, manakala menurunkan entalpi pengelatinan kanji jagung amilosa tinggi ($p<0.05$), menggambarkan KST mengurangkan ketersediaan air untuk pengelatinan kanji. Penambahan 1.5% dan 0.1% KST masing-masing didapati meningkatkan retrogradasi kanji jagung waxy dan kanji jagung amilosa tinggi ($p<0.05$). Sifat pengelatinan tepung gandum didapati tidak dipengaruhi oleh KST ($p>0.05$). Kesan KST terhadap retrogradasi tepung gandum dikaji dengan Kalorimeter Pengimbasan Perbezaan menunjukkan keputusan yang tidak konsisten. Namun, penambahan 0.1% KST menggalakkan retrogradasi tepung gandum berprotein rendah ($p<0.05$). Penambahan KST berjaya merencatkan sineresis semasa kitaran pembeku-cairan dan penyimpanan sejuk (4°C) selama seminggu untuk kedua-dua kanji jagung dan tepung gandum ($p<0.05$). Secara keseluruhan, kesan KST terhadap pengelatinan dan retrogradasi kanji dipengaruhi oleh kandungan amilosa dalam kanji jagung. Namun begitu, ciri-ciri ini kurang dipengaruhi oleh kandungan protein dalam tepung gandum komersil.

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AACC	American Association of Cereal Chemists
AGU	Anhydroglucose units
ANOVA	Analysis Of Varians
AOAC	Association of Analytical Communities
DMSO	Dimethyl sulfoxide
DP	Degree of polymerisation
DSC	Differential Scanning Calorimetry
e.g.	For example
FTC	Freeze-thaw cycle
k	kappa
NRS	Normal rice starch
rpm	Revolutions per minute
RVA	Rapid Visco Analyser
RVU	Rapid viscosity units
SD	Standard deviation
SOL	Solubilty index
SRC	Semi-refined kappa-carrageenan
TPA	Texture profile analysis

LIST OF SYMBOLS

%	percentage
$<$	less than
$\pm$	more or less than
Å	angstrom
ca.	circa
cm^{-1}	per centimetre
g	gram
G′	storage modulus
G″	loss modulus
J/g	Joule per gram
kDa	kiloDalton
mg	microgram
ml	millilitre
mm/min	millimetre per minute
nm	nanometre
^{o}C	degree Celsius
^{o}C/min	degree Celsius per minute
w/w	weight by weight
α	alpha
β	beta
δ	delta
ΔH_g	gelatinisation enthalpy
ΔH_r	retrogradation enthalpy
μm	micrometre

LIST OF APPENDIXES

CHAPTER 1

INTRODUCTION

1.1 Introduction

Starch is one of the most abundant storage polysaccharides in plants and at the same time the most important energy source for human. Starch is widely used in the food industry not only as a main ingredient of staple foods but also as a thickener, gelling agent and stabilizer (Imeson, 2010).

Starch consists of amylose and amylopectin molecules. Different starches of different amylose and amylopectin ratio have been used for different functions and have shown to exhibit different properties. Waxy starch (low amylose) develops higher viscosity and good clarity after cooking, besides having improved resistance to gel formation and syneresis during cold storage. Cooked paste of normal starch develops thick viscosity and translucent appearance but form opaque, resilient, short textured gel on cooling. On the other hand, high-amylose starch gels more rapidly to form stronger but more brittle gels (BeMiller and Whistler, 2009).

Starch is also a major component in wheat flour. There are different types of wheat flour as classified according to protein content and their application: (i) low protein content ideal for tender, fine crumb of crackers, cakes, and pastries (ii) medium protein content suitable for yeast bread (iii) high protein content for production of pasta (Brown, 2011). Differing protein content in wheat flour has shown to exhibit different retrogradation properties, with wheat flour of higher protein content showed lesser retrogradation (Salehifar, 2011).

Native starches are sensitive to shear and heat damage during processing (Appelqvist and Debet, 1997). After gelatinization, the starch gels formed are metastable in non-equilibrium states (Biliaderis and Zawistowski, 1990). Structure transformation (further chain aggregation, recrystallization) occurs during storage accompanied by gradual increases in rigidity and phase separation between polymer and solvent, causing syneresis, which is a negative and unwanted attribute of starch (Morris, 1990).

Function of starch has been shown to improve with chemical and physical modification (Jacobs and Delcour, 1998). Nowadays, the food industry tend to favour more natural food components, avoiding as much as possible the chemical treatments. Therefore, it is of interest to find new ways to improve the properties of native starches without using chemical modification. At present, many researchers are exploring the use of polysaccharides to improve the function of starch-based products (BeMiller, 2009).

Carrageenan, obtained from red seaweeds, is frequently used in combination with starch in various food products. There are three main types of carrageenan available commercially, that is kappa-carrageenan (k-carrageenan), iota-carrageenan (*i*-carrageenan), and lambda-carrageenan (λ-carrageenan) which differs in the proportion and location of ester sulphate groups and the proportion of 3,6-anhydrogalactose, and thus their chemical and physical properties. k-Carrageenan forms thermo-reversible, rigid, and brittle gel.

k-Carrageenan is obtained from *Kappaphycus alvarezii* (Imeson, 2010). Total production of *Kappaphycus* in Sabah at 2005 was 50,000 tonnes fresh weight (around 6,250 tonnes dry weight) (Neish, 2008). *K. alvarezii* is presently cultivated in Sabah for the extraction of semi-refined carrageenan (Phang, 2006). The monthly production of semi-refined from Semporna was around 60 to 100 tonnes dry weight per month (Phang, 1998).

Starch with the addition of k-carrageenan has shown to lower pasting temperature and increase peak viscosity when investigated using Rapid Visco Analyzer (RVA) (Funami *et al.*, 2005). k-Carrageenan also allows starch system to recover its preshear viscosity as investigated by Tye (1988) because addition of k-carrageenan restricted granule swelling, thus making starch granules less fragile. Therefore, k-carrageenan can be used to protect a starch system against shear degradation (Appleqvist *et al.*, 2006).

Addition of k-carrageenan was also found to decrease pasting temperature and peak viscosity as measured by RVA because its addition increases the capacity of the starch to swell. k-Carrageenan also decreased setback viscosity, therefore promoting delay in bread crumb firming. When measured with Brabender Amylograph, k-carrageenan exhibited the largest bump area as compared with using other hydrocolloids which is associated with softened effect of bread crumb. Therefore, addition of k-carrageenan wheat flour is able to produce the softest bread crumb, with retardation of setback, making it the best candidate as antistaling additive in bread formulation (Rojas *et al.,* 1999)

When k-carrageenan was added to waxy and low amylose rice starch with varied amylose content (0.9 – 11.9%) to investigate its effect on the textural properties of rice starch, interaction between amylose and k-carrageenan was observed resulting in different modification of textural properties for rice starch with different amylose content. Addition of k-carrageenan significantly increased the hardness and adhesiveness of the normal rice starch paste. On the other hand, addition of k-carrageenan to waxy rice starch resulted in a significant decrease in the hardness and but there was no significant effect on the adhesiveness of the paste. This could be because k-carrageenan could not form a gel network structure due to absence of amylose from waxy rice starch and thus had no effect on the texture of the mixed paste (Techawipharat *et al.*, 2008).

Most of the previous studies only focused on the effect of k-carrageenan on the properties of a single type of starch, such as the freeze-thaw stability of sweet potato starch (Lee *et al.*, 2002), rheological properties with normal corn starch (Tye, 1988) and potato starch (Tecante and Doublier, 2002), thermophysical and rheological properties of tapioca starch (Babić *et al.*, 2006), textural properties of rice starch (Huang *et al.*, 2007), and enzymatic digestibility of high amylose corn starch (Autio *et al.*, 2007). Only one study to date has investigated the effect of k-carrageenan on the different amylose content (Techawhipharat *et al.*, 2008). Even then, rice starch with a low range of amylose content (0.9 – 11.9%) was employed in the study and is less representative of the range of amylose content of starch products usually used in the food industry (0 – 70% amylose content).

For the effect of k-carrageenan on wheat flour, only one study to date has been performed by Rojas *et al.* (1999). It is well known that wheat flour of different protein content has been used for different applications in the food industry (Brown, 2011), and that wheat flour with different protein content exhibits different pasting (Singh *et al.*, 2011) and retrogradation properties (Salehifar, 2011). In addition to the research gaps mentioned above, the effect of semi-refined carrageenan, a food hydrocolloid derived from tropical seaweeds of Sabah, on starch and wheat flour has yet to be investigated by any researcher.

1.2 Objectives

The objective of this study is to determine the effect of semi-refined k-carrageenan on the gelatinization and retrogradation of corn starch with different amylose content and wheat flour with different protein content through investigating the following properties:

i. Pasting properties as determined by Rapid Visco Analyser (RVA),
ii. Thermal properties as determined by Differential Scaninng Calorimetry (DSC),
iii. Freeze-thaw stability, and
iv. Syneresis.

1.3 Importance of study

This study could disclose the interaction between semi-refined k-carrageeanan (SRC) and starch with different amylose content or wheat flour with different protein content. This information will be useful in knowing the potential application of SRC in a diverse range of starch-based foods with different amylose and protein content.

Besides that, semi-refined k-carrageenan is a natural food component obtained from the tropical seaweed, which is widely cultivated in Sabah. With the cheaper processing methods employed to produce semi-refined k-carrageenan, semi-refined k-carrageenan may serve as a cheaper and better alternative to improve functional properties of starch compared to chemical or physical modification of starch or using other hydrocolloids as the price of semi-refined carrageenan is lower.

CHAPTER 2

LITERATURE REVIEW

2.1 Starch

Starch occurs in the form of tiny white granules in various sites of plants, for example in cereal grains (maize, rice, wheat, barley, oat, sorghum), in roots (sweet potatoes, cassava, arrowroots, yam), in tubers (potatoes), in stems (sago palm), and in legume seeds (peas, beans) (Whistler and BeMiller, 2009). Starch consists of two major polymers, namely amylose and amylopectin. Waxy starches consist of mainly amylopectin and 0–8% amylose, normal starch consists of about 75% amylopectin and 25% amylose, and high amylose starches consist of 40–70% amylose. Amylose is present in an amorphous structure in the granule while amylopectin chains are primarily responsible for the crystallinity of starch. In addition to amylose and amylopectin, most cereal normal starches also contain lipids and phospholipids, which have profound impacts on the pasting property of the starch.

The amylose and amylopectin molecules, the granule structure in all shapes (spheres, ellipsoids, polygons, platelets, irregular tubules) and sizes (ranging from 0.1 to at least 200 μm), and the natures and amounts of the lipid and protein molecules present in granules vary with the botanical source of the starch, that is, they are unique to each type of starch. Native starch granules have a crystallinity varying from 15% to 45%, thus exhibiting a Maltese cross when observed under polarised light. In addition, native starch granules also yield x-ray diffraction patterns that can be classified into A-, B-, and C-type patterns. Most cereal starches yield A-type pattern, tuber starches and high-amylose cereal starches give B-type pattern, whereas legume starches give C-type pattern (Pérez *et al.*, 2009).

2.1.1 Amylose and Amylopectin

Amylose, a primarily linear polymeric molecule, consists of α 1-4 linked D-glucopyranos with a few branches. Molecular weight of amylose varies from ca. 500

anhydroglucose units (AGU) of high-amylose maize starch to more than 6000 AGU of potato starch. Amylose can have a high degree of polymerisation (DP) as high as 600 (Pérez *et al.*, 2009).

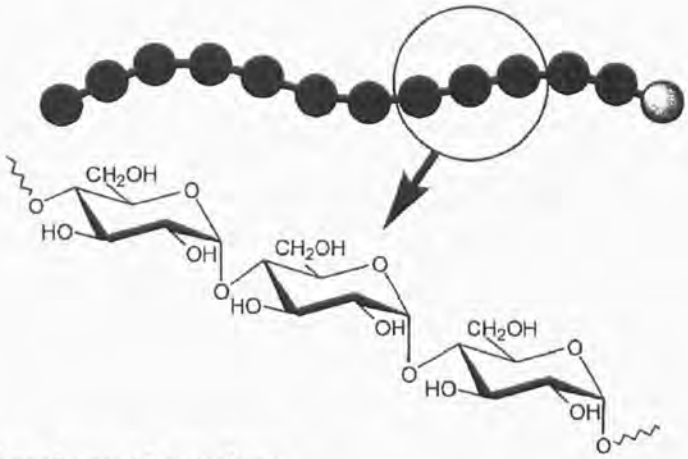

Figure 2.1 : Structure of amylose
Source : Knill and Kennedy (2004)

In a freshly prepared aqueous solution, amylose is present as a random coil conformation that is not stable. Amylose tends to form either single-helical (inclusion) complexes with suitable complexing agents (e.g. hydrocarbon chains of fatty acids) or form double helices among themselves when no suitable complexing agent is available. The transition from coil to a single or double helix is attributed to the structure of the (1→4) linked α-D-glucopyranosyl chains. With a complexing agent readily available in an aquoues solution, the hydrophobic side of the linear portion of starch molecule will face the cavity of the helix and interact with the non-polar moiety of the complexing agent, thus forming a single-helical complex.

Differential Scanning Calorimetry (DSC) thermograms of normal cereal starches (corn and wheat starch) reveal an additional thermal transition peak at a higher temperature than the starch gelatinisation peak, which is not found in waxy starches, attributed to the melting of the amylose-lipid complex. Amylose-lipid complex formation is instantaneous and reversible. The amylose-lipid complex is melted with an endothermic peak during heating to the melting temperature, but the complex is reformed during cooling, reflected by an exothermic peak at a similar temperature.

Without any complexing agent present, the linear portion of starch molecules will pair up to have their hydrophobic sides folded inside the double helix. The two chains in the double helix can be arranged in either parallel or antiparallel orientation. Double helices of amylose chains are packed in a hexagonal unit that consists of six double helices and an empty channel at the center of the packing unit and has ca. 36 water molecules in each unit. Formation of a double helix requires an alignment of two molecules and thus is a time-consuming process. Both single and double helices result in lower energy states and are thermodynamically favourable.

Amylose leaches easily from swollen granules at a temperature slightly above the gelatinisation temperature. The molecular weight of the extracted amylose increases with temperature. Amylose does not contribute to the total crystallinity of starch granules.

Normal corn starch containing amylose maintains granule integrity during cooking much better than waxy corn starch, suggesting that linear amylose molecules are intertwined with amylopectin to maintain the integrity of the granule when heated in water. Amylose is crosslinked onto amylopectin molecules in the granule, suggesting that amylose is located adjacent to or intertwined with amylopectin, but not in close proximity (4-7.5 Å) to other amylose molecules (Jane, 2009).

Amylopectin is a highly branched molecule, consisting of α 1-4 linked D-glucopyranose chains that are connected by α 1-6 branch linkages. Amylopectin has a very high molecular weight, varying between 10^5 to 10^7 AGU. Molecular weights of waxy starch amylopectin are higher than those of normal starch counterparts.

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