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Processing and quality of glutinous rice

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Abstract

The loss of stickiness of aged glutinous rice is one of the quality issues. This thesis examined the various factors that could affect the loss of stickiness of glutinous rice and potential pre-treatments and storage conditions that could be helpful to maintain the stickiness during storage. In this work, the effect of modified atmospheric packaging and various pre-processing treatments such as alkali washing, starch modification, and parboiling were assessed.

Initially, the effect of different rehydration temperatures (30 to 50ºC) and cooking times (2.7 to10.7 min) at 95ºC on the pasting properties of flour of three glutinous varieties (TDK11, TDK8, and Hom Mali Niaw) was investigated using the RVA. Increased soaking temperature and time resulted in reduced pasting temperature for all varieties. Extended holding at a cooking temperature (95ºC) had a more significant ($P<0.05$) effect on final gel viscosity.

Rice is usually consumed in the form of whole grains. Therefore, it is important to study the cooking kinetics of whole rice grain. To study the water uptake rate and cooking kinetics of TDK8 (fresh and aged) and TDK11, a novel *in situ* method using Thermal Mechanical Compression Test (TMCT) attached to a texture analyzer was developed. The TMCT cooking method was found valid for *in situ* analysis of rice cooking by using sample sizes as small as 0.50 g.

A new method (X-ray photoelectron spectroscopy) employed to quantify the surface composition of raw and cooked rice grains showed that the surface of uncooked grains of glutinous (TDK11) rice had a higher content of protein and lipids (49.7 and 36.2 %, respectively) to starch (13.4 %) compared the bulk composition (protein ~ 6.6 %, lipids ~ 0.8 %, and starch ~ 92.6 %). Protein was hypothesized to contribute to the loss of stickiness. An alkali washing with different concentrations of NaOH (0 to 0.2 %) of milled grains of TDK8 and Doongara resulted in a significant ($P<0.05$) increase in the stickiness and hardness of cooked rice grains, and final viscosity of rice flour, and a decrease in the amount of retrograded starch. In another study, the acetylation of starch in the whole grain of TDK8 and Doongara was achieved using various acetic anhydride concentrations (1-7 g per 100 g of milled grains in 225 mL of water). Results showed that acetylation reduced the crystallinity of starch with a significant ($P<0.05$) reduction in peak and final viscosities, and reduced thermal transition temperatures and enthalpy. Furthermore, the texture of cooked grains was softer and more adhesive. A significant reduction in the glycemic index (GI) of acetylated samples was also observed using the *in vitro* digestion method.

Parboiling of TDK8 and TDK11 was undertaken using various soaking mediums water (control), 3 % NaCl solution and 0.2 % acetic acid solution. These saline and acetic acid soakings improved the milling efficiency (39 to 41 % HRY for TDK8 and 48 to 53 % HRY for TDK11) when compared to the control (26 % HRY for TDK8 and 29 % HRY TDK11). Saline and acetic acid soaking resulted in reduced crystallinity and thermal endotherms. When compared to the control, saline soaking improved water absorption, resulting in a higher peak (~10 % increase) and final viscosity (~5 % increase), whereas, the acetic acid soaking restricted swelling, resulting in a reduced peak (~10 % decrease) and final viscosity (~15 % decrease). Furthermore, parboiling increased hardness (2.6 to 5 N for TDK8 and 2.3 to 3.5 N for TDK11) and adhesiveness (-0.2 to -0.5 N.s for TDK8 and -0.5 to -0.7 N.s for TDK11) of glutinous rice in saline and acetic acid soaking as compared to the control (-0.3 to -0.5 N.s for TDK8 and -0.6 to -0.7 N.s for TDK11). Also, parboiling improved the nutritional quality of glutinous rice by reducing the GI from 116.5 to 100.4 for TDK8 and 94.8 to 72.2 for TDK11. Overall, pre-process treatments of glutinous rice with alkali washing, acetylation of intact starch and parboiling showed improvement in the grain quality and which can have commercial potential.

The effect of modified atmospheric packaging (MAP) (control, vacuum, CO_2 and N_2) on the aging-induced changes in the physicochemical properties of TDK8 and TDK11 was also assessed. N_2 and CO_2 induced an increase in pasting temperature (72.6ºC ~ control, 73.7ºC ~ N_2 and 74.2ºC ~ CO_2) and a significant ($P<0.05$) reduction in final viscosity (2276 mPa-s ~ control, 2157 mPa-s ~ N_2 and 2216 mPa-s ~ CO_2) after 12 months of storage. The *in situ* TMCT cooking and texture analysis revealed that MAP slightly slowed the aging-induced changes in the cooking quality and stickiness of glutinous rice. Overall, among all the storage conditions used, the vacuum was considered the best to maintain the quality of the glutinous rice.

Declaration by author

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Publications during candidature

Peer-reviewed papers

Nawaz, MA, Fukai, S, Prakash, S & Bhandari, B 2018, 'Effect of soaking medium on the physicochemical properties of parboiled Laotian glutinous rice', *International Journal of Food Properties*, vol. 21, pp. 1896-1910.

Nawaz, MA, Fukai, S, Prakash, S & Bhandari, B 2018, 'Effect of starch modification in the whole white rice grains on physicochemical properties of two contrasting rice varieties', *Journal of Cereal Science*, vol. 80, pp. 143-149.

Nawaz, MA, Fukai, S, Prakash, S & Bhandari, B 2018, 'Effects of three types of modified atmospheric packaging on the physicochemical properties of selected glutinous rice', *Journal of Stored Products Research*, vol. 76, pp. 85-95.

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Research involving human and animal subjects

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Rice varieties used in the present study

Doongara (DG)

Hom Mali Niaw (HMN)

IR64

Thadokkham-8 (TDK8)

Thadokkham-11 (TDK11)

List of abbreviations used in the thesis

AACC	American Association for Cereal Chemists
AAC	Apparent amylose content
AC	Amylose content
approx.	Approximately
ACIAR	Australian Centre for International Agricultural Research
BD	Breakdown viscosity
CLSM	Confocal Laser Scanning Microscopy
df	Degree of freedom
DG	Doongara
DMSO	Dimethyl sulfoxide
DOM	Degree of milling
DSC	Differential Scanning Calorimetry
F´ 30s	1st derivative for every 30 sec
g	Gram/Grams
GI	Glycemic index
hr/hrs	hour/hours
Hg	Mercury
HMN	Hom Mali Niaw
Jg^{-1}	Joules per gram
kPa	Kilo Pascal
kV	Kilovolts
MAP	Modified Atmospheric Packaging
mg	Milligram/Milligrams
min	Minute/Minutes
mL	Milliliters
mm	Millimeters
mPa-s	Millipascal-second

N	Newton
n	Number of independent replicates
NAFRI	National Agriculture and Forestry Research Institute
Na_2CO_3	Sodium carbonate
NaOH	Sodium hydroxide
nm	Nanometers
N.s	Newton second
NSW DPI	New South Wales Department of Primary Industries
PB	Protein body
PBs	Protein bodies
pi	Point of inflection
PT	Peak time
P_{temp}	Pasting temperature
r	Correlation
R %	Percentage of retrogradation
RDS	Rapidly digestible starch
RH	Relative humidity
RRAPL	Rice Research Australia Pty Ltd
RVA	Rapid Visco Analyzer
SB	Setback viscosity
SD	Standard deviation
SE	Standard error
sec	Second/Seconds
SEM	Scanning Electron Microscopy
TDK8	Thadokkham-8
TDK11	Thadokkham-11
TMCT	Thermal Mechanical Compression Test
TN	Total nitrogen

TPA	Texture Profile Analysis
T_o	Onset temperature of gelatinization
T_p	Peak temperature of gelatinization
T_c	Conclusion temperature of gelatinization
$T_{o(r)}$	Onset temperature of retrogradation
$T_{p(r)}$	Peak temperature of retrogradation
$T_{c(r)}$	Conclusion temperature of retrogradation
V_f	Final viscosity
V_p	Peak viscosity
V_t	Trough viscosity
v/v	volume by volume
WBPR	weight of brown parboiled rice
WBR	weight of brown rice
WMPR	weight of milled parboiled rice
w/v	weight by volume
w/w	weight by weight
WWR	weight of white rice
XPS	X-ray Photoelectron Spectroscopy
ΔH	Enthalpy of starch gelatinization
$\Delta H_{(r)}$	Enthalpy of retrograded starch
µL	Microliter
µm	Micrometer

Chapter 1 General Introduction

1.1. Background

The humans have consumed rice for ages. There is evidence that the rice has been cultivated in China and Thailand dating from about 6000 BC (Zhang & Hung 2013). Rice (*Oryza sativa* L.) is one of the primary food crops in the world and the staple food for almost half of the world's population (Childs 2004). In general, rice is classified into two different types, namely glutinous and non-glutinous based on the native starch present in the endosperm. Glutinous rice primarily contains amylopectin, and non-glutinous rice contains amylose as well as amylopectin. Glutinous, waxy or sweet rice is characterized by its opaque appearance and very low amylose content (Wang & Wang 2002). The pasting and cooking properties of glutinous and non-glutinous rice are quite different.

Glutinous varieties are grown in many countries, including Lao PDR, Thailand, China, Myanmar, Vietnam, Cambodia, Japan, Bangladesh, and India (Calingacion et al. 2014; Mar et al. 2015). It is a staple food of Laotian people. It is usually consumed as a desert, as a breakfast cereal or as steamed rice in banana leaves in Thailand, Myanmar, Cambodia, India, China and Vietnam (Schiller et al. 2006). Glutinous rice possesses unique functional and processing properties mainly due to its distinct starch composition (Kim et al. 2013). The glutinous rice is consumed as milled raw rice (brown or polished). Milling efficiency and grain quality of rice can be improved by various preprocessing treatments such as post-harvest tempering and parboiling to reduce the internal fissures and increase head rice yield (Iguaz et al. 2006).

Usually, rice is harvested at 16-20 % (wet basis) moisture content, dried to 12-14 % moisture, milled and stored for one year or longer (Truong 2008). However, glutinous rice cannot be stored for a longer period, as longer storage deteriorates cooking time and the texture by making it harder and fluffier (Cheaupun et al. 2005). This is a key factor limiting the production efficiency of rice products such as rice tamale, rice pudding, and rice snacks from glutinous rice (Sung et al. 2008). Therefore, it is a challenge for rice processing industry to achieve the best quality aged glutinous rice having smooth and sticky texture after cooking.

Considerable studies have been conducted to investigate the mechanisms of aging (Perdon et al. 1997; Zhou et al. 2002a), storage temperature (Chung & Lim 2003), roles of protein and starch during aging (Teo et al. 2000). Most of these works have focused on non-waxy rice with very few studies on waxy rice (Sodhi et al. 2003). Majority of the reported studies on waxy rice elaborate

the physicochemical properties (Nicholas et al. 2013; Chun et al. 2015) of waxy rice, influence of amylopectin chain length (Mar et al. 2015), protein bodies composition (Kim et al. 2013) and, starch-protein interaction (Zhou et al. 2003a) on the textural attributes of fresh cooked rice. Very few researchers (Chrastil 1990b; Likitwattanasade & Hongsprabhas 2010; Huang & Lai 2014) have reported the aging-induced structural changes of macromolecules (starch, protein, and lipid) in waxy rice cultivars. During rice aging, the enzymatic activity of α- and β-amylase decrease; however, protease, lipase, and lipoxygenase become more active, increasing the amount of free fatty acids (FAs) and free amino acids (Dhaliwal et al. 1991). Because of this reason, the surface of polished aged rice was decreased in pH level (Rehman 2006). In another study, Martin & Fitzgerald (2002) hypothesised that the polymerisation of rice proteins caused by the disulphide bond formation during storage, resulting in the decreased protein solubility, increased slurry viscosity, and reduced cooked aged rice stickiness. Starch is usually considered as an inert macromolecule; thus the changes of starch properties are considered to be insignificant over the time during rice aging (Rehman 2006). However, Patindol et al. (2005) proposed enzymatic degradation of starch might occur during aging, which could cause an increase in the percentage of short chains (DP 6-12), resulting in harder texture while cooking.

The mechanism of harder texture formation during due to aging reduced water uptake and swelling ability of aged waxy rice and reduction in the stickiness of cooked waxy rice is still not very clear. The surface of the grains usually perceives the stickiness of cooked rice grains. Surface composition of rice can influence the textural attributes especially stickiness of the cooked glutinous rice. However, the surface composition of cooked rice has not been studied earlier. The effects of various storage conditions, potential pre-treatments to maintain the stickiness of waxy rice have not been sufficiently studied. Thus, the present study is mainly focused on the effect of various factors on the quality of glutinous rice. Effect of various pre-processing techniques such as surface protein washing by alkali treatment, acetylation of intact starch in the whole grain, and parboiling on the quality of glutinous rice have been investigated in this study. In addition to this, the effect of modified atmospheric packaging to slow down the aging-induced quality deterioration of aged glutinous rice is also included in the study.

1.2. Objectives

This project was aimed to investigate the mechanism of textural development and stickiness of the glutinous rice during cooking and examine the effect of pre-processing treatments and storage on the cooking quality of glutinous rice.

Following specific objectives were set to target above mentioned broad aim:

I. To investigate the effect of rehydration time and temperature and extended holding during cooking on the pasting properties of common Laotian glutinous rice cultivars.
II. To study the rehydration and cooking kinetics of glutinous rice by *in situ* Thermal Mechanical Compression Test (TMCT) cooking.
III. To study the surface composition of the raw and cooked glutinous rice grains as against the bulk composition.
IV. To study the effect of modified atmospheric packaging during aging on the physicochemical properties of glutinous rice.
V. To investigate the effect of the removal of protein bodies from the surface of milled glutinous rice by alkali washing on the stickiness of the cooked grains.
VI. To study the effect of starch modification by acetylation in the whole rice grains on the physicochemical properties of glutinous rice.
VII. To study the efficacy of soaking medium on the physicochemical properties of parboiled glutinous rice.

1.3. Hypothesis

Following specific hypothesis were tested in this project:

I. Different rehydration conditions (time and temperature) and cooking times have a distinct effect on the pasting properties of glutinous rice as a longer rehydration time at higher temperature, and extended cooking can result in more water absorption, breakdown, and reduced final viscosity.
II. The rate of cooking of glutinous and non-glutinous rice can be estimated by grain softening using the novel *in situ* TMCT device attached to a texture analyzer.
III. The aging can affect the rate of water uptake and cooking because of aging-induced physicochemical changes.

IV. The surface composition, in particular, protein of the grain may influence the stickiness of cooked glutinous rice. By washing the surface proteins and fat with dilute alkali can expose more starch on the surface may lead to making sample stickier during cooking.

V. Acetylation of starches in the grain can slow down the recrystallisation of gelatinised starch. Therefore, reduced retrogradation due to acetylation may help in maintaining the stickiness of glutinous rice.

VI. Thermal treatment such as wet parboiling of fresh glutinous paddy may help in maintaining to improve quality of parboiled glutinous rice by increasing head rice yield and cooking quality.

1.4. Expected outcomes and significance

The main expected outcome of this research project is to improve the knowledge of processing and to optimise the pre-process and storage treatments and influential factors on the stickiness property of cooked glutinous rice. Also, these findings could assist in understanding the role of various macromolecules (starch, protein, and fat) on the functional properties of glutinous rice. The overall outcome of the study is to help rice-producing countries in Asia to improve the quality of glutinous rice and also to enhance its export.

1.5. Outline of the dissertation

This thesis consists of 10 chapters, starting with a general introduction (Chapter 1), followed by a literature review (Chapter 2). The research undertaken in the project is described in 7 consecutive chapters (Chapter 3 to 9) in a format of journal manuscripts. Chapters 3 to 5 are based on the methodology development and effect of rehydration time and temperature and extended cooking on the pasting properties of glutinous rice flour. Chapters 6 to 8 are based on the study of the effects of preprocessing treatments on the physicochemical properties of glutinous rice. Chapter 9 is based on the study of the effects of modified atmospheric packaging on the physicochemical properties of glutinous rice. Chapter 10 provides the general conclusions and recommendations from the research.

Chapter 1 describes the background understanding relating to the aging-induced changes in the physicochemical properties of glutinous rice.

Chapter 2 presents a review of literature relating to the general classification of rice based on the starch type, physicochemical properties of glutinous rice flour and grains. Current literature on the aging-induced changes of glutinous rice is also reported. Moreover, the literature on the storage techniques and preprocess treatments of glutinous rice to slow down the aging-induced changes are also reviewed.

Chapter 3 presents the role of rehydration time and temperature in the gelatinization properties of glutinous rice flour. This chapter also deals with the effects of extended cooking time on the pasting profiles of glutinous rice flour.

Chapter 4 illustrates a new procedure of *in situ* TMCT cooking to study the rate of grain softening during soaking and cooking. This chapter also deals with the changes in the cooking kinetics due to the aging of glutinous rice.

Chapter 5 describes a new method of surface analysis of rice grains and flour of glutinous and non-glutinous varieties by using x-ray photoelectron spectroscopy (XPS).

Chapter 6 deals with preprocessing of alkali washing of milled glutinous rice to remove the alkali soluble protein from the surface glutinous rice to maintain the stickiness of cooked glutinous rice.

Chapter 7 presents the effect of acetylation pre-processing for starch modification in the whole polished grain of glutinous rice to maintain the stickiness of cooked glutinous rice. This chapter also deals with *in vitro* digestion of acetylated glutinous rice.

Chapter 8 presents the preprocessing of parboiling of selected glutinous rice cultivars using the various soaking medium. This chapter deals also with *in vitro* digestion of parboiled glutinous rice.

Chapter 9 deals with the efficacy of various modified atmospheric packaging techniques on the physiochemical properties of glutinous and non-glutinous rice. In this chapter, the novel methods of *in situ* TMCT cooking and surface analysis using XPS described in Chapters 4 and 5, respectively are also used on fresh and aged samples to study the changes in cooking kinetics and surface composition of fresh and aged samples and how the modified atmospheric packaging (MAP) can affect the said attributes.

Chapter 10 provides overall conclusions and recommendations for further studies in the future.

Chapter 2 Literature review

2.1. Introduction

Rice production is geographically concentrated in Eastern and Western Asia with more than 90 percent of world output (Prasad et al. 2017) but the recent increased interest and consumption of rice in the West, has prompted further research to discover the basis of the distinctive properties of different cultivars and associated technology (Flor et al. 2016). China and India, which account for more than one-third of the global population, supply over half of the world's rice (as shown in Fig. 2.1). Brazil is the biggest non-Asian producer, followed by the United States of America. Italy is the leading producer in Europe. World production has shown a significant and very stable growth, mainly due to increased production in Western and Eastern Asia (Muthayya et al. 2014).

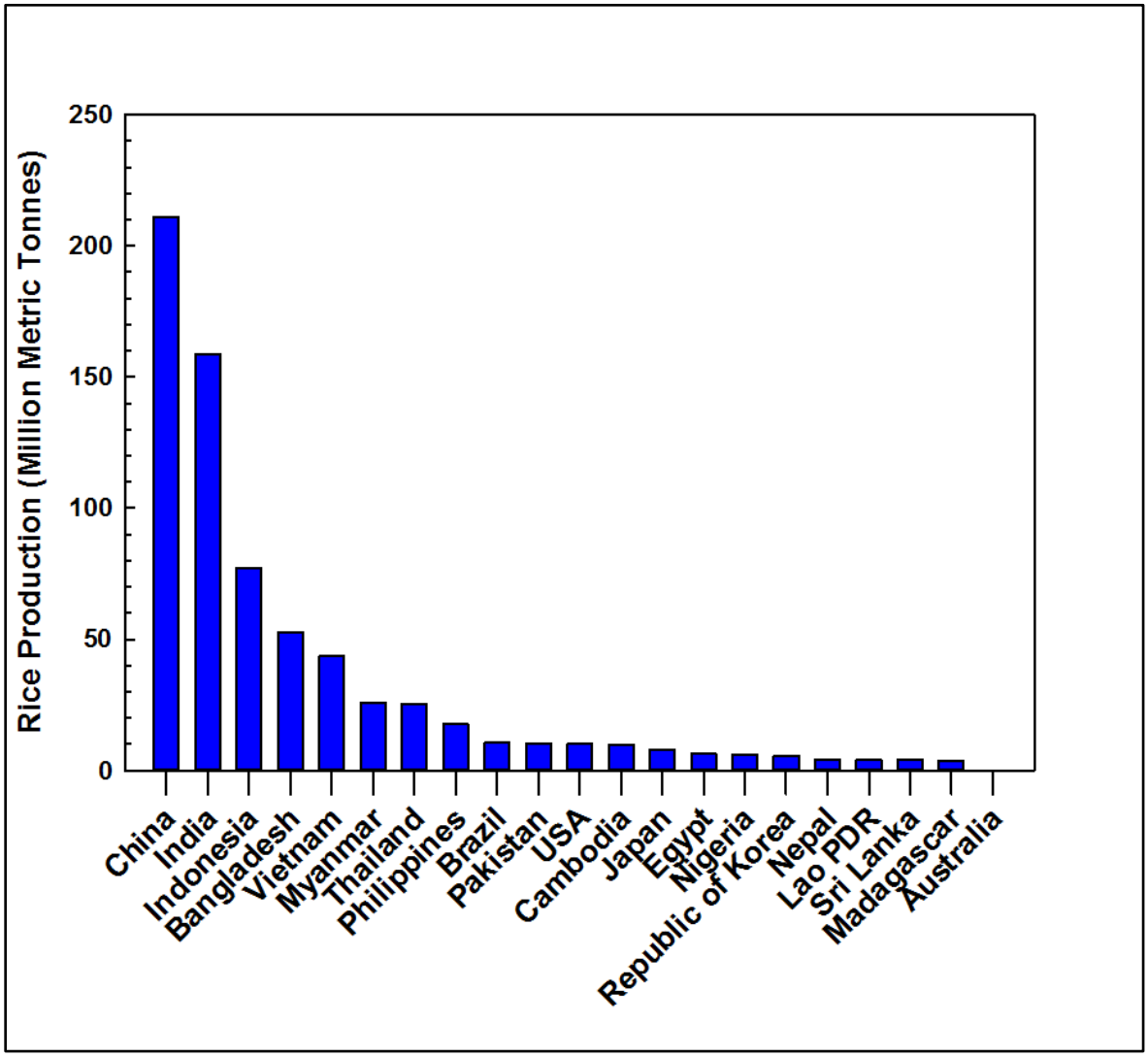

Figure 2.1 Rice production worldwide in the year 2015-16 (Food and Agriculture Organization 2018)

Rice is considered as a semiaquatic, annual grass plant and member of the grass family, *Poaceae*. This family is divided into some genera or subfamilies, one of which is *Oryzoideae*. This genus is further divided into some sections, one of which is *Sativae*. There is a further subdivision into a species, the most relevant of which are: *Oryza sativa* and *Oryza glaberrima* (Soreng et al. 2015). *O. sativa* is the world's most widespread species because it is used for human consumption. *O. glaberrima*, although used as human food, is grown only in Africa on a very small scale. The three most common subspecies of *O. sativa* are japonica, javanica, and indica (Wang et al. 2014). Japonica varieties are mainly found in Japan and Korea. Javanica varieties are commonly found in Indonesia and the Philippines. Indica varieties are the majority of rice grown all over Asia including India. Some indica varieties were also brought to America for large scale production (Awan et al. 2017).

2.2. Grain Structure

The anatomy of mature rough rice (complete grains with husks intact) consists of a brown kernel enclosed by the husk. The most visible part of a rough rice grain is the husk also known as the hull. The hull is the outer layer covering the caryopsis and, although inedible, it makes up about 20-25 % of the total grain weight (Ogawa et al. 2002). The hull serves as a protective barrier against infestation and environmental fluctuations as shown in Fig. 2.2. The hull is comprised of sterile lemmas, rachilla, palea, and lemma. The lemma covers two-thirds of the seed, with the edges of the palea fitting inside so that the two close tightly around the seed. The caryopsis contains the embryo and starchy endosperm, surrounded by the seed coat (tegumen) and the pericarp (Moldenhauer et al. 1998; Hoogenkamp et al. 2017).

The caryopsis consists of three fibrous bran tissues: pericarp, tegumen, and aleurone. Endosperm and embryo are also parts of the caryopsis. The bran portion accounts for one-tenth of the weight of the rough grain and has a high nutritional value because it contains proteins, lipids, and dietary fibres. The pericarp is made of thin bran layers of proteins. The tegumen consists of arrays of fatty materials. The aleurone surrounds the endosperm and the embryo. Its tissues are rich in protein and cellulose. The embryo is the reproductive organ of the grain and is very rich in protein and fat.

The endosperm, the largest component of the grain, is mainly composed of starch granules, with minute amounts of proteins, lipids, and water (Champagne et al. 2004).

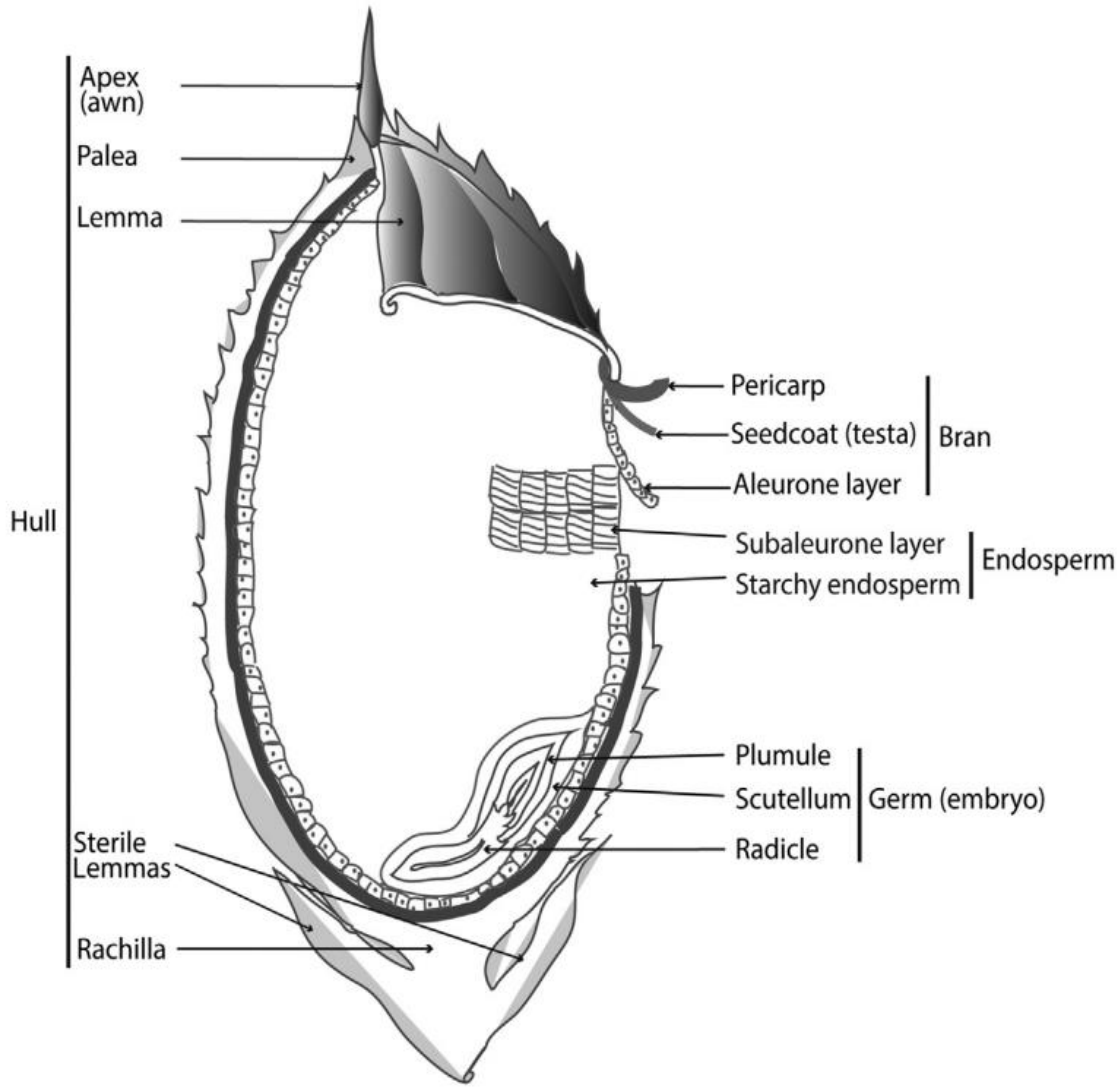

Figure 2.2 Cross section of a rice kernel (Hoogenkamp et al. 2017)

2.3. The general composition of rice grain

The chemical composition and properties of rice and its milling fractions are subject to the varietal, environmental, and processing variability (Bhattacharya 2017). A wide range of values is evident for all milling fractions (Bond 2004). As shown in Table 2.1, among the milling fractions of rice, the bran fraction has the highest energy and protein content and the hull fraction has the lowest (Champagne et al. 2004). Only the brown rice fraction is eatable. Abrasive or friction milling is

usually done to remove the pericarp, seed-coat, testa, aleurone layer and embryo to yield milled rice results in loss of fat, protein, crude and neutral dietary fibre, ash, thiamine, riboflavin, niacin and α-tocopherol (as shown in Table 2.2). Available carbohydrates, exclusively starch, are higher in milled rice than in brown rice (Table 2.1) (Belitz et al. 2009). The gradients for the various nutrients are not identical as evidenced by analysis of successive milling fractions of brown rice and milled rice. Dietary fibre is highest in the bran layer (and the hull) and lowest in milled rice. Density and bulk density are lowest in the hull, followed by the bran, and highest in milled rice because of the low oil content (Champagne et al. 2004).

Table 2.1 Range of mean proximate analysis and content (%) of organic fractions of rough rice and its milling fractions at 14 % moisture (Champagne et al. 2004)

Nutrient	**Rough Rice**	**Brown Rice**	**Milled Rice**	**Hull**	**Bran**	**Embryo**	**Polish**
Protein (N x 5.95)	5.8-7.7	4.3-18.2	4.5-10.5	2.0-2.8	11.3-14.9	14.1-20.6	11.2-12.4
Crude Fat	1.5-2.3	1.6-2.8	0.3-0.5	0.3-0.8	15.0-19.7	16.6-20.5	10.1-12.4
Crude Fibre	7.2-10.4	0.6-1.0	0.2-0.5	34.5-45.9	7.0-11.4	2.4-3.5	2.3-3.2
Crude Ash	2.9-5.2	1.0-1.5	0.3-0.8	13.2-21.0	6.6-9.9	4.8-8.7	5.2-7.3
Available Carbohydrates	64-73	73-87	77-89	22-34	34-62	34-41	51-55
Starch	53.4	66.4	77.6	1.5	13.8	2.1	41.5-47.6
Neutral Detergent Fibre	16.4-19.2	2.9-3.9	0.7-2.3	65.5-74.0	23.7-28.6	13.1	-
Pentosans	3.7-5.3	1.2-2.1	0.5-1.4	17.7-18.4	7.0-8.3	7.9-6.4	3.6-4.7
Hemicelluloses	-	-	0.1	2.9-11.8	9.5-16.9	9.7	-
Cellulose	-	-	-	31.4-36.3	5.9-9.0	2.7	-
1,3:1,4 β-glucans	-	0.11	0.11	-	-	-	-
Polyuronic Acid	0.6	-	-	-	1.2	0.4	-
Free Sugars	0.5-1.2	0.7-1.3	0.22-0.45	0.6	5.5-6.9	8.0-12	-
Lignin	3.4	-	0.1	9.5-18.4	2.8-3.9	0.7-4.1	2.8
Energy (kJ/g)	15.8	15.2-16.1	14.6-15.6	11.1-13.9	16.7-19.9	-	17.9

Table 2.2 Vitamin and mineral content of rough rice and its milling fractions at 14 % moisture (Champagne et al. 2004)

Rice Fraction	Thiamine (mg)	Riboflavin (mg)	Niacin (mg)	α - Tocopherol (mg)	Calcium (mg)	Phosphorus (g)	Phytin P (g)	Iron (mg)	Zinc (mg)
Rough rice	0.26-0.33	0.06-0.11	2.9-5.6	0.90-2.00	10-80	0.17-0.39	0.18-0.21	1.4-6.0	1.7-3.1
Brown rice	0.29-0.61	0.04-0.14	3.5-5.3	0.90-2.50	10-50	0.17-0.43	0.13-0.27	0.2-5.2	0.6-2.8
Milled rice	0.02-0.11	0.02-0.06	1.3-2.4	75-0.30	10-30	0.08-0.15	0.02-0.07	0.2-2.8	0.6-2.3
Rice bran	1.20-2.40	0.18-0.43	26.7-49.9	2.60-13.3	30-120	1.1-2.5	0.9-2.2	8.6-43.0	4.3-25.8
Rice hull	0.09-0.21	0.05-0.07	1.6-4.2	0	60-130	0.03-0.07	0	3.9-9.5	0.9-4.0

2.3.1. Starch

Starch is the primary constituent of milled rice at about 90 % of the dry matter. Starch is a polymer of D-glucose linked α-(1-4) and usually consists of an essentially linear fraction, amylose, and a branched fraction, amylopectin. Various physicochemical properties of starch fractions are shown in Table 2.3. Branch points are α-(1-6) linkages. Innovative techniques have now shown rice amylose to have two to four chains with a number-average degree of polymerisation (DP_n) of 900 to glucose units and a ß-amylolysis limit of 73-87 % (Belitz et al. 2009). It is a combination of branched and linear molecules with DP_n of 1100 to 1700 and 700 to 900, respectively. The branched fraction constitutes 25-50 % by number and 30-60 % by weight of amylose. The iodine affinity of rice amyloses is 20-21 % by weight.

Rice amylopectin has ß-amylolysis limits of 56-59 %, chain lengths of 19 to 22 glucose units, DP_n of 5,000 to 15,000 glucose units and 220 to 700 chains per molecule (Belitz et al. 2009). The iodine affinity of rice amylopectin is 0.4-0.9 % in low- and intermediate-amylose rice but 2-3 % in high-amylose rice. Isoamylase-debranched amylopectin showed longest chain fractions ($DP_n > 100$) (9-14 %) in high-amylose samples with higher iodine affinity than in low and intermediate-amylose samples (2-5 %) and waxy rice amylopectin (0 %), (Champagne et al. 2004).

Based on colorimetric starch-iodine color absorption standards at 590 to 620 nm, milled rice is classified as waxy (1-2 %), very low amylose (2-12 %), low amylose (12-20 %), intermediate (20-25 %) and high (25-33 %), (Juliano & Bechtel 1985; Gayin et al. 2015). Recent studies showed that the maximum true amylose content is 20 % and that additional iodine binding is due to the long linear chains in amylopectin (Champagne et al. 2004). Hence colorimetric amylose values are now termed "apparent amylose content" (Delwiche et al. 1995).

Table 2.3 Properties of starch fractions in rice endosperm (Zobel 1988)

Property	**Amylose**	**Amylopectin**
Molecular Structure	Linear (α-1-4)	Branched (α-1-4; α-1-6)
Dilute Solutions	Unstable	Stable
Gels	Stiff, irreversible	Soft, reversible
Films	Coherent	-
Complex Formation	Favourable	Unfavourable
Iodine Color	Blue	Red-Purple
Digestibility, β-Amylase	100 %	60 %
Degree of Polymerization	1500-6000	$3 \text{ X } 10^5 – 3 \text{ X } 10^6$

The waxy endosperm is opaque and shows air spaces between the starch granules, which result in lower density than the non-waxy endosperm. The structure of the starch granule is still not well understood, but crystallinity and staling are attributed to the amylopectin fraction (Champagne et al. 2004). It is broadly accepted that amylopectin molecules are composed of short-amylose-chains consisting of 6~100 glucosyl residues, and it is very difficult to elucidate the fine structure assembled these chains because of high molecular weight (Hisamatsu et al. 1996).

2.3.2. Protein

Protein plays an important role in determining the physicochemical properties of rice (Cornejo & Rosell 2015). As compared to the majority of cereals, the protein content of rice is typically in the lowest range. It also has low fibre and lipid contents (Table 2.4). However, the net protein utilisation and digestible energy in rice are the highest among the other cereal grains (Hoogenkamp et al. 2017).

Table 2.4 Composition and energy balance data of selected whole-grain cereals (Nadathur et al. 2017)

Property	**Brown rice**	**Wheat**	**Corn**	**Barley**	**Millet**	**Sorghum**	**Rye**	**Oat**
Protein (N x 6.25) (%)	7.3	10.6	9.8	11.0	11.5	8.3	8.7	9.3
Fibre (%)	0.8	1.0	2.0	3.7	1.5	4.1	2.2	5.6
Net protein utilization (%)	73.8	53.0	58.0	62.0	56.0	50.0	59.0	59.1
Digestible energy (kJ $(100 \text{ g})^{-1}$)	1550	1360	1450	1320	1440	1290	1330	1160

Protein contents range from 6.6 to 7.3 % for brown rice (Basak et al. 2002; Hoogenkamp et al. 2017), 6.2 to 6.9 % for milled rice (Singh et al. 1998), and 8.2 to 8.4 % for milled basmati rice (Deka et al. 2000). Some wild varieties of China and North America have high protein contents. Zhai et al. (2001) figured out that protein content in such varieties could be as high as 12.0 to 15.0 %. The protein (and fat) contents reduced linearly with increase in the degree of polish, as these constituents were mainly concentrated in the peripheral layers of the kernel (Pal et al. 1999).

Endosperm (milled rice) protein comprises of several fractions comprising of 15.0 % albumin (water soluble) plus globulin (salt soluble), 5.0 to 8.0 % prolamin (alcohol soluble) and the rest glutelin (alkali soluble), (Juliano & Bechtel 1985). On the other hand, Basak et al. (2002) found that the solubility fractions of rice proteins comprised of 9.7 to 14.2 % albumin, 13.5 to 18.9 % globulin, 3.0 to 5.4 % prolamin and 63.8 to 73.4 % glutelin for non-basmati aromatic, basmati aromatic and non-aromatic rice samples, respectively. Average protein fractions in milled rice are shown in Fig. 2.3. Several studies have been conducted by using sequential protein extraction (Chrastil 1990a; Kato et al. 2000; Shigemitsu et al. 2013; Hoogenkamp et al. 2017). Huebner et al. (1990) reported that the mean ratio for 33 samples was found to be 9 % prolamin, 7 % albumin plus globulin and 84 % glutelin.

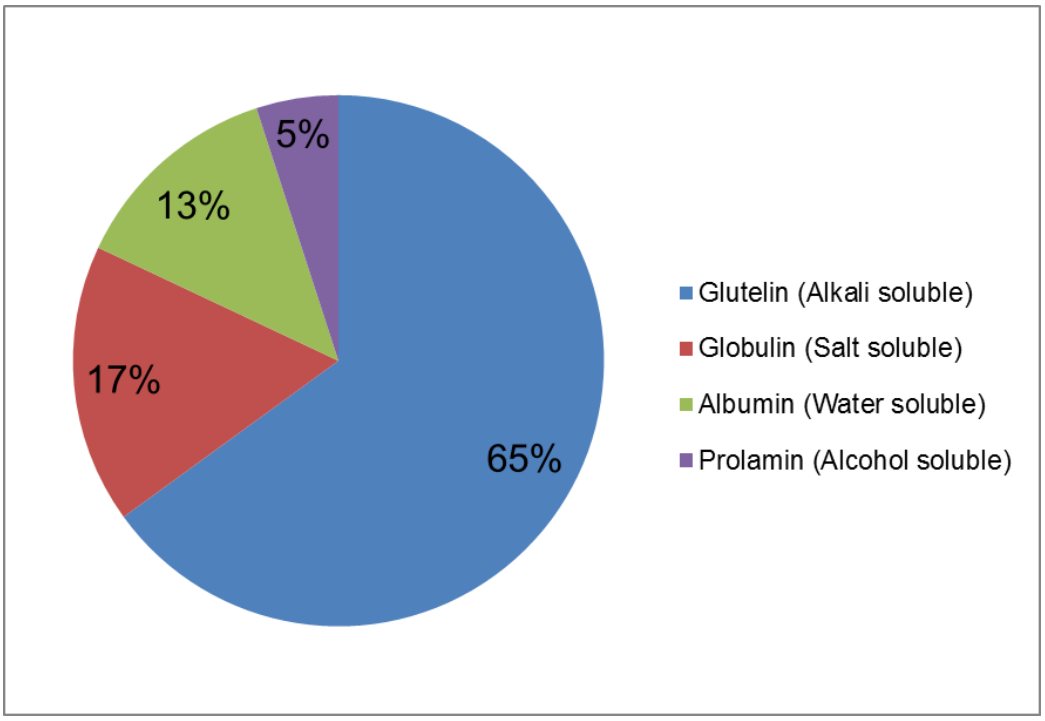

Figure 2.3 Average protein fractions in milled rice samples (Basak et al. 2002)

Protein is most ample in the subaleurone layers and is also present in aleurone cells (Azhakanandam et al. 2000). Rice bran proteins are richer in albumin than endosperm proteins and are found as distinct protein bodies containing globoids in the aleurone layer and the germ. These structures are not similar to endosperm protein bodies (Hoogenkamp et al. 2017).

2.3.2.1. Protein bodies

The endosperm protein is localised mainly in large spherical protein bodies (PB). The estimated size of proteins is usually around 0.5 to 4 µm. The crystalline (PB-II) protein bodies are rich in glutelin, and the large spherical protein bodies (PB-I) are rich in prolamin (Masumura et al. 2015). Ogawa et al. (1989) reported that both PB-I and PB-II were distributed throughout the rice endosperm. Moreover, storage proteins in endosperm were composed of 60-65 % PB-II proteins, 20-25 % PB-I proteins and 10-15 % albumin and globulin in the cytoplasm (Ogawa et al. 1987). Saito et al. (2012) proposed the model for the internal structure of mature PB-I in rice starchy endosperm by using immunofluorescence microscopy. PB-I in rice endosperm consists of a core region containing 10 kDa prolamin, 13 kDa prolamin-rich inner layer, 13 and 16 kDa prolamin-rich middle layers, and a 13 kDa prolamin-rich outermost layer. The 13 kDa prolamins were a major group of rice prolamins and were grouped into distinct sub-classes (13a and 13b) (Fig. 2.4).

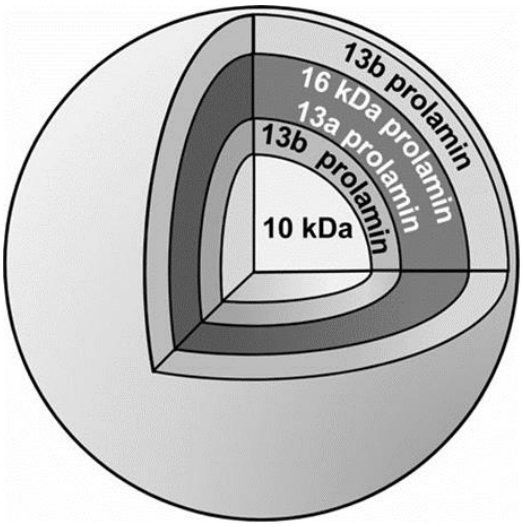

Figure 2.4 Model for the internal structure of the mature protein body PB-I in rice starchy endosperm (Saito et al. 2012)

2.3.2.2. Waxy gene protein

Rice starch granule amylose binds up to 0.7 % protein that is mainly the waxy gene protein or granule-bound starch synthase, with a molecular mass of about 60 kDa, (Villareal & Juliano 1989). Rice glutelin consists of three acidic or α-subunits of 30 to 39 kDa and two basic or ß-subunits of 19 to 25 kDa (Belitz et al. 2009). The two kinds of subunits are formed by cleavage of a 57 kDa polypeptide precursor. Prolamin consists mostly (90 %) of the 13 kDa subunit plus two minor subunits of 10 and 16 kDa (Belitz et al. 2009).

The waxy gene protein has a huge number of disulphide linkages and mostly presents in greater proportion in high-amylose compared with low-amylose rice varieties (Zhou et al. 2002b). Hamaker at al. (1991) reported the correlation of waxy gene protein with amylose contents ($r = 0.95$) and cooked rice stickiness ($r = -0.85$). Protein with intact disulphide bonds makes the swollen granules less prone to break down. When protein disulphide bonds were uninterrupted, rice starch granules swelled to a huge size, thereby increasing the degree of gelatinization and gel strength (Hamaker & Griffin 1993). So, the low concentration of waxy gene protein makes the waxy rice soft, smooth and develops low viscosity while cooking. Traore et al. (2011) figured out that the presence of a rice waxy gene single nucleotide polymorphism (SNP) marker is associated with elevated Rapid Visco Analyzer (RVA) properties in specific high amylose rice cultivars.

2.3.3. Lipids

Fat contents or lipids in cereal grains are diverse. Cereal lipids can be divided into neutral lipids, glycolipids, and phospholipids (Mano et al. 1999). Both japonica and indica rice contain different lipid classes in almost same ratios (Kang et al. 2011), but they are not uniformly distributed within the grain. Moreover, the endosperm lipids contained a higher proportion of polar lipids (Kang et al. 2009). The lipid or fat content of rice is mostly in the bran fraction (20 %, dry basis), specifically as lipid bodies or spherosomes (0.1-1 µm) in the aleurone layer and bran; however, about 1.5-1.7 % is present in milled rice, mostly as non-starch lipids extracted by ether, chloroform-methanol and cold water-saturated butanol (Belitz et al. 2009; Zhou et al. 2002b).

Resurreccion et al. (1979) estimated the crude oil in rice at various stages of processing. It was found that 2.9 % crude oil in brown rice, of which 51 % was found in the germ, 32 % in the polish, and only 17 % in the endosperm. Lipids are not evenly distributed in the endosperm, with the

highest amount in the outer layer and decreasing progressively towards the core of the kernel (Normand et al. 1966; Houston 1967 and Hogan et al. 1968). Moreover, in protein bodies, especially the core, are rich in lipids. The key fatty acids of these lipids are linoleic, oleic and palmitic acids. Essential fatty acids in rice oil are about 29-42 % linoleic acid and 0.8-1.0 % linolenic acid. The content of essential fatty acids is likely to be increased with temperature during grain development but at the expense of a reduction in total oil content (Champagne et al. 2004). Starch lipids (lipids complexed with amylose) are primarily monoacyl lipids (fatty acids and lysophosphatides). The starch-lipid content is lowest for waxy starch granules (<0.2 %). It is highest for inter-mediate amylose rice (1.0 %) and may be slightly lower in high-amylose rice. Waxy milled rice has more non-starch lipids than non-waxy rice. However, starch lipids contribute little to the energy content of the rice grain. The key fatty acids of starch lipids are palmitic and linoleic acids, with lesser amounts of oleic acid (Belitz et al. 2009).

2.3.4. Non-starch polysaccharides

Non-starch polysaccharides comprise of water-soluble polysaccharides and insoluble dietary fibre. They can make a complex with starch and may have a hypocholesterolemic effect. The endosperm has a lower content of dietary fibre than the rest of brown rice. Reported values for neutral detergent fibre are 0.7-2.3 %. Also, the endosperm or milled rice cell wall has low lignin content but a high content of pectic substances or pectin. Endosperm pectin has a higher uronic acid content but a lower arabinose-to-xylose ratio than the other grain tissues. The hemicellulose of endosperm also has lower arabinose to xylose ratio than the three other grain tissues (Belitz et al. 2009).

2.3.5. Volatiles

The volatiles characteristic of cooked rice is ammonia, hydrogen sulphide and acetaldehyde. Upon cooking, all aromatic rice contain 2-acetyl-1-pyrroline as the major aromatic principle. Volatiles characteristics of fat rancidity are aldehydes, predominantly hexanal, and ketones (Belitz et al. 2009). A wide range of volatiles such as alcoholic, aldehydes, and ketone can be detected in different rice varieties (Tsugita 1986). Lin et al. (2010) studied the volatile compounds in different indica and japonica varieties of rice. The amplest volatile alcohols in indica rice were n-hexyl alcohol, n-octanol, and 2-hexyl-1-octanol, while in Japonica they were n-octanol, 2-hexyl-1-octanol and 3,7,11-trimethyl-1-12 alcohol.

Both indica and japonica contained pentanal, hexanal, heptanal, 2-heptene aldehyde, octanal, nonanal, decyl aldehyde and benzene formaldehyde. The most abundant aldehyde was hexanal, which on average accounted for 13.31 % of the aldehydes (averaging 14.69 % for indica and 1.93 % for japonica), followed by nonanal which accounted for an average of 7.93 %. Pentanal, hexanal, heptanal, octanal, nonanal, decyl aldehyde and benzene formaldehyde were present at relatively high levels. A total of twenty-three different volatile ketones were identified of which there were nineteen in indica and thirteen in japonica rice. The ketones content was much lower than the aldehyde content (Lin et al. 2010).

2.4. Classification of rice

Several varieties of rice have been developed in the past few decades, which are widely grown in the world, with immense diversity in physicochemical properties. Therefore, it is very necessary to classify different varieties. There are different ways of rice classification (Kambo & Yerpude 2014). Rice can be classified either by the color of caryopsis or kernel length or type of starch (Fig. 2.5).

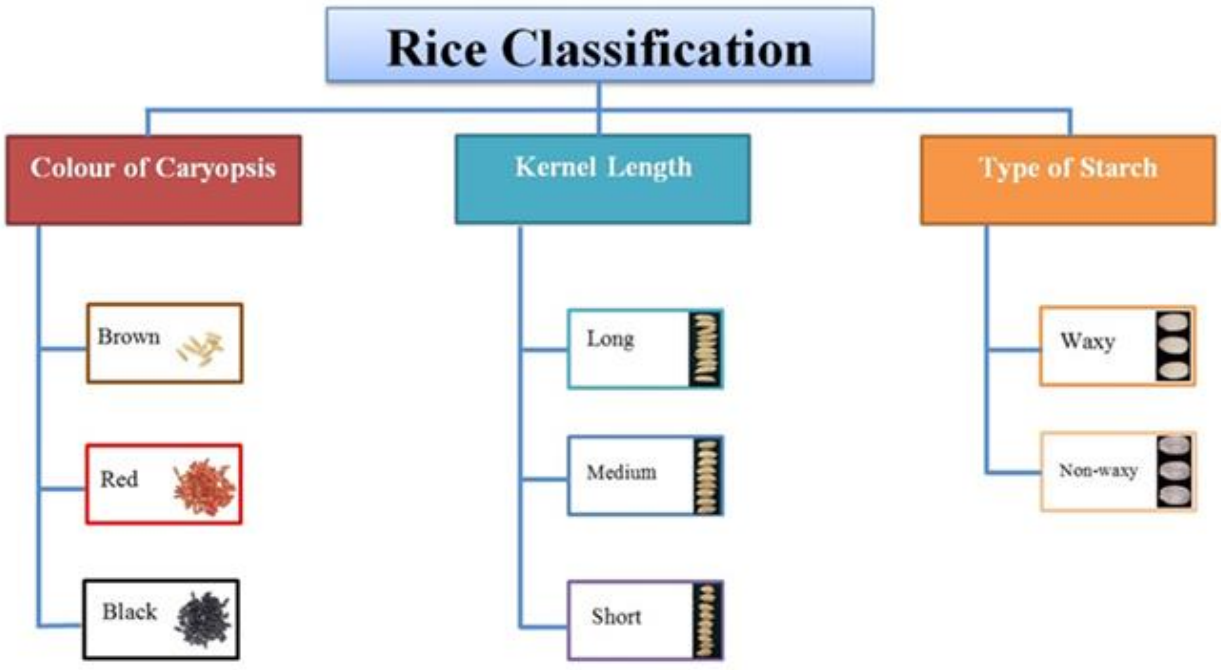

Figure 2.5 Different ways of rice classification

2.4.1. Classification by color caryopsis

Rice can be classified on the color of the caryopsis; it can be brown, red or black. White rice is usually obtained from brown rice by removing the bran layers through a process known as milling. The red and black varieties are less common and essentially only available in Thailand and the Philippines (Parrinello 2008).

2.4.2. Classification by kernel length and length/width ratio

According to Codex Alimentarius Commission (1995), rice is categorised as long grain, medium grain or short grain by one of the following specification:

Option 1: Kernel length/width ratio.

Long/slender grain rice

- Husked rice or parboiled husked rice with a length/width ratio of 3.1 or more.
- Milled rice or parboiled milled rice with a length/width ratio of 3.0 or more.

Medium grain rice

- Husked rice or parboiled husked rice with a length/width ratio of 2.1-3.0.
- Milled rice or parboiled milled rice with a length/width ratio of 2.0-2.9.

Short/bold grain rice

- Husked rice or parboiled rice with a length/width ratio of 2.0 or less.
- Milled rice or parboiled milled rice with a length/width ratio of 1.9 or less.

Option 2: Kernel length.

Long grain rice

- Kernel length of 6.6 mm or more.

Medium grain rice

- Kernel length of 6.2 mm or more but less than 6.6 mm.

Short grain rice

- Kernel length of less than 6.2 mm.

Option 3: A combination of kernel length and length/width ratio.

Long grain rice has either;

- Kernel length of more than 6.0 mm and with a length/width ratio of more than 2 but less than 3.
- Kernel length of more than 6.0 mm and with a length/width ratio of 3 or more.

Medium grain rice

- Kernel length of more than 5.2 mm but not more than 6.0 mm and a length/width ratio of less than 3.

Short grain rice

- Kernel length of 5.2 mm or less and a length/width ratio of less than 2.

2.4.3. Classification by type of starch

Rice can also be classified by starch type found in the endosperm. There are two types of starch, namely, amylose and amylopectin. As mentioned in Fig. 2.6, amylose consists predominantly of linear chains of carbohydrates, whereas amylopectin has a more branching tree-like structure. The proportion of amylose and amylopectin strongly affects the appearance as well as the cooking characteristics of the grain (Ahromrit et al. 2006).

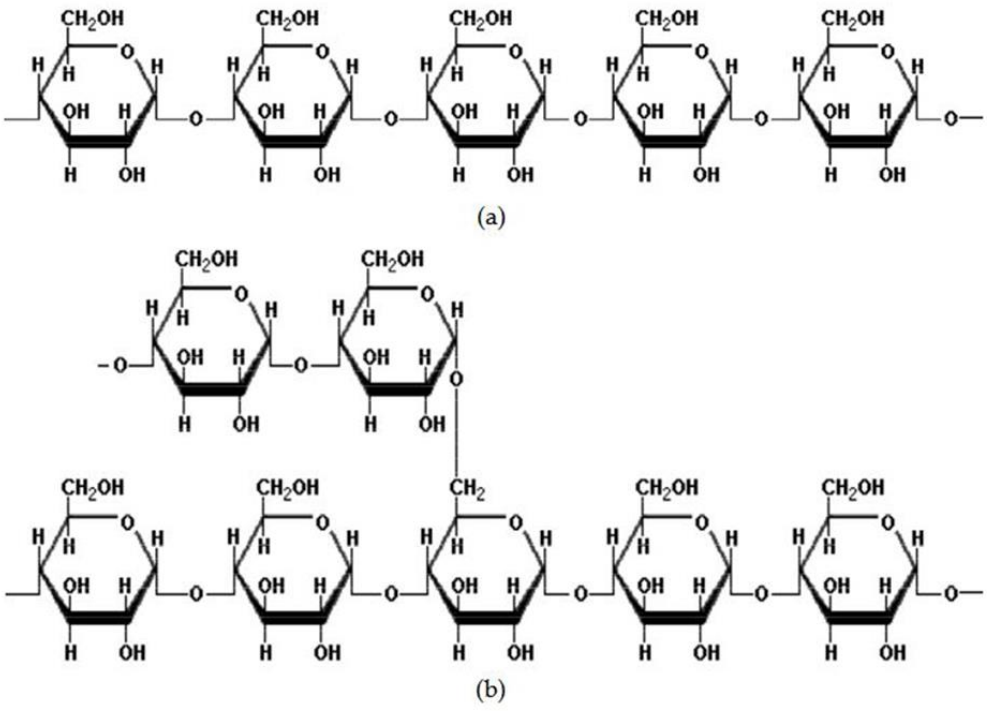

Figure 2.6 Different type of starch found in rice endosperm: (a) Linear structure of amylose, (b) Branched structure of amylopectin (Belitz et al. 2009)

2.4.3.1. Glutinous rice

Glutinous rice, also known as sticky or waxy rice, has a white and opaque endosperm because of the air spaces between the starch granules (Zhou et al. 2002a). Its starch consists almost entirely of amylopectin. When cooked, the grain usually loses its original shape and becomes very sticky (Noosuk et al. 2003).

2.4.3.2. Non-glutinous rice

Non-glutinous or non-waxy rice has a translucent appearance and contains amylose as well as amylopectin. The cooked grain tends to retain its shape and is less sticky (Kang et al. 2009).

2.5. Glutinous, waxy or rice variety

The conventional method for determining amylose content has shown that waxy rice could have up to 5 % amylose. However, including a 0 % amylose standard in the standard curve causes the amylose content of these varieties to become 0-2 % (Cuevas 2008). The composition of glutinous rice is depicted in Table 2.5.

Table 2.5 Proximate composition and nutrition facts of brown and white glutinous rice (Amornsin 2003)

Nutrition Facts Servings: 100 g	**Brown Glutinous Rice**	**White Glutinous Rice**
Calories, kcal	362	355
Moisture, g	11.2	11.7
Carbohydrates, g	77.7	81
Protein, g	7.4	6.3
Total Fat, g	2.4	0.6
Dietary Fibre, g	2.8	0
Niacin, g	5.5	1.8
Phosphorus, mg	255	63
Potassium, mg	326	0
Calcium, mg	12	7
Sodium, mg	12	0
Vitamin B1, mg	0.26	0.08
Vitamin B2, mg	0.04	0.03

Zeng et al. (2009) investigated the volatile composition of three different varieties of glutinous rice (Tatsukomochi, Kinunohada, and Miyakadoganemochi) using combined gas chromatography-mass spectrometry with modified headspace solid-phase microextraction method. Altogether, 96 different volatile compounds were identified, of which 27 volatile compounds have not been previously reported in rice. The volatile components detected in the three waxy rice cultivars during cooking belong to the chemical classes of aldehydes, ketones, alcohols and heterocyclic compounds, as well as fatty acids and esters, phenolic compounds, hydrocarbons, etc.

2.5.1. Global cultivation and consumption of glutinous rice

Glutinous rice or sticky rice (*Oryza sativa* L.) is a kind of rice commonly cultivated in Thailand, Cambodia, Lao PDR, Myanmar, Vietnam, Indonesia, Bangladesh, Northeast India, Japan, Korea, Taiwan, and China (Fig. 2.7). Waxy rice has regional importance in Lao PDR and Thailand, and a large majority of the population consumes it as the main component of their diet (Roder et al. 1996). The cultivation of sticky rice has been recorded in the region for at least 1100 years.

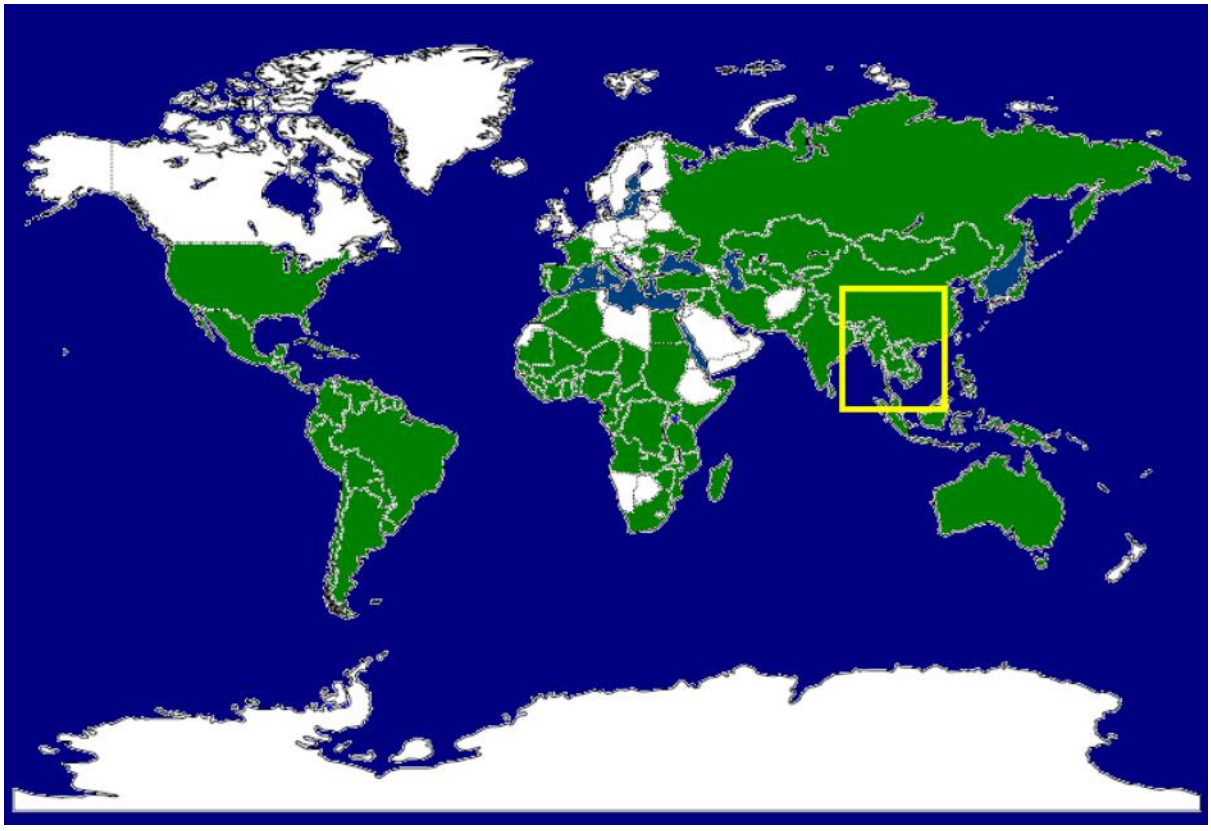

Figure 2.7 Rice-producing nations are denoted in green. The yellow box highlights the centre of waxy rice production. Countries in white did not produce rice (Nguyen & Tran 2002)

2.5.2. Quality characteristics of traditional glutinous rice

2.5.2.1. Hydration and gelatinization behavior of glutinous rice

Glutinous rice absorbs very little water during soaking and cooking and therefore have low volume expansion (Schiller et al. 2006). The grain of most high-amylose rice cultivars shows high volume expansion (up to 400 %) during cooking (Shinde et al. 2014). Low-amylose rice is moist, sticky, and glossy when cooked (Dela Cruz & Khush 2000). The texture of the cooked rice is affected by gelatinization temperature (Peñaflor et al. 2014). Rice that gelatinizes at high temperatures (>74ºC) takes a longer time to cook and, when finally cooked, is excessively soft; it also collapses when overcooked (Briffaz et al. 2014). Such rice generally requires more water and time for cooking than does rice that has low (<70ºC) or intermediate (70-74ºC) gelatinization temperatures. Such rice is undesirable in all rice markets (Schiller et al. 2006). Low gelatinization temperature is the most common property of the preferred waxy rice varieties (Amornsin 2003; Horigane et al. 2000).

2.5.2.2. The opaque endosperm of waxy rice varieties

Another characteristic feature of glutinous rice that distinguishes it from the non-glutinous form is that, if the moisture content of the glutinous form is reduced to about 15 %, the endosperm becomes opaque and its color changes to milky white or paraffin-like. This white appearance comes as a result of the way light is differentially refracted in the starch crystals in the absence of amylose. In non-glutinous rice, the endosperm remains translucent regardless of the moisture content of the grain (Archer et al. 2008).

2.5.2.3. Nutritional value of waxy rice and consumer preference

There is relatively little specific information available relating to the relative nutritional value of waxy and non-waxy rice (Schiller et al. 2006). It has been suggested that the higher content of amylopectin in glutinous rice and the associated larger molecular size and its branched molecular structure result into a longer stay in the digestive system than non-glutinous rice (Benmoussa et al. 2007). This, in turn, is reflected in the belief by waxy rice consumers that they only feel “full” when they consume glutinous rice; they complain that the consumption of non-waxy rice results in their becoming hungry again within a short time (Schiller et al. 2006).

The low volume expansion of glutinous rice relative to non-glutinous rice on being cooked, and the resulting higher weight to volume ratio of glutinous vs. non-glutinous rice on consumption,

probably explains the effects of perceived differences in “fullness and hunger” between the two types of rice (Ngaosyvanthn & Ngaosyvanthn 1994).

2.5.3. Cooking process of rice

Rice can be cooked by boiling in excess water or using a fixed rice: water ratio in a rice cooker. Depending on the cooking conditions (temperature, time, water-to-rice ratio, etc.); the rice grain undergoes specific structural and physicochemical transformations (Briffaz et al. 2014). During cooking, several stages occur to transform a raw rice grain into a cooked grain of pleasing textural attributes. These include glass transition, gelatinization, swelling, pasting and leaching of amylose, and retrogradation. It is now quite accepted concept that the cooking attributes, texture, water absorption ability, stickiness, volume expansion, hardness and even the shine and whiteness of the cooked milled rice are greatly affected by the composition of starch (amylose and amylopectin contents) (Jie et al. 2010) and protein contents (Teo et al. 2000). Glutinous rice varieties having high amylopectin contents in endosperm are non-gelling because of the lack of amylose (Jane at al. 1999). The main stages of the cooking process of glutinous rice are shown in Fig. 2.8.

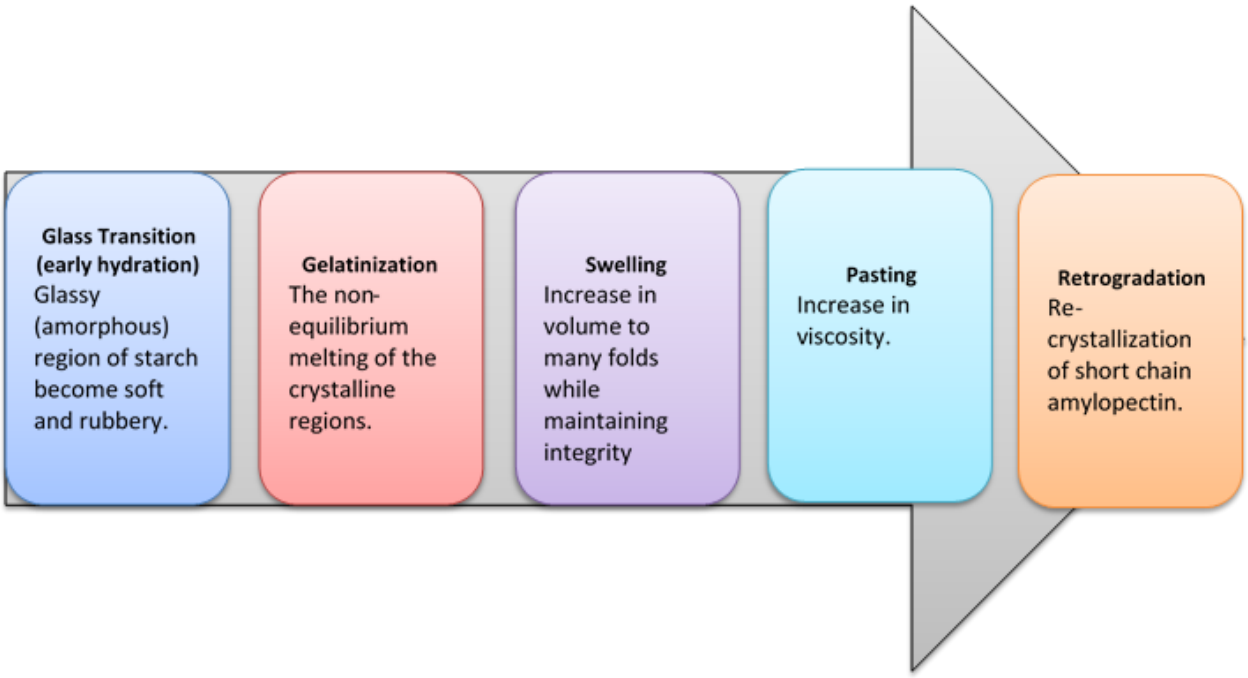

Figure 2.8 Main stages of the cooking process of glutinous rice

2.5.3.1. Glass transition

The concept of 'glass transition' has been applied to food science and technology for the last five decades (Truong 2008). The glass transition or glass-liquid transition is a reversible transition in amorphous materials (or in amorphous regions within semicrystalline materials) from a hard and relatively inelastic state into a molten or rubber-like state. It is a branch of material science which considers food components like starch and protein to be biopolymers (Truong 2008). Before gelatinization of starch can occur, the amorphous (glassy) regions of the starch must become soft and rubbery as they go through the glass transition temperature (T_g). The physical and thermal properties changes in the amorphous regions of the starch during glass transition (which is a second order transition), while the crystalline regions in starch are disrupted during the first-order transition, i.e., melting (Roos & Karel 1991).

As shown in Table 2.6, below the glass transition temperature (T_g), the amorphous regions of the starch are in a glassy state, but they become flexible, viscous and rubbery above the glass transition temperature (T_g). In the glassy state, molecular movement is quite limited, making it quite viscous, having low specific volume and low thermal expansion coefficient. During the glass transition from glassy to rubbery states, many changes in physical properties are observed; like a discontinuous change in heat capacity increases in thermal expansion coefficient, diffusivity, and specific volume, and a decrease in viscoelasticity. Some factors including free volume, water content, average molecular weight, the degree of crystallinity, and degree polymerisation can affect these properties (Bhandari & Howes 1999; Roos & Karel 1991).

Water is considered as a strong plasticiser, thereby depressing T_g of the amorphous regions at low water content (<30 %) (i.e., T_g increases with decreased water content) (Biliaderis et al. 1986; Huang et al. 1994). The relationship between the glass transition temperature and moisture content hypothesised by Cnossen et al. (2001) and Perdon et al. (2000) is shown in Fig. 2.9. Water reduces the glass transition temperature, and so at higher moisture contents, glass transition occurs at lower temperatures (Roos & Karel 1991). The milled rice at 12 % moisture content has a glass transition temperature (T_g) of about 50ºC (Cnossen et al. 2001). In another study, Thuc et al. (2010) reported the glass-rubber transition temperature ($T_{g\text{-}r}$) of rice flour (12-16 % moisture content) ranging from 41.6 to 56.7ºC. During the early hydration stage of cooking, there will be a transition from amorphous to rubber state facilitating the gelatinization of crystalline starch.

Table 2.6 Properties of glassy and rubbery states of starch (Truong 2008)

State of amorphous regions	GLASSY	T_g (glass transition temperature)	RUBBERY
Microstructure	Semi-crystalline structure		Disordered and short-range orders
Molecular mobility	Highly limited		Increased
Molecular motion	Vibration Localized translation and Rotation		Vibration Rotation Translation
Physical and thermal properties	High viscosity (>10^{12} Pa.s) Low specific volume Low thermal expansion coefficient Low diffusivity High thermal conductivity		Low viscosity (<10^{7} Pa.s) High specific volume High thermal expansion coefficient High diffusivity Low thermal conductivity

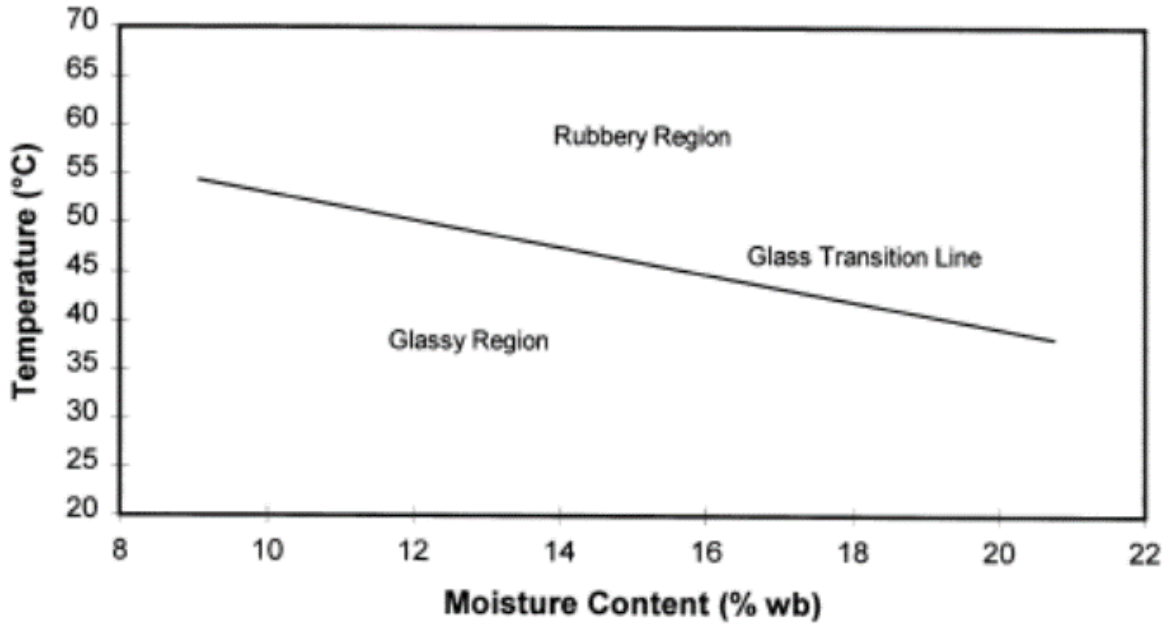

Figure 2.9 State diagram describing the glassy and rubber regions of rice (Cnossen et al. 2001)

2.5.3.2. Gelatinization

Gelatinization is the non-equilibrium melting of the crystalline regions, and prerequisite is the pre-softening of the amorphous regions (glass transition). Starch gelatinization is an endothermic

process that corresponds to the loss of starch crystallinity in the starch granules under particular heat and moisture conditions. Not all granules in any rice sample gelatinise at the same temperature; rather gelatinization occurs over a temperature range of about 8-15ºC (Shih et al. 2007). Pictorial representation of starch gelatinization is shown in Fig. 2.10.

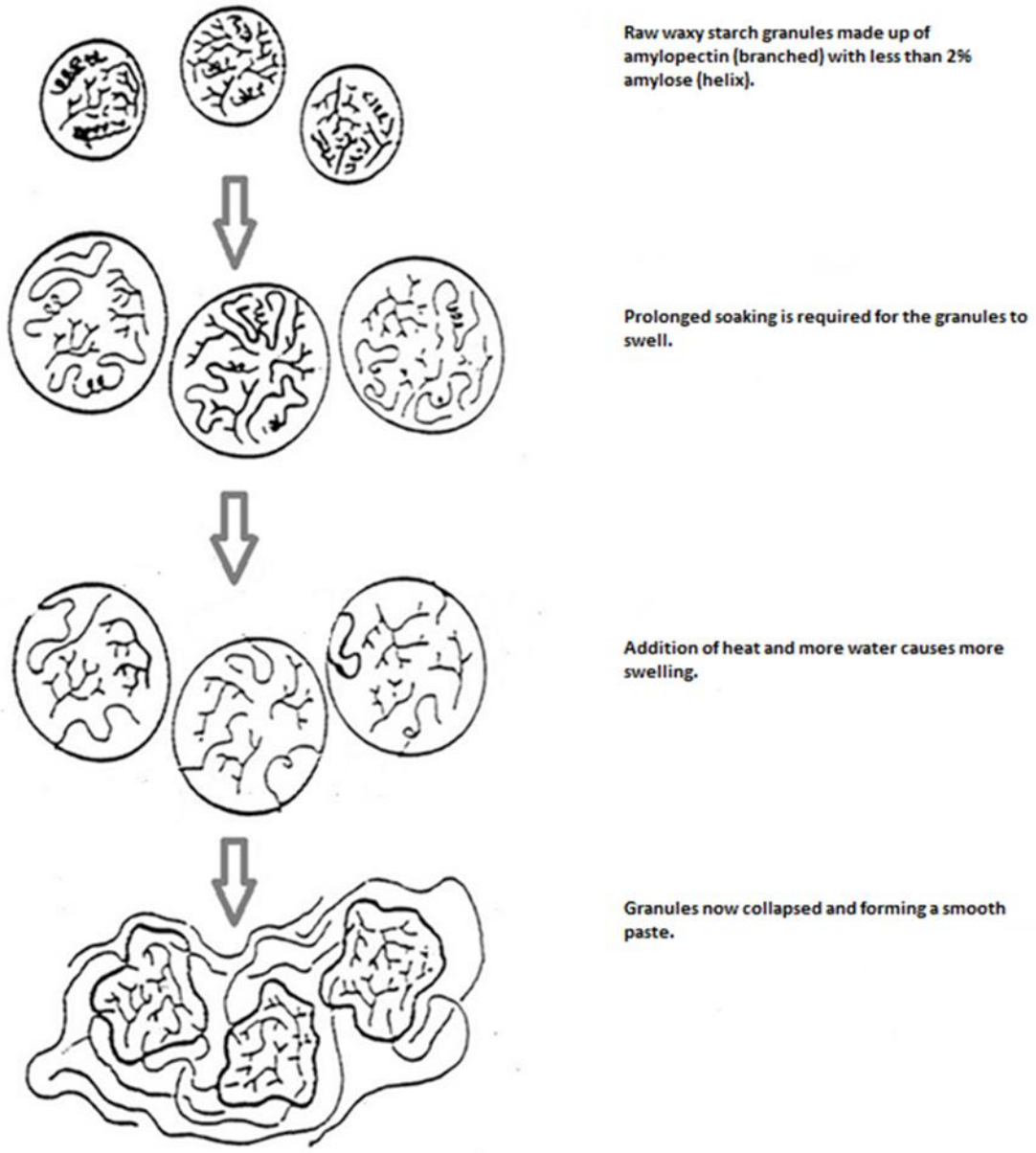

Figure 2.10 Process of starch gelatinization (Singh et al. 2007; Ahmed et al. 2008)

2.5.3.3. Gelatinization temperature

Gelatinization temperature is a key property of rice because it correlates strongly with the cooking time and the texture of the cooked product. McGuiness et al. (2000) worked on the relationship between moisture content and gelatinization temperature. They found that if the moisture content is too low, then gelatinization cannot take place. Also, the higher the moisture content, the lower the temperature at which the reaction can occur.

This can be clarified by the plasticizing effect of water on the melting of starch; rice starch gelatinization endpoint temperature is thus over 100ºC when the water content is less than 50 % (Briffaz et al. 2012). Moreover, gelatinization behavior also directly affects the perceived texture of cooked rice; the higher the gelatinization temperature of the grain, the firmer the core of cooked rice will be (Mestres et al. 2011).

2.5.3.3.1. Rice starches and gelatinization temperature

The composition of rice starches has a great effect on the gelatinization. Rice starches having short average amylopectin branch chain lengths displayed low gelatinization temperatures (Kalichevsky et al. 1990).

2.5.3.4. Swelling and pasting

After starch gelatinization, the starch granules begin to swell and, in the absence of shear, can swell and increase in volume to many folds while maintaining their integrity (Parker & Ring 2001). Leaching of amylose molecules accompanies the swelling of the starch granules into the liquid phase only in non-waxy rice. In waxy starches, after swelling granules disrupt, resulting in a smooth paste.

2.5.3.5. Retrogradation

Gelatinized starch contains no crystalline regions, but under certain conditions of storage and temperature, the molecules in a starch gel can re-associate into an ordered structure. Retrogradation describes the rapid recrystallisation of amylose and the slow recrystallisation of amylopectin. The degree of retrogradation and the nature of newly formed crystals can depend on the time and temperature of storage (Li et al. 2014), the source of starch (Lian et al. 2015), and the presence of other molecules (Likitwattanasade & Hongsprabhas 2010) in the system.

2.5.3.5.1. Recrystallization of amylose

Recrystallization of amylose is essentially the rapid formation of double helices in parts of the amylose chains followed by aggregation of these helices. The first stage of retrogradation depends on the amylose that is free, rather than complex with lipids (Yao et al. 2002). Further, hot-water-soluble components of rice starch with high molecular weight promote retrogradation more than lower-molecular-weight polymers. Tsai & Lii (2000) suggested that the molecular weight distribution of the amylose contributes significantly to the first phase of retrogradation. Retrogradation due to amylose is not reversible at temperatures less than 100ºC because amylose crystals melt only above 100ºC.

2.5.3.5.2. Recrystallization of amylopectin

Recrystallization of short-chain amylopectin chains constitutes the second process of retrogradation (Baik et al. 1997). Several studies have been conducted to study the retrogradation behavior of amylopectin. It is revealed that the fine structure or the chain-length distribution of amylopectin contributes to differences in the degree of retrogradation by amylopectin, in particular, the proportion of short A chains in amylopectin (Yao et al. 2002; Tsai & Lii 2000 and Silverio et al. 2000). The interaction of amylose with amylopectin increases the rate of amylopectin retrogradation. Retrogradation due to amylopectin is reversible if the retrograded gel is exposed to a temperature greater than the gelatinization temperature of crystalline amylopectin (Yao et al. 2002).

2.6. Pre-treatment processes

The cooking process of rice involves wetting of the kernels up to a moisture content of 65-70 %. This wetting of grains results in swelling. Consequently, the melting of amylopectin crystals takes place, the release of amylose from the starch granules (only in non-waxy rice starches), and gelatinization takes place. Cooking process leads to the softness of rice and makes it easily masticateable product, ready for consumption. The food industry has engineered several pre-treatment processes for consumers' convenience, for example, shorter cooking or preparation times (Mohoric et al. 2009). These processes include wet processing and puffing. During wet processing, polished rice is cooked and dried afterward, resulting in reduced cooking time with poor texture product (Lee et al. 2000). However, puffing raw rice results in a product which still

requires cooking, but cooking time is reduced with the good texture final product (Mohoric et al. 2009).

2.6.1. Alkali treatment

Alkali treatment of cereals is widely used in food industry to produce many value-added food products such as tortillas, waxy rice dumplings, and yellow alkaline noodles (Nadiha et al. 2010; Lai et al. 2002; Guo et al. 2017). Alkali application to the starch leads to reduced swelling power and water binding capacity (Karim et al. 2008; Wang & Copeland 2012). Moreover, starch particles display Donnan-potential in the presence of water due to its weak acidic ion-exchanging behavior. The starch particles have a negative charge; therefore, penetration of Na^+ into the amorphous regions of starch granules is promoted (Upma et al. 2017). Moreover, under alkaline conditions, hydroxyl groups of starch might have a greater tendency to ionize and create even more binding sites for cations. It is hypothesized by Oosten (1990) that anions might tend to destabilize starch granules by breaking hydrogen bonds. However, such destabilizing effects of anions might be much weaker than the stabilizing effects of cations. This electrostatic interactions between hydroxyl groups of starch and Na^+ ions result in increased gelatinized temperature (Lai et al. 2002). Also, alkali treatment induces structural changes in amylopectin possibly due to alkali-induce de-polymerization, resulting in reduced retrogradation (Abhari et al. 2017).

2.6.2. Starch modification

Starch can be modified by using various chemical agents. These chemical agents can be classified as monofunctional or bi-functional reagents based on their chemical properties (Wolf et al. 1999). A non-ionic, cationic, hydrophobic or covalently reactive substituent group are provided by monofunctional reagents (Sui & BeMiller 2013). Etherification modification method or hydroxypropylation is a common example of starch modification by monofunctional reagents (Clasen et al. 2018). Tri-meta-phosphates and phosphoryl chlorides are the common examples of bi-functional reagents as they can react with more than one hydroxyl group and can thus reinforce starch granules (Masina 2016; Włodarczyk-Stasiak et al. 2017). Moreover, these bi-functional reagents allow crosslinking of the polymers, resulting in increased starch stability and modify its swellability, solubility, and mobility (Xiao 2013). Such modifications usually alter the physicochemical (gelatinization and thermal) properties of starch. In the recent years, great focus has been given to study the chemical modification of starch and a variety of different starch

molecules have been synthesized, each with its distinct chemical property and functionality. However, there are very few methods applicable to starch particles as most reactions require the sample to be in solution or slurry (Masina et al. 2017). The three main types of chemical starch modifications and their derivatives are summarized in Fig. 2.11.

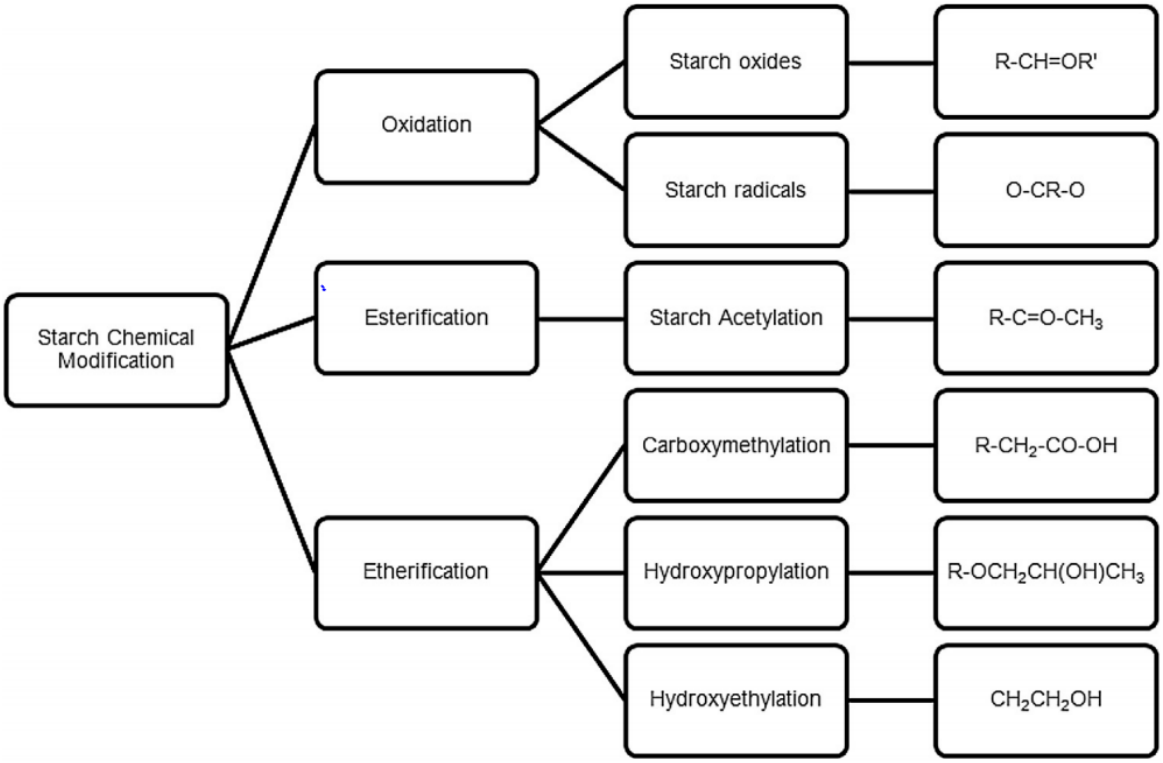

Figure 2.11 Schematic summarization of the classical chemical methods for starch modification (Masina et al. 2017)

The esterification of starch is the most common chemical modification used in the food industry. It involves the conversion of the available hydroxyl groups to alkyl or aryl derivatives (Morán et al. 2011; Ačkar et al., 2015). Esterified starch has reduced retrogradation ability and glycemic index response (Masina et al. 2017). Therefore, starch esterification can be a potential solution to maintain the stickiness. However, no reported data is available on the intact starch esterification in the milled rice.

2.6.3. Parboiling of rice

The parboiling of rice is the major processing technique has been used in South Asian developing countries especially Indian subcontinent for decades (Islam et al. 2001; Bhattacharya 2004).

According to an estimate, 20 % of the world's rice is parboiled, and production seems to be growing day by day (Buggenhout et al. 2014).

2.6.3.1. Process of parboiling

Conventionally parboiling is a three-step hydrothermal process given to rough rice. The paddy or rough rice is first soaked in excess water at a temperature below gelatinization temperature to increase average moisture contents to 25-35 %. Excess water is drained off, and the soaked paddy is steamed at 100-130^{o}C for 5-30 min to gelatinize the starch. Steaming of paddy is followed by cooling and drying of paddy with or without tempering to a moisture content below 14 %. After drying, the broken husk is removed during milling and followed by polishing (Derycke et al. 2005a; Delcour & Hoseney 2010; Buggenhout et al. 2013).

Rice industrial research has engineered various parboiling techniques in recent years to enhance the quality of head rice and ready to use products. Use of pressurized steam and roasting of paddy with or without sand are the latest parboiling techniques introduced by the researchers in the rice industry (Dutta & Mahanta 2014). The schematic flow diagram of conventional and latest parboiling techniques is shown in Fig. 2.12.

2.6.3.2. Superheated steam drying

Superheated steam dryers are designed to use superheated steam as a heating source for drying instead of hot of air. This drying technique is environmentally friendly and economical, as there are no pollution or odor emissions, and the steam can be recovered (Mujumber 1995). The drying mechanism of the superheated steam dryer is the condensation of steam onto the surface of rice grain and a rise in grain temperature to saturation point (100^{o}C) during the initial approximately 30 min. In the second phase of the drying mechanism, there is a reduction in moisture content due to the high heat transfer rate (Taechapairoj et al. 2003).

Taechapairoj and co-workers (2004) found that the reduction in moisture content from 41 to 25 % (dry basis) using superheated steam at 150-170^{o}C was initially linear with drying time followed by the exponential decay when the moisture content was below 25 % (dry basis). The SEM results revealed that the use of high temperature along with condensation steam enabled the development of the gel layer. This gel layer caused the very low effective diffusivity when compared with the case of no gel formation in paddy.

Taechapairoj et al. (2003) also reported the similar characteristics of paddy dried by superheated steam and parboiled rice. The paddy is firmer and tougher after drying in superheated steam, as a result of gelatinization taking place for paddy with high initial moisture content. Consequently, head rice production is improved, while a lower value of rice whiteness is usually observed as a result of the high drying temperature. The schematic diagram of the superheated steam dryer is shown in Fig. 2.13.

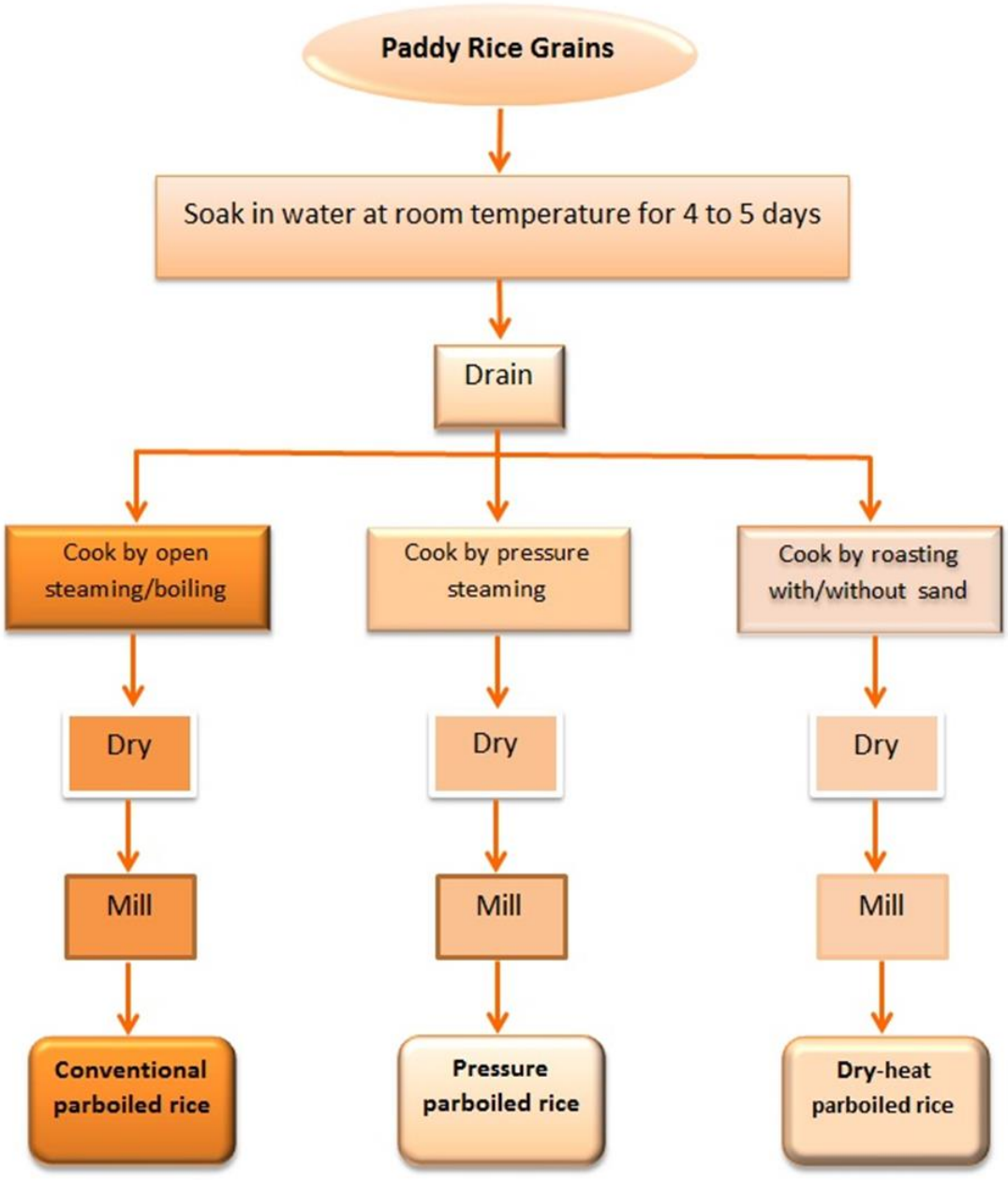

Figure 2.12 A schematic flow diagram of various parboiling techniques (Dutta & Mahanta 2014)

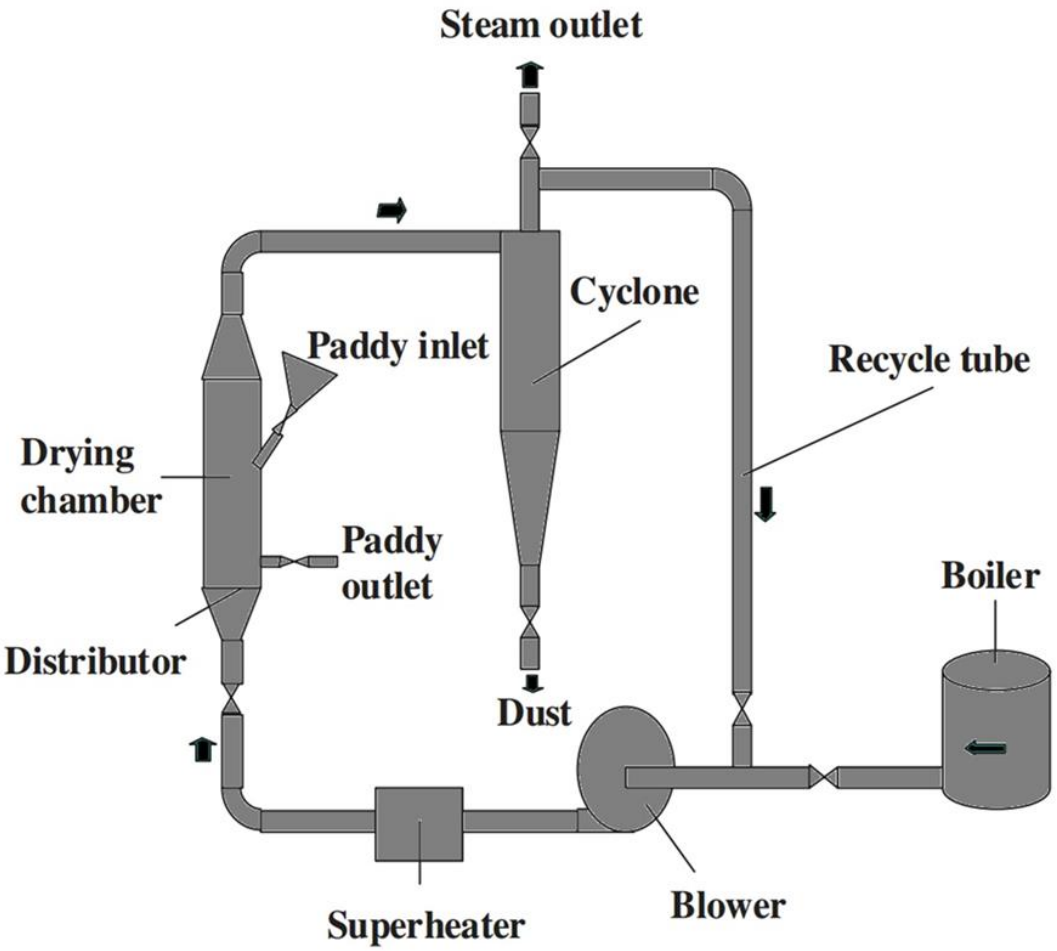

Figure 2.13 A schematic diagram of the superheated steam dryer (Rordrapat et al. 2005)

2.6.3.3. Effect of parboiling on rice breakage

The main advantage of parboiling is the reduced level of breakage during dehulling and milling (Delcour & Hoseney 2010), this only cases when the process of parboiling is carried out properly. Indeed, the Head Rice Yield (HRY) of parboiled rice depends on the parboiling conditions and the resulting changes in physicochemical and mechanical properties. The starch gelatinization and kernel fissuring during parboiling have a great impact on hardness. The more compact and homogeneous ultrastructure obtained as a result of starch gelatinization increases the grain hardness (Islam et al. 2001; Islam et al. 2002a, Islam et al. 2002b, Islam et al. 2004; Jagtap et al. 2008; Oli et al. 2014), while the presence of fissures has the opposite effect.

The physicochemical changes in paddy can also influence breakage susceptibility during steam heating; these changes include protein polymerization, amylose-lipid complex formation, and

retrogradation of amylopectin (Buggenhout et al. 2013). The process of starch gelatinization and fissuring during parboiling are discussed below.

2.6.3.4. Starch gelatinization during parboiling

Both hydration and heating conditions impact the extent of swelling of the starch granules and degree of gelatinization. Water is absorbed by the rice grains during rehydration and starch granules inside the endosperm swell. When these swelled starch granules are heated over gelatinization temperature, their structural order is irreversibly destroyed. These irreversible changes during gelatinization include loss of birefringence and crystalline melting (Dercyke et al. 2005).

Limited water is available, therefore the gelatinization temperature shifts to a higher value. As the moisture content and the extent of heating increase, the degree of starch gelatinization increases by 100 % (Patindol et al. 2008). However, during hydration, moisture content gradients exist inside the rice grain. It is assumed that without equilibration, the moisture contents at the core of endosperm may be too low for the starch to gelatinize. As a result, parboiled rice grains have translucent surfaces with opaque cores known as white bellies (Buggenhout 2013).

Therefore, it is suggested that the starch granule swelling and the degree of gelatinization are the key factors to determine whether air spaces and fissures, present before parboiling or induced during hydration, remain present in the parboiled rice grain or are sealed upon parboiling. As a consequence, conditions adopted during parboiling result in either increased or decreased the Head Rice Yield (HRY) (Marshall et al. 1993; Miah et al. 2002a; Miah et al. 2002b; Patindol et al. 2008).

2.6.3.5. Fissuring during parboiling

Kernel fissuring can increase the extent of the hardness of parboiled rice. During hydration and drying, fissures develop as a result of moisture absorption and desorption, respectively. There is no evidence in the literature on the development of fissures during the heating step in the parboiling process (Buggenhout et al. 2013).

2.6.4. Effect of parboiling on the quality attributes of waxy rice

Waxy rice varieties contain 0 % to very low amylose contents in the endosperm. Amylopectin is the main component of starch in such rice varieties. The quality attribute of such varieties is to

produce smooth paste after cooking. Conventional and pressurized steam parboiling result in the hardness of the grains and deteriorate the final product quality. Researchers have engineered a new technique known as dry heat parboiling (Dutta et al. 2016). It involves conduction heating of fully soaked paddy at high temperature for shorter durations using sand or hot air. Steam parboiling causes starch gelatinization during steaming followed by retrogradation during extended drying. Gelatinization and rapid loss of water result in grain during dry heating which does not allow retrogradation (Mahanta & Bhattacharya 2010).

Dutta et al. (2016) worked on the cooking quality of traditional Indian waxy rice to make a speciality product called Bhoja chaul. It was observed that by the use of dry heat parboiling the cooking time in waxy can be reduced. No white bellies were observed in the grains. After processing the kernel became bolder. RVA and DSC endotherms suggested molecular damage and amylose-lipid complex formation by the linear β-chains of amylopectin, respectively.

2.7. Aging-induced changes

Rice is mostly consumed as cooked grains while a small amount of the rice crop is used as ingredients in processed foods. This varying consumption pattern results in the need to store rice over varying periods (Zhou et al. 2002a). Moreover, some markets (e.g., India, Pakistan, Sri Lanka, and Nepal) have a preference for aged rice while other (e.g., Japan, China) favor fresh crop. Japanese people are so keen about the freshness of rice that tests are devised for its measurement (Matsukura et al. 2000).

2.7.1. Aging

Aging is a complexed terminology mostly used for the number of physicochemical and physiological changes occur during storage, potentially leading to both desirable and undesirable effects on functional properties (Chrastil 1992; Howell & Cogburn 2004). These changes mostly include pasting properties, color, flavor, and composition (Zhou et al. 2002b). Different researchers have reported the storage induced changes in both waxy (Tulyathan & Leeharatanaluk 2007) and non-waxy rice (Tananuwong & Malila 2011). These storage-induced changes affect the rice quality (Katekhong & Charoenrein 2012).

2.7.2. Mechanism of aging

The actual mechanisms involved in the process of rice aging have yet to be understood fully (Truong 2008), and are described in a number ways (Chrastil 1992; Sowbhagya & Bhattacharya 2001; Howell & Cogburn 2004);

i. The polymerization of protein and the oxidation of esters in non-starch polysaccharides which resulting in cross-linking and extended strength of cell walls.
ii. The binding starch to the denatured oryzenin (one type of rice protein).
iii. Changes in non-soluble amylose.

2.7.3. Cooked rice texture of the aged rice

Aged rice when cooked, the texture becomes fluffier and harder (Suzuki et al. 1999). Three months of storage is considered as the least period for major changes to occur in the hardness of cooked rice, gel consistency, and amylograph viscosity values (Perez & Juliano 1981). During the last couple of decades, several researchers published the pasting properties of different non-waxy and waxy rice cultivars and the subsequent effect of storage on quality attributes. It has been observed that the peak viscosity and breakdown of fresh rice were higher than the aged rice (Fig. 2.14) (Noomhorm et al. 1997; Zhou et al. 2002a; Zhou et al. 2003a; Tulyathan & Leeharatanaluk 2007 and Tanauwong & Malila 2011). Moreover, data from Differential Scanning Calorimetry (DSC) and Rapid Visco Analyzer (RVA) indicated that longer storage (usually over six months) at room temperature resulted in extended gelatinization and pasting temperature and setback. All these changes in the pasting attributes predict the harder texture and decreased stickiness especially in sticky or waxy rice. This is usually not desirable depending on consumer preference (Sowbhagya & Bhattacharya 2001).

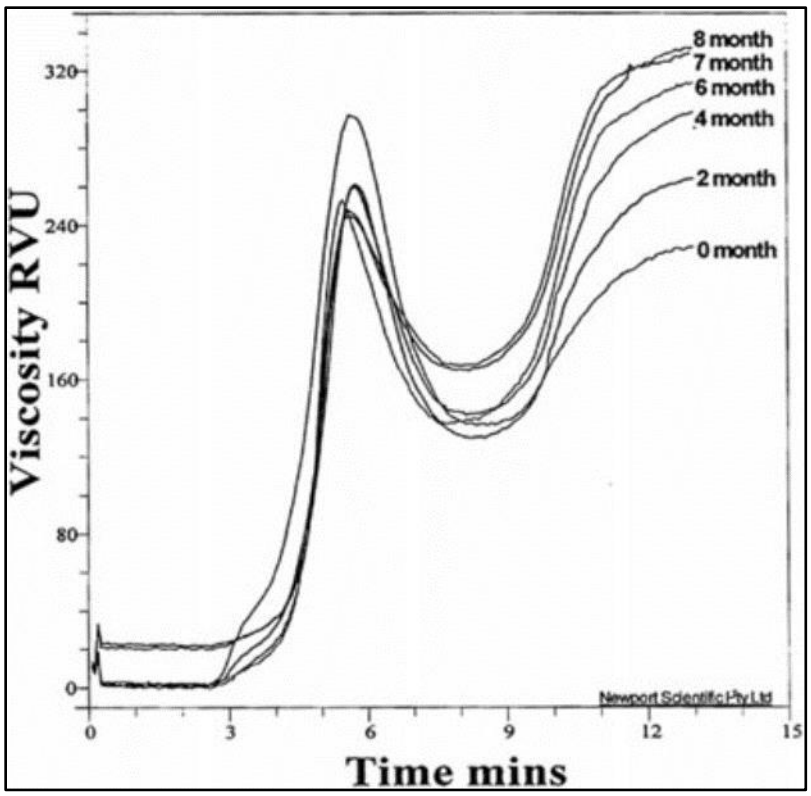

Figure 2.14 Rapid Visco Analysis curves for rice flour following grain storage for up to 8 months (Tulyathan & Leeharatanaluk 2001)

2.7.4. Effect of storage conditions on physicochemical properties

Storage conditions affect the hydration and cooking behavior of rice. Among storage conditions, storage temperature is of great importance. Zhou and co-workers (2007a) investigated the changes in hydration and cooking attributes of different rice cultivars stored at 4ºC and 37ºC. Researchers concluded that the positive correlation between storage temperature and water uptake, higher temperature storage led to greater water uptake, while the negative correlation between storage temperature and pH and turbidity of residual cooking liquid.

2.8. Conclusions and perspectives

This literature review has given an account of and the reasons for the attention to the cooking quality of glutinous and non-glutinous rice. For waxy rice, cooking and eating qualities are dictated, not by amylose, but by amylopectin. Rice being the staple food of half of the world's population has given huge importance in food industries, and several researches have been conducted in the past to streamline the cooking process of rice. However, maximum researches focused only on non-glutinous cultivars neglecting waxy cultivars.

Stickiness and smoother texture of the cooked rice is the quality attribute of glutinous rice. Unfortunately, stickiness of glutinous is reduced during aging. This deterioration in the cooking quality has been associated with the starch type and other macromolecules (like protein bodies and lipid) in the rice endosperm. Controlled storage conditions can slow down the macromolecules shift in the endosperm, resulting in improved stickiness and smooth texture in cooked aged glutinous rice.

Pre-process treatments of freshly harvested rice are very common in the Indian subcontinent. Processers mostly parboiled paddy to partially gelatinize the starch, resulting in a reduced cooking time of milled rice. However, these techniques mostly employed for non-glutinous rice cultivars and very little work has been in the past on the pre-processing of sticky rice. Therefore, it is thought that pre-processing of paddy could help in improving the cooking quality of glutinous rice or can avail rice with different quality characteristics.

Chapter 3 Effect of different cooking conditions on the pasting properties of flours of glutinous rice varieties from Lao People's Democratic Republic

This chapter has been published in the International Journal of Food Properties;

Nawaz, MA, Fukai, S & Bhandari, B 2016, 'Effect of different cooking conditions on the pasting properties of flours of glutinous rice varieties from Lao People's Democratic Republic', *International Journal of Food Properties*, vol. 19, pp. 2026-2040.

3.1. Abstract

The effect of different rehydration temperatures (30, 40 and 50ºC) and cooking times (2.7, 4.7, 6.7, 8.7 and 10.7 min) at 95ºC on the pasting properties of three glutinous varieties (TDK11, TDK8, and Hom Mali Niaw) from Lao PDR was investigated using Rapid Visco Analyzer (RVA). Non-glutinous varieties (IR64 and Doongara) were also analyzed to compare glutinous (amylose < 4.5 %) and non-glutinous (amylose > 15 %) varieties. All rice flours took up water at significantly ($P<0.05$) higher rates in the case of increased temperature and soaking time, resulting in a decrease in the onset temperature for pasting. Among the glutinous rice, TDK8 showed a significant ($P<0.05$) decrease in peak viscosity in response to increased rehydration time and temperature. For this variety maximum viscosity (2403.3 mPa-s) was observed at 1 min of rehydration at 30ºC and minimum viscosity (1852.0 mPa-s) at 15 min of rehydration at 50ºC. The viscosity values of TDK11 and Hom Mali Niaw varieties increased to their highest values (1608.7 and 1477.7 mPa-s, respectively) with an increase in temperature to 40ºC for 1 min. In general, the glutinous rice produced weaker gel than non-glutinous rice. Extended holding at a cooking temperature (95ºC) had a more significant ($P<0.05$) effect on the glutinous varieties TDK8 and TDK11 than on the non-glutinous varieties (IR64 and Doongara) used in this study.

3.2. Introduction

Rice (*Oryza sativa* L.) has been a staple food in the majority of countries in the Asian continent, and its consumption is also growing in other parts of the world following changes in demography and eating habits of the population. It has been reported that rice has been cultivated in China and Thailand dating from about 6000 BC (Zhou et al. 2002a; Childs 2004). There are different cultivars of rice grown throughout the world (Kambo & Yerpude 2014), each cultivar exhibiting distinct physicochemical properties, and the type of the starch present influencing the cooking quality (Yu et al. 2009). Consumer preference is normally dependent on the growing location, and this is a crucial factor for the selection and utilisation of rice cultivars in a given location (Allahgholipour et al. 2006).

Rice is classified into glutinous and non-glutinous categories by the type of starch found in the endosperm. There are two types of starch found in rice, namely, amylose and amylopectin. Amylose consists predominantly of linear chains of α-D-glucose units, whereas amylopectin has a more branching tree-like structure (Yu et al. 2015). The proportion of amylose and amylopectin

strongly affects the appearance, as well as the cooking characteristics of rice grain (Ahromrit 2006). When the amylose content in rice is lower than 5 %, the rice is classified as glutinous (Prathepha et al. 2005). Glutinous rice, also known as sticky or waxy rice, has a chalky and opaque endosperm because of the presence of air spaces between the starch granules (Zhou et al. 2002a). When cooked, the grain usually loses its shape and becomes very sticky (Noosuk et al. 2003). On the other hand, non-glutinous or non-waxy rice kernels have a translucent appearance and contain amylose as well as amylopectin. The cooked grain of non-waxy rice tends to retain its shape and is less sticky (Kang et al. 2009).

Waxy varieties of rice are grown in many countries, including Lao PDR, Thailand, China, Myanmar, Vietnam, Cambodia, Japan, Bangladesh, and India (Calingacion et al. 2014; Mar et al. 2015). It is consumed as a sweet dish, as a breakfast cereal or as specialty steam rice in banana leaves in Thailand, Myanmar, Cambodia, India, China, and Vietnam. In Lao PDR, waxy rice is a staple food which consumed by most of the population on a daily basis. Lao PDR has the highest per capita consumption of rice in the world (Schiller et al. 2006), and 85 % of the rice produced is the glutinous type (Food and Agriculture Organization 2011). Among the wide range of improved glutinous rice varieties grown in lowland farming systems in Lao PDR are Hom Mali Niaw (HMN), Thadokkham-8 (TDK8) and Thadokkham-11 (TDK11) (Sengxua et al. 2014).

A knowledge of pasting properties is a key indicator of the processing quality of cereals including rice and rice products. For example, an understanding of the pasting behavior can help a processor in optimising ingredient concentrations and temperature-pressure-shear limits when producing the desired product (Dang & Copeland 2004). Pasting properties are often estimated from pasting curves obtained using a Rapid Visco Analyzer (RVA), a temperature controlled viscometer that monitors the resistance of a cereal grain sample to a specified shear. In the beginning, the RVA was introduced in the 1980s as a means of rapidly measuring the extent of sprout damage in wheat affected by rain before harvesting (Ross et al. 1987). Originally, it was built to operate at a constant temperature (95°C), but the addition of variable and controlled heating and cooling made the instrument more versatile and enabled its use in the measurement of the pasting properties of other cereal starches under variable conditions (Walker et al. 1988). Currently, the RVA is an industry-wide instrument which is used extensively for product development, quality and process control and quality assurance of various cereals and starches (Doutch et al. 2012). Standard methods for

measuring starch pasting properties have been developed and have also been approved by the American Association of Cereal Chemists (AACC) (Doutch et al. 2012). However, the ability of RVA to differentiate between samples and to predict the quality of products may vary under various operating conditions (Konik et al. 1992; Batey et al. 1997; Batey et al. 2000). The pasting properties measured by RVA can indicate the cooking characteristics of rice to a certain extent (Champagne et al. 1999). Cooking time is normally the time required for 90 % of the kernels to become completely translucent when cooked by immersion in distilled water at 95±1^{o}C (Ranghino 1966). However, for RVA analysis the grain is ground to mm size. Therefore, it is not the same as cooking whole grains, but the technique is still found to provide useful information on the cooking properties of rice (Zhu et al. 2013). Numerous studies have been done aimed at predicting rice cooking behavior (Mohapatra et al. 2006; Han & Lim 2009). In most of these studies, the focus has been to develop a link between cooking time and rice physicochemical characteristics (Vidal et al. 2007). It has been widely reported that kernel size and shape (especially thickness) are the key factors influencing cooking time, but cooking time is also dependent on the composition of the rice kernel, as rice with high protein (Martin & Fitzgerald 2002) and amylose contents (Yu et al. 2009) has been found to have a longer cooking time. Glutinous rice varieties with low amylose content have been found to have different pasting properties compared to non-glutinous varieties (Huaisan et al. 2009; Bao et al. 2004).

Before cooking, the soaking (rehydration) makes the grain softer, enabling water uptake by the starch during gelatinization (Kashaninejad et al. 2007). Rehydration is a slow and diffusion limited process. Besides the inherent effect of rice kernel composition and internal structure, the diffusivity of water is a function of time and temperature (Bello et al. 2010). Warm water rehydration is a common method used to shorten the soaking time because a higher temperature will increase the hydration and diffusion rates. Rehydration should be done below the starch gelatinization temperature to reduce the leaching of solids and unintended gelatinization during this process (Han & Lim 2009). Thus, to improve the cooking properties and quality, rice is sometimes soaked in water for hours before cooking; however, there is limited data available relating to the effects of soaking temperature and soaking time for different types of rice (Lee at al. 2001). This also applies to glutinous varieties of rice, as systematic studies on the effects of soaking time and temperature on glutinous varieties are very limited in the literature. Moreover, no such information is available on the glutinous varieties that are widely grown and consumed in Lao PDR. This study was aimed

to investigate the effect of rehydration time and temperature and extended holding during cooking, on the pasting properties of the common Laotian glutinous rice cultivars. Two non-glutinous rice varieties were used as reference rice samples for comparison purposes. The results of the study provide a new data in the literature on the pasting properties of common Laotian glutinous rice varieties.

3.3. Materials and methods

3.3.1. Materials

Three cultivars of glutinous rice (TDK11, TDK8, and Hom Mali Niaw) were used in this study. The freshly harvested and milled TDK8 was provided by National Agriculture and Forestry Research Institute (NAFRI), Lao PDR, while about eight-month-old TDK11, Hom Mali Niaw (HMN) and the reference non-glutinous rice varieties, IR64 and Doongara (DG), were provided by Rice Research Australia Pty Ltd (RRAPL), Mackay, QLD, Australia.

3.3.2. Grinding of rice kernels

All rice samples were ground to flour using a hammer mill equipped with a plate of 0.75 mm size. The samples which passed through this plate were used for RVA analysis.

3.3.3. Apparent amylose content

The apparent amylose content (AAC) of rice samples was determined by the iodine colorimetric method (Hoover & Ratnayake 2005). This method is based on the fact that iodine-amylose complex formation gives a blue color, but iodine-amylopectin gives a purple color. This means that the iodine-amylose complex absorbs other light spectrum but reflects the blue. Measuring the absorbance in the non-blue region (peak absorbance at 600 nm) will provide the amount of amylose present in a sample (Knuston & Grove 1994). Samples (20±0.1 mg) in a tube (round bottom with Teflon cap) were dispersed with 8 mL 90 % DMSO (v/v) and thoroughly mixed with vortex for 5 min. To establish a standard curve, a series of potato amylose (Sigma A-0512) and waxy maize amylopectin (Sigma S-9679); mixtures at different ratios at the same solids concentrations were dissolved in 90 % DMSO (v/v) in the same way as the test samples. The tubes containing samples were heated in a water bath at 85ºC for 15 min with intermittent mixing, allowed to cool to room temperature (~ 45 min) and then diluted to 25 mL with deionised water. Then, 1 mL of the diluted solution was mixed with 40 mL of deionised water and 5 mL iodine reagent (2.5×10^{-3} M I_2/6.5 x

10^{-3} M KI) in a 50 mL volumetric flask. The tubes with these diluted solutions were vortexed for thorough mixing and left for 15 min at room temperature for color development. The absorbance of the standards and samples was measured at 600 nm against a blank reagent as the reference. The apparent amylose content was determined from the amylopectin/amylose standard curve.

3.3.4. Pasting properties

The pasting properties of rice flours were determined according to the AACC International Method 61-02.01(AACC 1999) by using a Rapid Visco Analyzer (RVA-4D model Thermocline Windows Control and analysis software, Version 1.2 (New Port Scientific, Sydney, Australia)). To calculate the sample size for RVA, the moisture contents of all the samples were measured according to the AACC International Method 44-40.01(AACC 1999) by using a vacuum oven. Based on the moisture contents, the sample and deionised water required for each cultivar were calculated as follows: TDK11 (3.00 g sample and 25 mL deionised water), TDK8 (3.01 g sample and 25 mL deionised water), HMN (3.01 g sample and 25 mL deionised water), IR64 (3.02 g sample and 25 mL deionised water), and DG (3.00 g sample and 25 mL deionised water). Deionised water was dispensed in RVA canisters. Accurately weighed samples were transferred onto a water surface in the canisters. Subsequently, an RVA paddle was placed in each canister and firmly inserted into the RVA. Samples were mixed at 960 RPM for 10 sec to make a homogeneous solution. After 10 sec the RPM was reduced to 160, which was maintained until the end of the run. A standard program of heating and cooling cycles was: holding the sample at 50ºC for 1 min, followed by heating to 95ºC in 3.45 min; holding at 95ºC for 2.7 min, then cooling to 50ºC in 3.91 min and holding at 50ºC for 1.24 min.

3.3.5. Effect of rehydration time and temperature

The effect of rehydration time and temperature on the pasting properties of the rice flours was investigated by altering the standard AACC International method. The rice flours were rehydrated at different temperatures for different periods of time: *control* (50ºC for 1 min)), t_1 (50ºC for 15 min), t_2 (50ºC for 30 min), t_3 (40ºC for 1 min), t_4 (40ºC for 15 min), t_5 (40ºC for 30 min), t_6 (30ºC for 1 min), t_7 (30ºC for 15 min) and t_8 (30ºC for 30 min). The remainder of the procedure was left unaltered. These temperature conditions were chosen based on the practical rehydration temperature of rice before cooking.

3.3.6. Effect of holding time at 95ºC

The effect of holding time at 95ºC on the pasting properties of rice flours was investigated by altering the standard AACC International Method. This was intended to determine the effect of prolong shear on the rice at the cooking temperature. The rice flours were held at 95ºC for different time periods, *control* (2.7 min), T_1 (4.7 min), T_2 (6.7 min), T_3 (8.7 min) and T_4 (10.7 min). The rest of the procedure was left unaltered.

3.3.7. Statistical analysis

All treatments were replicated three times to get mean values. The reported data for the Pasting temperature (P_{temp}), Peak viscosity (V_p), Trough viscosity (V_t), Breakdown (BD), Final viscosity (V_f) and Setback (SB) for all rice flours were analyzed by analysis of variance (Two-Factor Factorial Design) using Minitab R16 (Minitab® for Windows Release 16, Minitab Inc, Chicago) in order to determine significant differences. The data was then analyzed using Tukey's pair-wise comparison, at 5 % level of significance, to compare the results between different treatments.

3.4. Results and discussion

3.4.1. Apparent amylose content

The rice cultivars selected for this study represented a wide variation in AAC, ranging from 3.72 % to 19.71 % (Table 3.1). Previous studies reported that AAC of various waxy and non-waxy rice genotypes ranged from 0 % to as high as 29.2 % (Dang & Copeland 2004; Kong et al. 2015). As expected TDK8, TDK11 and HMN, contained very low levels of amylose, and are therefore classified as glutinous rice varieties, while IR64 and DG (with amylose contents in the range of 14.92 and 19.71 %) are classified as non-glutinous rice varieties. The variation in AAC has been reported to differ with the botanical source of the starch and is usually affected by the climatic and soil conditions during grain development (Tashiro & Wardlaw 1991; Singh et al. 2006; Wang et al. 2010). This study revealed how the glutinous and non-glutinous group would respond to an extension of rehydration and cooking time (holding at 95ºC). The results (Fig. 3.1), show a positive correlation between the AAC and P_{temp}; higher the AAC the highest P_{temp} will be. The onset of gelatinization is indicated by the pasting temperature (P_{temp}).The glutinous varieties had significantly ($P<0.05$) lower P_{temp} than the non-glutinous cultivars (IR64 and DG) used in this study.

Table 3.1 Apparent amylose content (AAC) of selected rice varieties

Variety	AAC (%)
TDK11	3.72±0.07
TDK8	3.77±0.15
HMN	3.82±0.07
IR64	14.92±0.29
DG	19.71±0.59

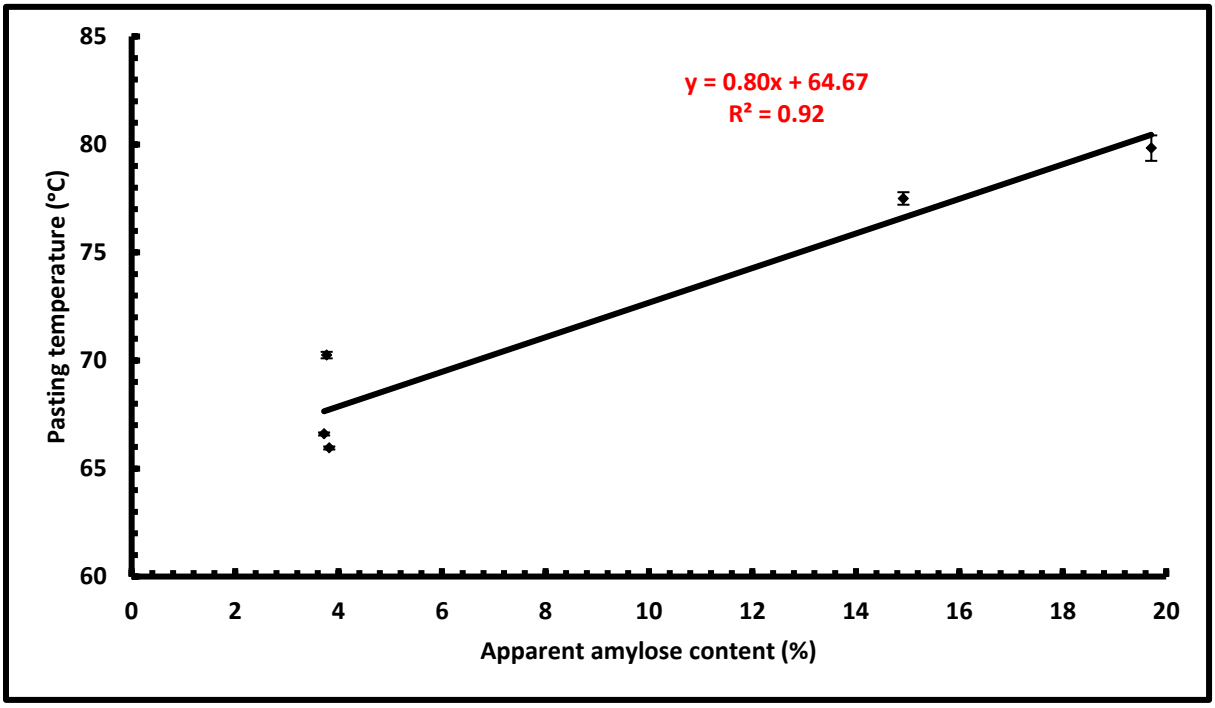

Figure 3.1 Change in pasting temperature (onset of gelatinization) with increasing apparent amylose content (AAC)

3.4.2. Effect of rehydration time and temperature on pasting properties

The viscograph is designed to study the three basic properties of starch. First, when starch particles are subjected to heat with water, they will swell, forms a paste and provides a body. The second important property of the starch is the stability of the body or paste or the viscosity. The third property is the extent of paste congelation while cooling. The representative RVA curves for selected glutinous rice varieties (TDK11, TDK8, and HMN) under standard analytical conditions showing all the pasting attributes, are presented in Fig. 3.2.

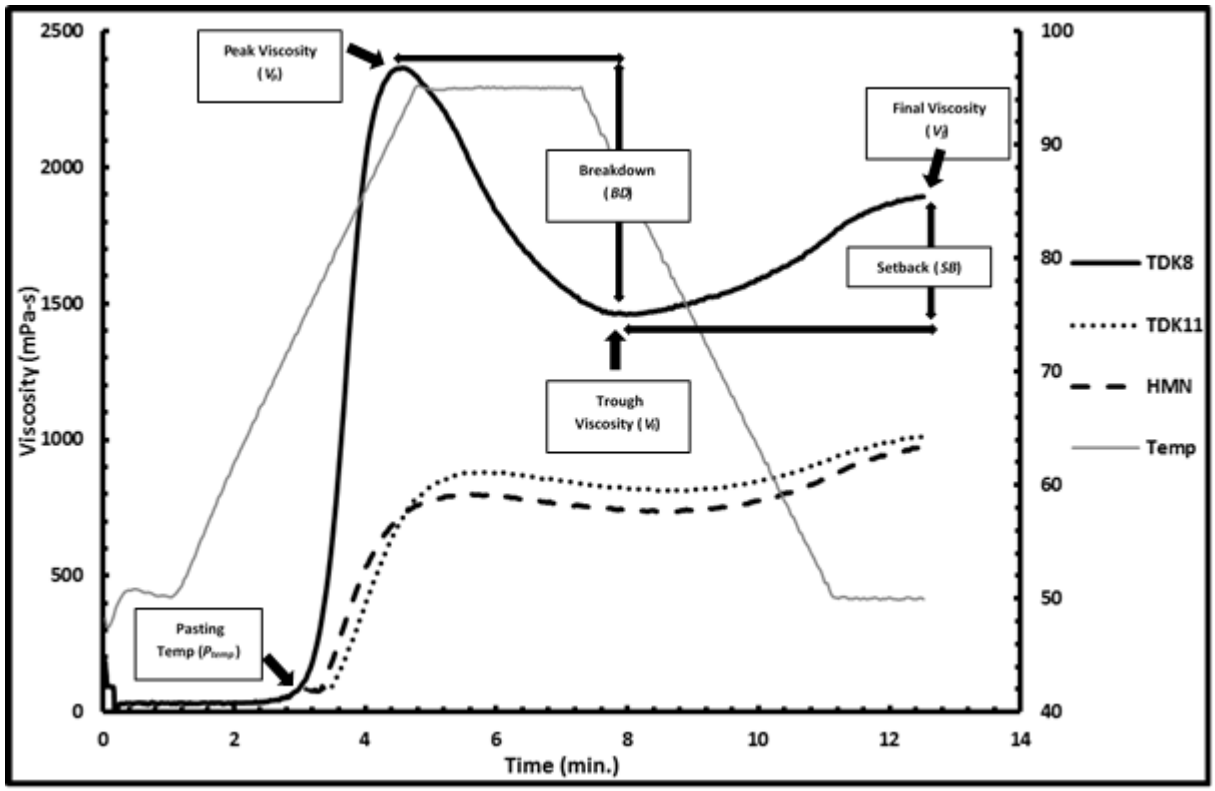

Figure 3.2 Representative RVA curves of selected glutinous varieties (TDK11, TDK8, and HMN) at standard RVA analysis

The temperature at the onset of the rise in the viscosity, known as pasting temperature (P_{temp}) is considered as an indication of the minimum temperature required for cooking (Yadav et al. 2014). The equilibrium point between starch granules is swelling, and polymer leaching is known as peak viscosity (V_p). This normally happens close to 95ºC in RVA analysis and can represent the cooking temperature of rice. It is the maximum viscosity attained by gelatinised starch during heating. V_p indicates the water binding capacity of starch granules (Shimelis et al. 2006) at this temperature. In fact, this viscosity value also depicts a balance between water holding capacity and breakdown of starch at the shear rate. During the hold period at this temperature, the samples are subjected to a period of constant mechanical shear stress. Continuous stirring of the RVA pedal at high temperature (95ºC) can result in disruption of starch granules and a reduction in viscosity, which eventually reaches to a minimum value, known as trough viscosity (V_t) (Song & Shin 2007). The difference between peak and trough viscosity is commonly referred to as breakdown viscosity (BD). Re-alignment of starch molecules occurs when the mixture is allowed to cool; consequently, a gel is formed. This leads to enhanced viscosity known as final viscosity (V_f). It is usually considered as a quality parameter to measure the strength of gel upon cooling (Cornejo-Villegas

et al. 2010). The range of the RVA curve between the trough and final viscosity is usually referred to as the setback region. The difference between the two ends of this region is known as setback viscosity (SB) (Zhu et al. 2013). The effect of rehydration time and temperature on the pasting properties of glutinous and non-glutinous rice cultivars with various amylose contents used in this study is shown in Fig. 3.3, 3.4, 3.5, 3.6, 3.7 and 3.8.

3.4.2.1. Pasting temperature (P_{temp})

A range of factors governs variations in the $P_{temp,}$ but the type of starch in the endosperm is the most significant factor (Thomas et al. 2014). It is now a very well established concept that water uptake by the starch granules is directly proportional to time and temperature of rehydration (Buggenhout et al. 2014; Briffaz et al. 2014). Higher water uptake will result in a decrease in the onset pasting temperature which is also a trend observed in all glutinous rice varieties investigated in this study (Fig. 3.3). This effect was not found in the non-glutinous variety IR64 but was found in the variety DG. This indicates that the effect is variety dependent and will certainly be influenced by the physicochemical characteristics of the grain (Bao et al. 2004).

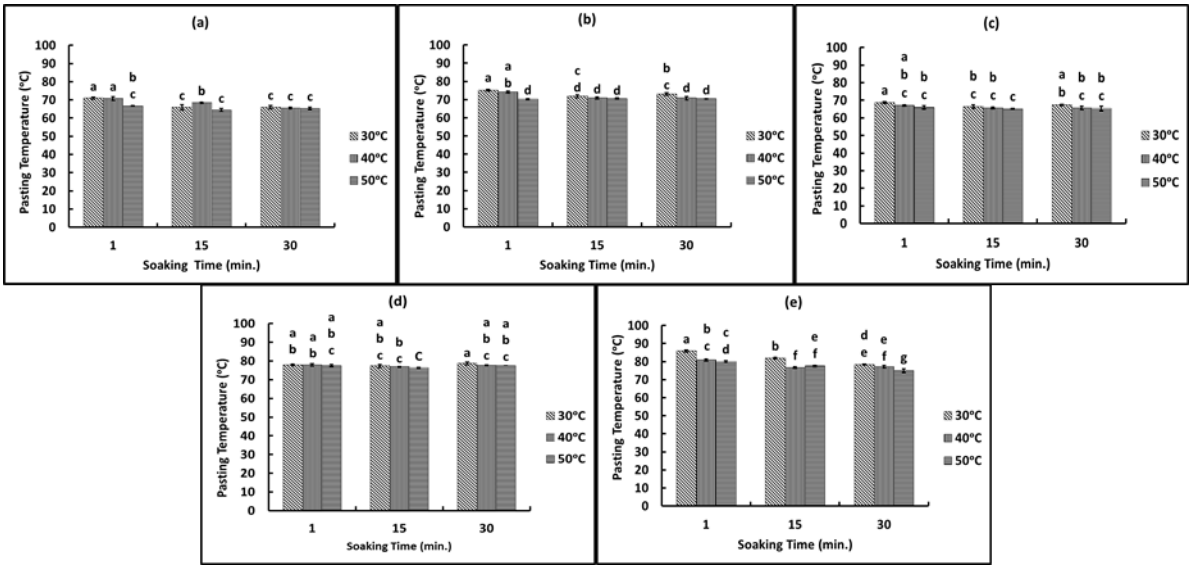

Figure 3.3 Effect of soaking time and temperature on the pasting temperature (P_{temp}) of three different glutinous rice varieties; (a) TDK11, (b) TDK8, (c) HMN, and two non-glutinous rice varieties; (d) IR64, and (e) DG

Pasting temperature (P_{temp}) for TDK11 after 1 min of soaking was more significantly ($P<0.05$) reduced at 50ºC when compared with 30ºC and 40ºC. However, soaking time did not have a significant ($P>0.05$) effect. A similar trend was observed for the varieties TDK8 and HMN. The

results revealed that 1 min of soaking of flour particles (≤750 µm) at ambient temperature is optimum for the onset of gelatinization (Song & Shin 2007). Among the waxy rice, TDK8 exhibited the highest P_{temp} during all rehydration conditions (Fig. 3.3b). Pasting properties are related to amylopectin branch chain length distribution. The presence of long chain branches contributes to increased pasting temperature (Jane et al. 1999). The higher proportion of long chains (DP≥37) in amylopectin results in a relatively high level of stabilised starch granules, leading to higher pasting temperature or the gelatinization temperature (Koroteeva et al. 2007). IR64 and DG (non-glutinous varieties) had higher P_{temp} than the glutinous varieties TDK11, TDK8, and HMN. This means that the absence or low amount of amylose can also facilitate the gelatinization of amylopectin. Similar observations have also been reported by Tran and co-workers (2001) for other rice varieties.

3.4.2.2. Peak viscosity (V_p)

The temperature of the soaking had a much greater impact on peak viscosity (V_p) than the duration of soaking. Different glutinous rice cultivars behaved in a diverse manner when treated with various soaking times and temperatures (Fig. 3.4). The peak viscosity (V_p) of TDK11 (Fig. 3.4a) showed a significant ($P<0.05$) increase when soaked at 40ºC, while a further increase in temperature had no significant ($P>0.05$) effect. It is also observed that the duration of soaking had no significant ($P>0.05$) effect on peak viscosity (V_p), except 30 min of soaking at 40ºC which resulted in a significant ($P<0.05$) decrease in peak viscosity (V_p). TDK8 showed a significant ($P<0.05$) decrease in the peak viscosity (V_p) with an increase in soaking temperature. Maximum viscosity (2403.3 mPa-s) was observed within 1 min of rehydration at 30ºC, and minimum viscosity (1852.0 mPa-s) was observed after 15 min of rehydration at 50ºC (Fig. 3.4b). HMN showed a significant ($P<0.05$) increase in peak viscosity (V_p) at 40ºC, with a further increase in temperature, resulting in a significant ($P<0.05$) decrease in viscosity (Fig. 3.4c). TDK8 showed a significant ($P<0.05$) decrease in the peak viscosity (V_p) with an increase in rehydration time and temperature. Maximum viscosity (2403.3 mPa-s) was observed after 1 min of rehydration at 30ºC, and minimum viscosity (1852.0 mPa-s) occurred after 15 min of rehydration at 50ºC (Fig. 3.4b). The V_p of TDK11 and HMN increased to highest values (1608.7 and 1477.7 mPa-s, respectively) with an increase in temperature up to 40ºC for 1 min (Fig. 3.4a and 3.4c). A further increase in either rehydration time or temperature decreased the V_p in both TDK11 and HMN. HMN showed

the lowest V_p for all treatments. You et al. (2014) suggested that the amylopectin with more short chains (DP6-12) would result in a lower pasting temperature and peak viscosity, as the short branch chains do not provide strong interactions (Chung et al. 2011) to maintain the integrity of the swollen granules, resulting in lower peak viscosity. Peak viscosity is the balance between starch swelling and breakdown at the given shear rate. In the non-glutinous rice, the variety DG showed susceptibility towards changes in rehydration conditions (Fig. 3.4e), with an increase in time and temperature of soaking increasing the equilibrium point of granules swelling and leaching of amylose (Hasjim et al. 2012). IR64 was quite stable in all soaking conditions, and no significant changes were recorded (Fig. 3.4d).

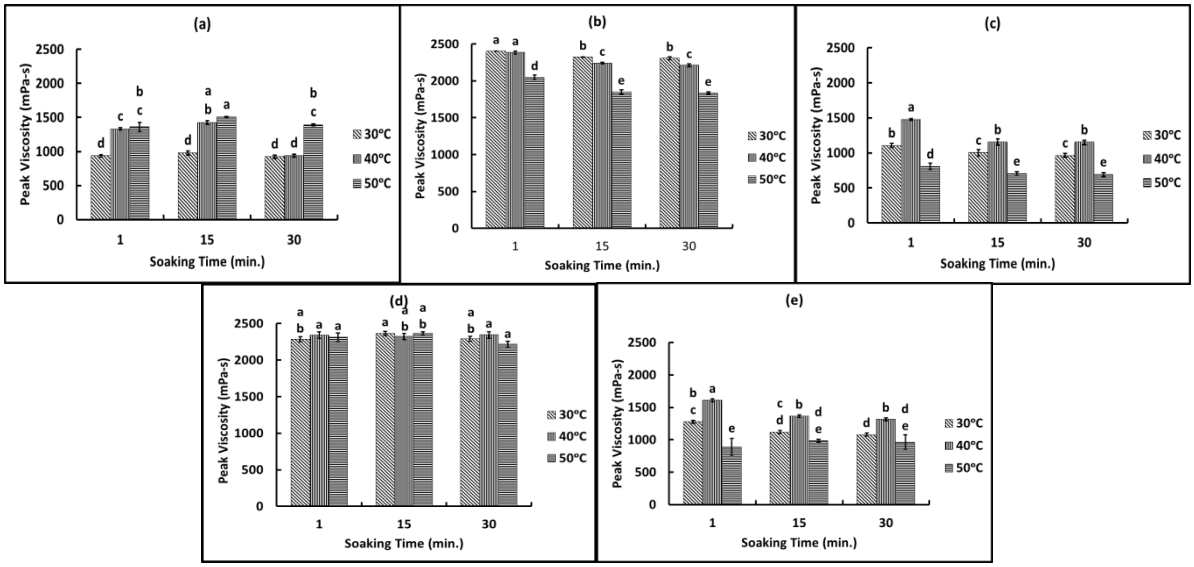

Figure 3.4 Effect of soaking time and temperature on the peak viscosity (V_p) of three different glutinous rice varieties; (a) TDK11, (b) TDK8, (c) HMN, and two non-glutinous rice varieties; (d) IR64, and (e) DG

3.4.2.3. Trough viscosity (V_t) and breakdown (BD)

TDK11 and HMN flours showed significantly higher breakdown at 40ºC (Fig. 3.5a and 3.5c). Among the waxy rice varieties, the higher breakdown was observed for TDK8 (Fig. 3.5b). Mostly breakdown is correlated with peak viscosity, the higher the peak viscosity, the greater the level of breakdown (Higley et al. 2003). In general, a longer soaking time was not reflected in significant differences in the trough viscosity of the rice samples (Fig. 3.6). However, the temperature of soaking showed a reduction in the trough viscosity in all rice varieties except TDK8 which showed an increasing trend. The reduction in trough viscosity was much greater for the samples soaked at 50ºC. Thus, the breakdown of starch granules exhibited a strong positive correlation with soaking

time and temperature among both the glutinous and non-glutinous rice cultivars evaluated (Fig. 3.5). A high level of breakdown is associated with a high degree of collapse of swollen starch granules (low trough viscosity). This may indicate a softer texture of the gel or cooked grain.

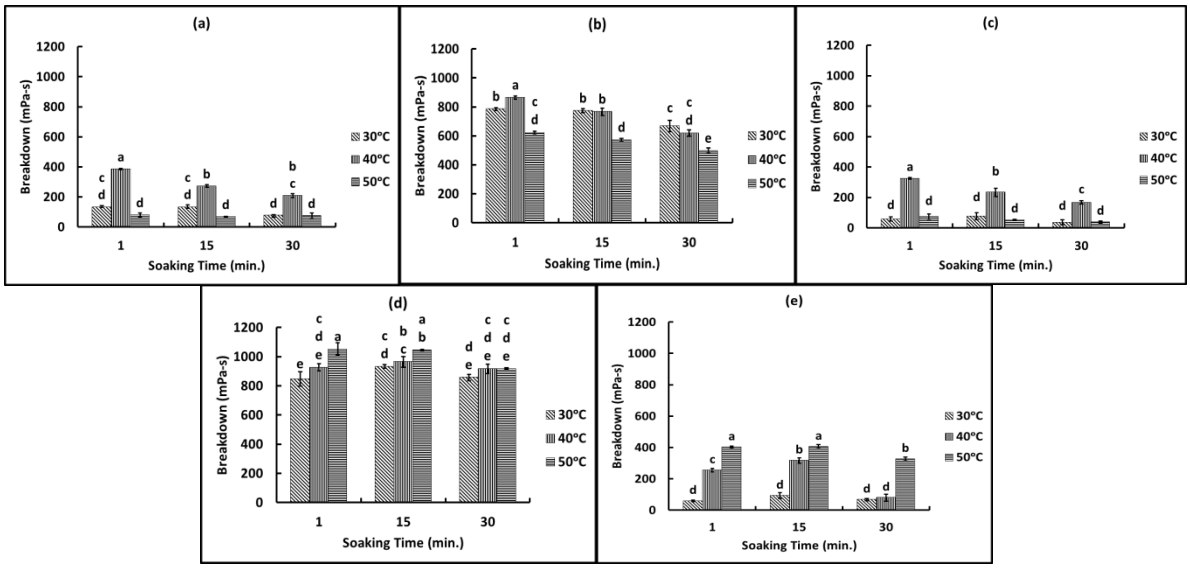

Figure 3.5 Effect of soaking time and temperature on the breakdown viscosity (BD) of three different glutinous rice varieties; (a) TDK11, (b) TDK8, (c) HMN, and two non-glutinous rice varieties; (d) IR64, and (e) DG

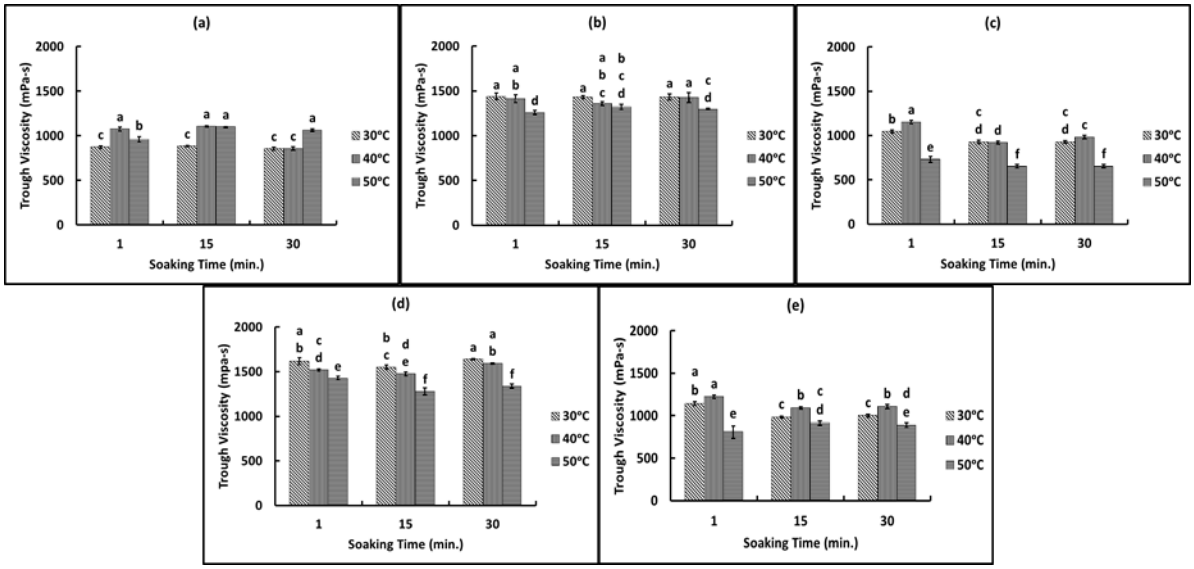

Figure 3.6 Effect of soaking time and temperature on the trough viscosity (V_t) of three different glutinous rice varieties; (a) TDK11, (b) TDK8, (c) HMN, and two non-glutinous rice varieties; (d) IR64, and (e) DG

3.4.2.4. Final viscosity (V_f) and setback (SB)

The final viscosity was measured by cooling the sample to 50ºC. The increase in viscosity due to cooling reflects the effects of temperature on viscosity and retrogradation of starch. Retrogradation ability of straight chain amylose molecules is much higher and faster than for branched amylopectin (Suzuki et al. 2006). The glutinous varieties containing a very low amount of amylose retrogrades very slowly (Singh et al. 2012). Similar observations were recorded in this study through the observation of reduced final viscosity of the gel (Fig. 3.7 and 3.8). Among the glutinous rice, TDK11 (Fig. 3.7a) and TDK8 (Fig. 3.7b) showed significantly ($P<0.05$) high setback (SB) at 40ºC from soaking for one min while HMN (Fig. 3.7c) showed significantly ($P<0.05$) high setback at 50ºC after soaking for 1 min. Non-glutinous rice IR64 and DG (Fig. 3.7d and 3.7e, respectively) showed significant ($P<0.05$) increase in setback (SB) with an increase in soaking temperature. For both varieties, maximum setback (SB) was observed at 50ºC. It is observed that among the soaking conditions only temperature significantly affects the setback (SB) rather than the time of soaking.

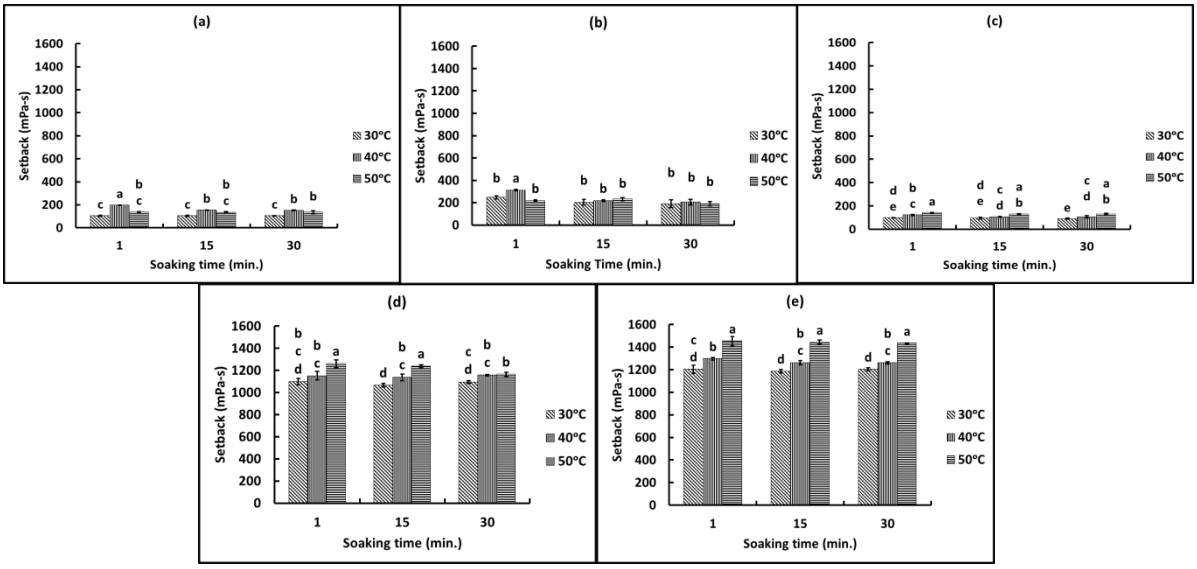

Figure 3.7 Effect of soaking time and temperature on the setback viscosity (SB) of three different glutinous rice varieties; (a) TDK11, (b) TDK8, (c) HMN, and two non-glutinous rice varieties; (d) IR64, and (e) DG

With a decrease in soaking temperature to less than 40ºC, strong resistance was recorded by the RVA paddle, resulting in high final viscosity (V_f). The soaking time did not show a strong effect on the final viscosity, but all glutinous varieties showed a decline in final viscosity (V_f) at higher

soaking temperature. Among the three varieties, TDK8 (Fig. 3.8b) showed the greatest sensitivity to soaking temperature. Similar effects were observed in the non-glutinous rice, (both IR64 and DG) but the retrogradation would be higher in non-glutinous rice due to the presence of higher amounts of amylose.

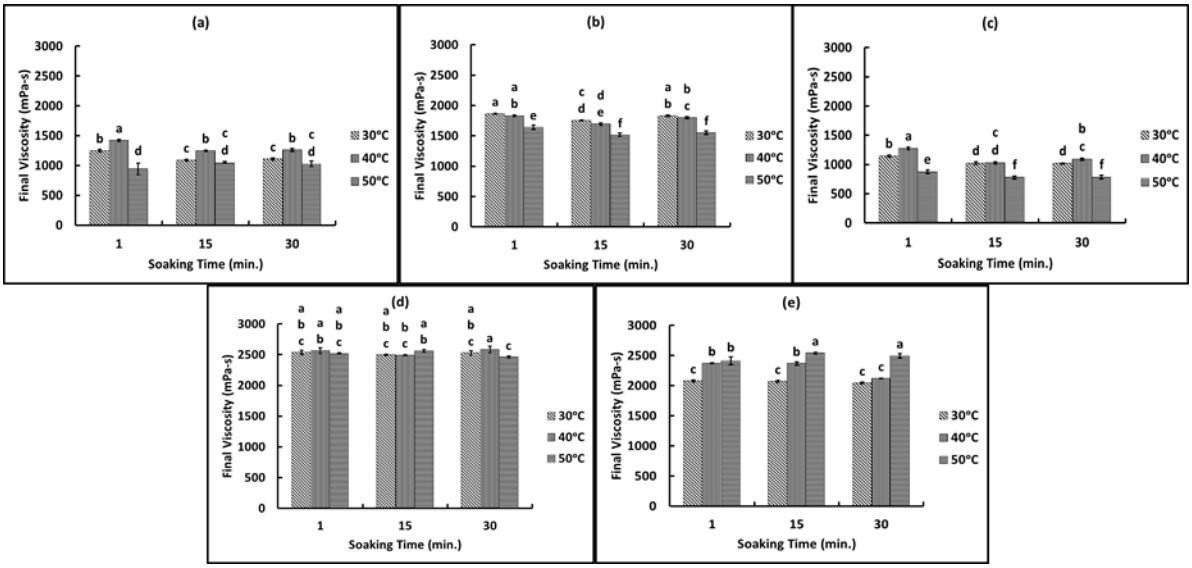

Figure 3.8 Effect of soaking time and temperature on the final viscosity (V_f) of three different glutinous rice varieties; (a) TDK11, (b) TDK8, (c) HMN, and two non-glutinous rice varieties; (d) IR64, and (e) DG

3.4.3. Effect of an extension of holding time at 95ºC on the viscosity

Increased shear at cooking temperature is expected to increase the breakdown, and reduce trough and final viscosities. The effect of extended holding time at 95ºC on the pasting properties of all rice cultivars used in this study is shown in Fig. 3.9. As expected, there was a significant difference ($P<0.05$) in the pasting properties of all five glutinous and non-glutinous rice cultivars used in this study. Peak viscosity (V_p) for all cultivars with various amylose contents was significantly ($P<0.05$) different (Fig. 3.9a). However, there was no significant ($P>0.05$) difference in holding time at 95ºC on the peak viscosity (V_p) of TDK8, HMN, DG and IR64 but peak viscosity (V_p) of TDK11 flour increased significantly ($P<0.05$) with an increase in holding time from the control to 4.7 min, but remained constant with any further increase in holding time. In this case, TDK11 was found to be more resistant than the other varieties to the breakdown of the swelled starch particles during shearing at 95ºC. This indicates that not only the amylose content but also the starch grain structure will contribute to the breakdown of gelatinised and swelled starch.

The disruption of starch granules followed the peak viscosity during holding at the same temperature. This period is usually accompanied by a reduction in the viscosity, which eventually reaches a minimum value. This minimum viscosity attained by the starch granules during RVA run is known as trough viscosity (V_t) (Dang & Copeland 2004). The loss of viscosity is caused by leaching of amylose and also a breakdown of the swelled granules due to the shear in RVA. A lot of variation was observed in trough viscosity (V_t) of the glutinous and non-glutinous cultivars used in the present study (Fig. 3.9b), with extended holding at a higher temperature (95^{o}C) having a significant ($P<0.05$) effect on the glutinous cultivars TDK11 and TDK8, and for non-glutinous cultivar IR64. The variety DG showed resistance towards extended heating up till 6.7 min, after which there was a significant ($P<0.05$) disruption of granules and possibly leaching of amylose from starch granules was observed, resulting in a decrease in V_t. While HMN flour was quite stable with extended holding at 95^{o}C, there was no significant ($P>0.05$) change in trough viscosity (V_t). Among the glutinous rice, maximum breakdown (BD) was observed in TDK8 followed by TDK11, while least breakdown was observed in HMN (Fig. 3.9c). Breakdown (BD) of TDK11 and HMN significantly ($P<0.05$) increased up to 4.7 min, with further cooking having no significant ($P>0.05$) effect on breakdown (BD). Significant ($P<0.05$) breakdown (BD) was observed in TDK8 up till 6.7 min of cooking, further cooking had no significant ($P>0.05$) effect. In the non-glutinous rice, IR64 showed higher breakdown than DG. Both varieties had significantly ($P<0.05$) higher breakdown (BD) and leaching of amylose up till 4.7 min of cooking, with further cooking having no significant ($P>0.05$) effect on breakdown (BD) in either of these varieties.

Flour of glutinous rice cultivars produced weaker gels at the final temperature of cooling (50^{o}C) than the non-glutinous flours (Fig. 3.9d). Among the glutinous rice, TDK8 had significantly ($P<0.05$) higher final viscosity (V_f) and setback (SB) than the other glutinous rice (TDK11 and HMN) (Fig. 3.9d and 3.9e). Significantly ($P<0.05$) higher final viscosity and setback was observed in TDK11 up till 4.7 min of cooking, with a further increase in cooking time affecting setback and final viscosity. TDK8 behaved in an entirely different manner to TDK11. Increased cooking time resulted in a significant decline in final viscosity (V_f) and greater setback (SB) up to 4.7 min. Further cooking resulted in low final viscosity (V_f), but the effect was non-significant. There was no significant ($P>0.05$) effect of extended cooking on the final viscosity (V_f) and setback (SB) for the variety HMN. Among the non-glutinous rice, IR64 showed a significant ($P<0.05$) decline in

final viscosity (V_f) and setback (SB) up till 4.7 min, with any further increase not resulting in further significant differences in either final viscosity and setback. The variety DG showed quite a stable behavior towards extended cooking, resulting in no significant differences in final viscosity (V_f) and setback (SB). Thus in general, the effect of holding at 95ºC and prolonged shear exhibited the same effects on glutinous and non-glutinous varieties and was variety dependent.

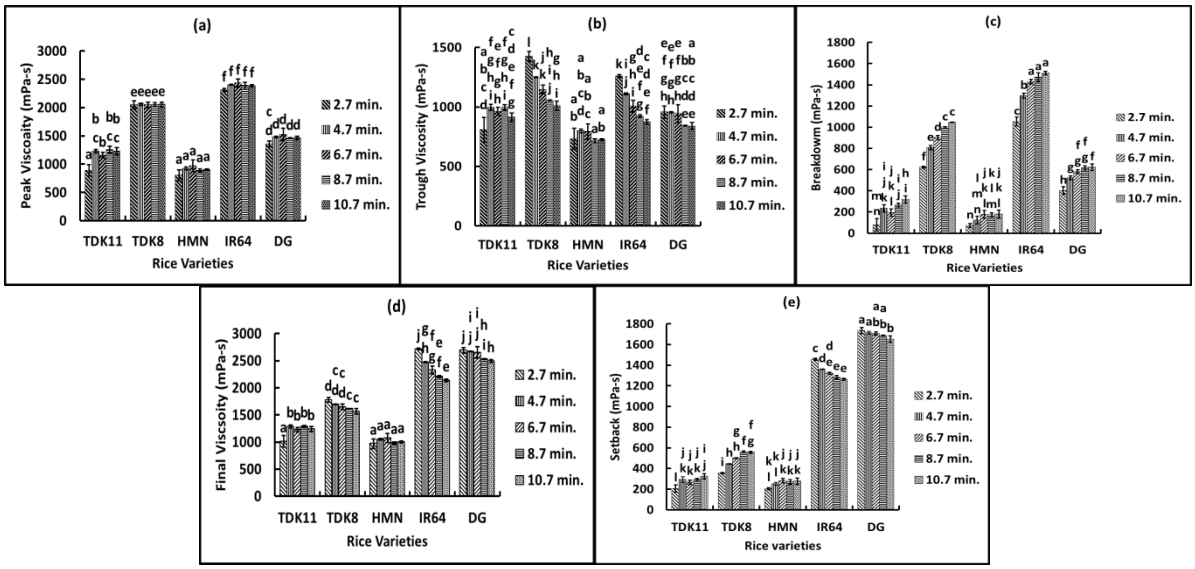

Figure 3.9 Effect of extended holding time at 95ºC on the pasting properties of glutinous and non-glutinous rice varieties; (a) Peak viscosity (V_p), (b) Trough viscosity (V_t), (c) Breakdown (BD), (d) Final viscosity (V_f) and (e) Setback (SB)

Hasjim and co-workers (2013) also reported that increasing the cooking time at 95ºC longer than 4 min caused only small changes in the trough and the final viscosity of polished long grain rice flour.

3.5. Conclusions

A knowledge of rehydration and cooking attributes of various glutinous and non-glutinous rice varieties is very important, as rice is a major ingredient in many processed foods. As expected, significant differences in the pasting properties of various glutinous varieties from Lao PDR were observed. Water uptake by the flour of all rice varieties was directly proportional to the time/temperature of rehydration. Pasting temperature depends on the amylose content, the higher the amylose, the higher will be the onset temperature of starch gelatinization. Among the glutinous rice varieties tested, TDK8 showed the greatest response to rehydration conditions, resulting in highest peak viscosity. The rice cultivars evaluated in the study showed diverse response when

treated with extended cooking. For the glutinous variety TDK8 and non-glutinous variety, IR64 starch granules showed a higher breakdown in response to extended cooking, resulting in reduced trough viscosity and reduced retrogradation. It is therefore recommended that for glutinous rice especially TDK8, extended cooking will result in a better-cooked product. This work has generated the pasting data for the most popular glutinous varieties consumed in Lao PDR. Further work is being undertaken on the cooking, and textural attributes of whole grain of these glutinous varieties as RVA analysis can only use ground samples.

Chapter 4 *In situ* analysis of cooking properties of rice by Thermal Mechanical Compression Test (TMCT) method

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4.1. Abstract

A procedure for *in situ* analysis of rice cooking was developed in this study. Grain softening during soaking and cooking of selected rice varieties (fresh and aged TDK8, TDK11, and Doongara) were subjected to *in situ* analysis by using a thermally controlled sample block (TMCT) attached to a texture analyser. This technique measures the changes in the mechanical properties of intact rice grain during cooking continuously. The results obtained from the TMCT technique were validated against two standard and conventional procedures viz. analysing the pasting properties of rice flours by Rapid Visco Analyzer (RVA) and microscopic observations during the cooking of rice grains. The technique developed in this study was found valid for *in situ* analysis of rice cooking. This technique can be used for a sample size as small as 0.50 g.

4.2. Introduction

Rice is one of the most popular staple cereals consumed around the globe. There are diverse varieties of rice, and their aromatic and textural properties can be very distinct. Consumer preferences for cooked rice vary from region to region, Laotian people like sticky glutinous rice as staple and the Japanese like it on special occasions (Mohapatra & Bal 2006; Boualaphanh et al. 2011), while Italians consume short grain varieties *Baldo* and *Arborio* rices with high amylopectin-content, which release starch during cooking making a creamy and smooth *Risotto* (Puri et al. 2013). Therefore, it is very difficult to find out a standard procedure for rice cooking. For example, traditionally, cooked waxy glutinous rice is produced by soaking overnight before steaming the rice. Prolonged soaking is required to soften the grains and increase the water content to accelerate the starch gelatinization to reduce the steaming (cooking) time. In some population, freshly harvested rice grain is preferred while other population may prefer the aged rice due to the difference in the texture of the cooked rice with aging (Tananuwong & Malila 2011).

Many studies have reported the effect of various cooking conditions such as temperature and pressure (Leelayuthsoontorn & Thipayarat 2006; Tian et al. 2014), water to rice ratio (Srisawas & Jindal 2007), steam cooking and stir-frying (Reed et al. 2013) on the texture of cooked rice. A significant number of researches describing rate and effect of water infusion into non-glutinous rice grains on their cooking quality have been carried out in the past (Bakshi & Singh 1980; Hendricks et al. 1987; Miah et al. 2002b; Tian et al. 2014; Rafiq et al. 2015). The cooking quality of these rice is analysed after the final cooking. This is mainly done by measuring the hardness of

the cooked rice using a texture analyser (Bello et al. 2006). Similarly, although the information about glutinous rice hydration and cooking are very limited, its soaking and softening properties are characterised by some researchers (Singh et al. 2000; Ahromrit et al. 2006; Peerapattana et al. 2010). Moreover, considerable research has been conducted to find out the mechanism of water movement and final cooking qualities of cereal grains to develop kinetics model (Bello et al. 2010). Gelatinization initiated major changes in the physical and chemical properties of starch. Therefore, it is important to get information on the evolution of cooking of rice during the cooking period. RVA analysis of the rice has been reported to interpret the pasting properties to the cooking qualities of rice (Nawaz et al. 2016a). However, during RVA analysis the grain needs to be ground to flour. The rate of moisture diffusion and gelatinization of starch during cooking can be different between flour and whole rice kernel. The cooking behavior and grain characteristic changes with time have also been analysed by microscopic observation such as the presence of un-gelatinised white belly at the interior of the cereal grains undergoing cooking (Lund & Lorenz 1984; Srikaeo et al. 2006). This analysis requires periodical sampling, slicing and microscopic observation during the cooking. The disappearance of the white belly is the indication of the cooking time required. The cooking quality of the rice is analysed by determining the texture (mainly hardness) of the cooked grain by the texture analyser. There are no methods which analyse the rate of cooking and the texture of the whole grain rice as a one-step analysis.

In this study, a new *in situ* method is introduced to study the textural properties of rice using the Thermal Mechanical Compression Test (TMCT) device attached to a texture analyser. The TMCT has been used previously for analysing the stickiness and glass-rubber transition temperature of various food materials (Liu et al. 2010) including spray dried orange juice powder, milk powders, spaghetti and rice (Shrestha et al. 2007b; Boonyai et al. 2007; Rahman et al. 2011; Thuc et al. 2010). This method is based on the measurement of the displacement of a probe compressing the rice grain under a constant force while cooking in a temperature controlled TMCT device. During hydration and gelatinization, the softening of the grain will cause probe movement to maintain a set constant force. This study was aimed to establish a standard procedure for *in situ* cooking analysis by using TMCT. This novel method will also allow the development of kinetic models of cooking and also determine the final cooking time of different rice varieties.

4.3. Materials and methods

Two *Oryza sativa* indica cultivars of glutinous rice from Lao PDR viz. TDK8 and TDK11 having 3.77 % and 3.72 % apparent amylose contents (AAC), respectively and one *O. sativa* japonica non-glutinous rice from Australia (Doongara, 19.71 % (AAC)) were used in this study. The milled TDK8 was provided by National Agriculture and Forestry Research Institute (NAFRI), Lao PDR, while TDK11 and Doongara were provided by Rice Research Australia Pty Ltd (RRAPL), Mackay, QLD, Australia.

4.3.1. The moisture content of rice grains

The moisture content of rice grain was measured according to the AACC International Method 44-40.01(AACC 1999) by using the modified vacuum-oven method. Two grams of well-mixed sample was accurately weighed in a covered dish, which was previously dried at 98-100ºC and, cooled in a desiccator to room temperature. The samples were heated at 98-100ºC to constant weight (for about 5 hrs) in a partial vacuum having pressure equivalent to 25 mm Hg or less. The dried dishes with sample were cooled in a desiccator and weighed soon after it reached room temperature.

4.3.2. Moisture uptake by rice grains

Rice grains (10±2 g, known initial moisture contents) were taken in a sieve (a tea filter). The sieve with the sample was fully immersed in water maintained at 22±1ºC, 40±1ºC, and 50±1ºC. After every 5 min of soaking, the sample was taken out from the sieve and wiped carefully with blotting paper to remove the surface moisture. The sample was weighed carefully, and weight gained by the sample was recorded. The sample was again soaked in water for 5 min. The weight gained by the sample for 45 min was recorded.

4.3.3. Measurement of grain softening during hydration and cooking by TMCT device

The TMCT device used in this work is shown in Fig. 4.1. This device is made-up of the temperature controlled aluminum block (50x50x25 mm) and attached to a Texture Analyser. A single layer of 1 g of rice kernels was soaked in 1 mL of deionised water in thermally controlled (TMCT) aluminum block. The soaked samples were compressed by a 35 mm Teflon probe at the steady force of 0.10 and 0.15 N. The hydration and gelatinization of the starch will cause the softening of

the grain resulting into the displacement of the probe to maintain the constant force. At a compression force of 0.10 N, the force was unstable, so the probe displacement was distorted (Fig. 4.2a). In the preliminary experiment, a load of 0.15 N was found appropriate as the compression of the sample was low (10 % of the grain height at the highest hydration level) and the probe movement signal was found stable (Fig. 4.2b). Thus, the sample deformation at 0.15 N during the measurement was not high. The change in distance of probe during soaking was recorded by the Texture Analyzer TA-XT*plus* (Stable Microsystems, UK) for 1.5 h.

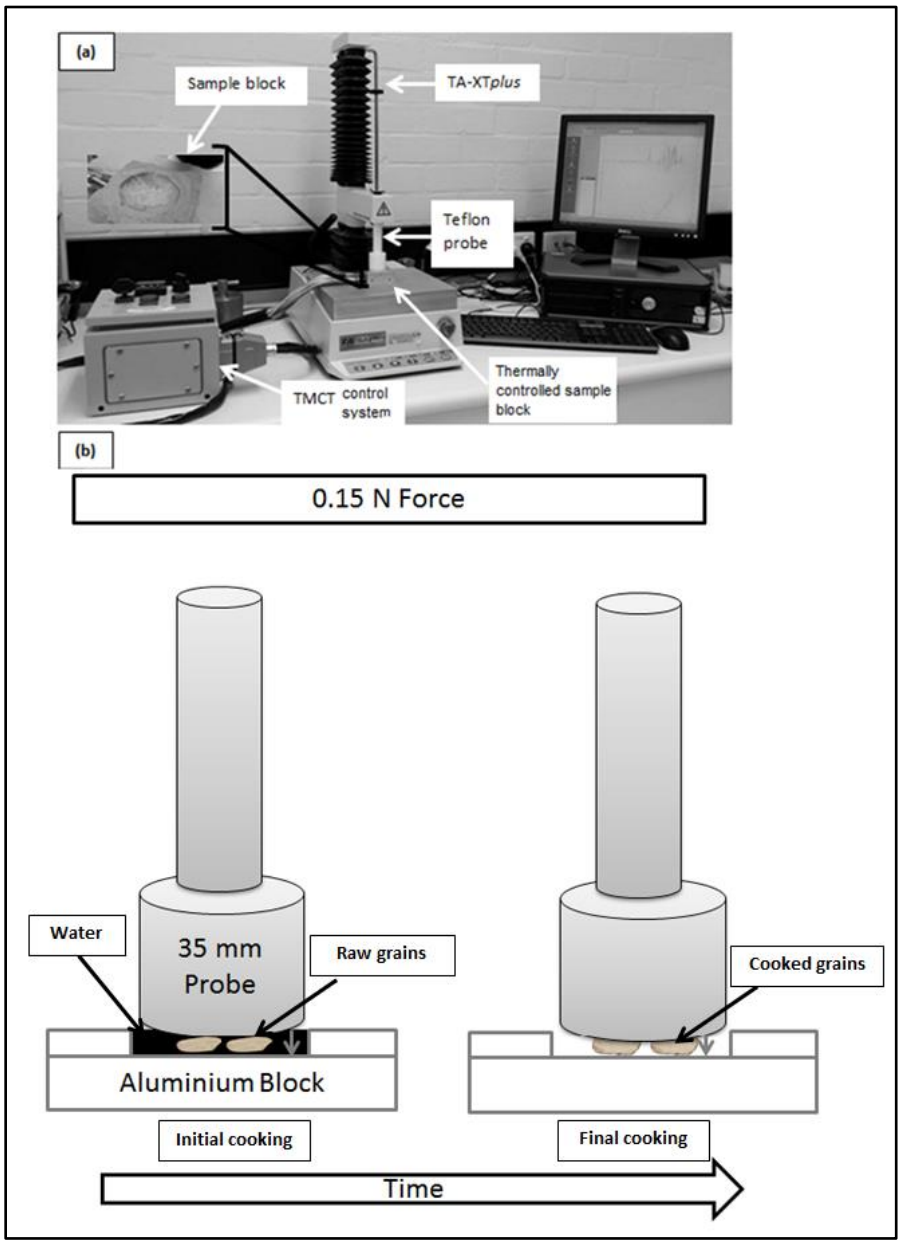

Figure 4.1 Illustration of grain softening during soaking and cooking, (a) Overall experiment assembly, (b) Pictorial representation of the measurement process

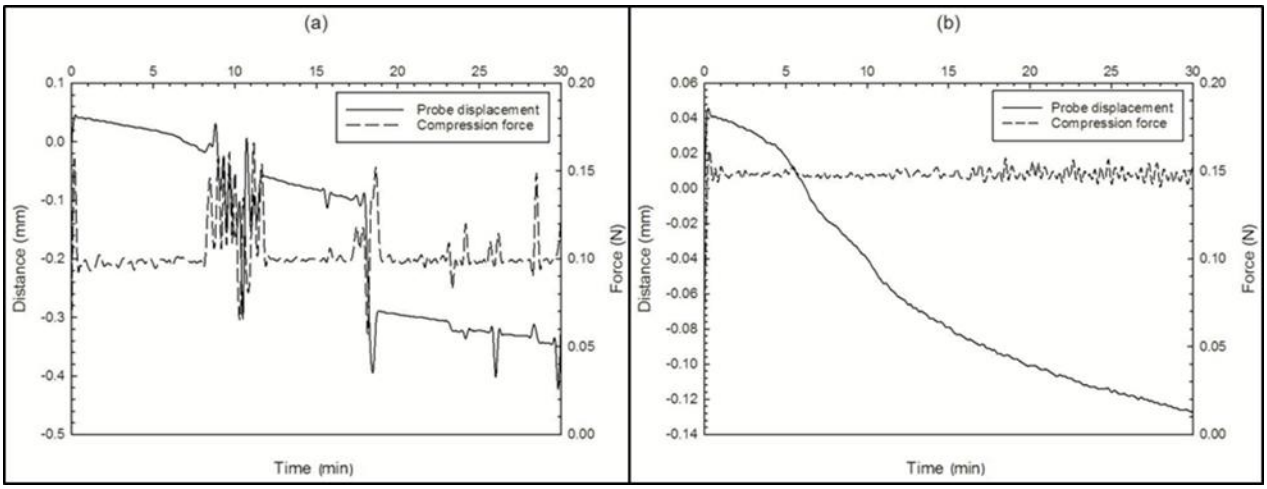

Figure 4.2 Probe displacement during *in situ* soaking of rice in a TMCT device at two compression forces, (a) 0.10 N, and (b) 0.15 N

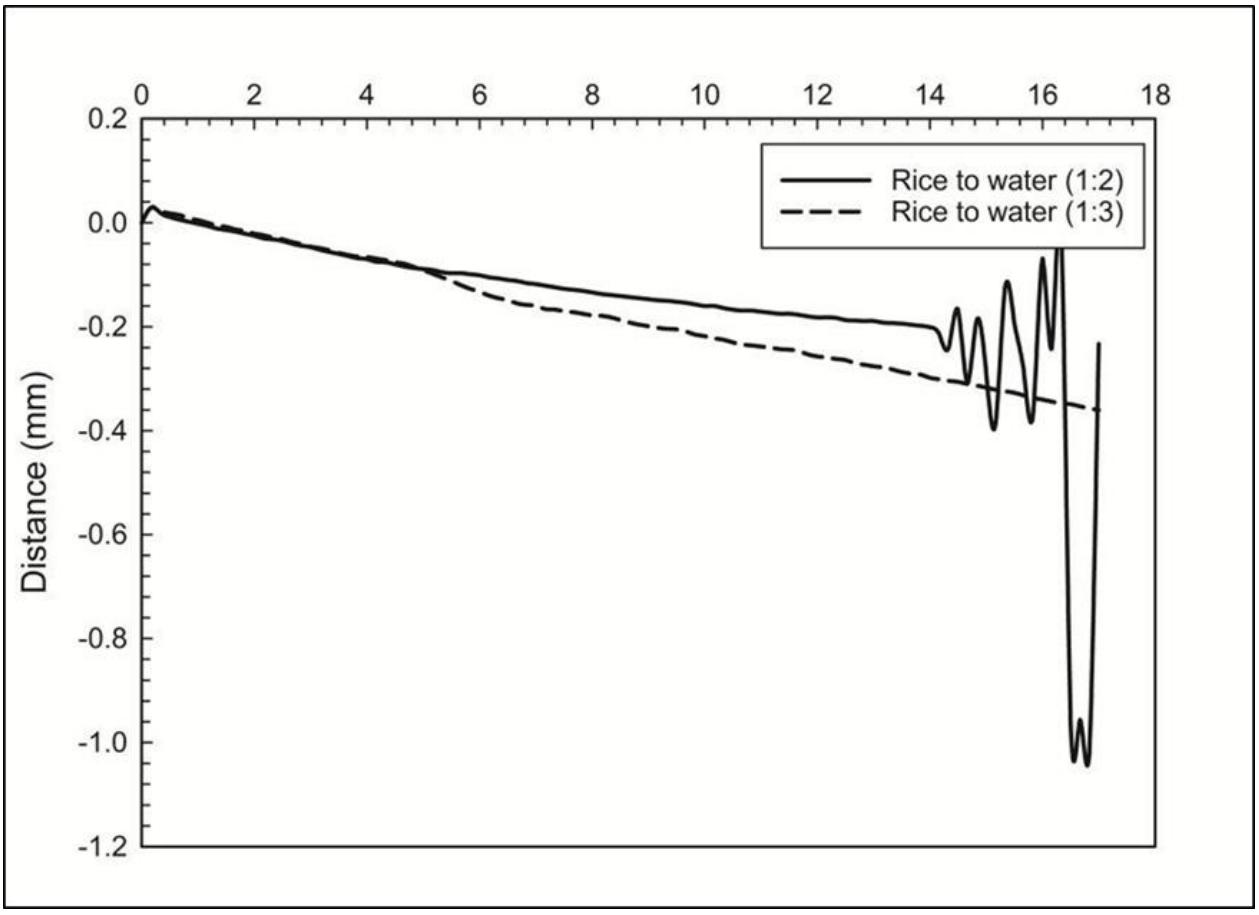

Figure 4.3 The *in situ* TMCT cooking of rice at 95±1°C with two different rice to water ratios (1:2 and 1:3)

A similar experiment was conducted during cooking of rice. The sample block was heated to a rice cooking temperature of 95±1ºC and held it at this temperature. In the preliminary trials various

rice to water ratios such as 1:2 and 1:3 were used to streamline the *in situ* TMCT cooking process. It was observed that 1:2 rice to water ratio was not stable and probe distortion was started within the initial 14 min due to evaporation of water from the sample container. However, it was found that 1:3 provided stable results (Fig. 4.3) with the compression of the sample less than 20 %. The amount of water required to cook the rice is higher due to loss of water from the annulus space between the probe and the sample block, although the gap was very small (<1 mm). Therefore, a single layer of 0.5 g of rice kernels and 1.5 mL of deionised water (rice: water ratio = 1:3) was put on the sample block. The change in distance of probe was recorded by the texture analyser until rice sample has taken up all available water and fully cooked (indicated by the vibration in probe due to the evaporation of water from the cooked grain). The cooking rate of the grains was estimated by measuring the slope of the initial linear part of the *in situ* TMCT cooking curves.

4.3.4. Pasting properties

Pasting properties of rice flour (particle size ~ 750 µm) were determined according to the AACC International Method 61-02.01 (AACC 1999) using a Rapid Visco Analyzer (RVA-4D model Thermocline Windows Control and analysis software, Version 1.2 (New Port Scientific, Sydney, Australia)). Rice flour (3.01 g, 12.4 % moisture basis) was mixed with 25.0 g MilliQ water in the RVA canister. A programmed heating and cooling cycle were used, the samples were held at 50ºC for 1 min, heated to 95ºC in 3.45 min, held at 95ºC for 2.7 min before cooling to 50ºC in 3.91 min and holding at 50ºC for 1.24 min. The peak time (PT), peak temperature (P_{temp}) and peak viscosity (V_p) were recorded.

4.3.5. Light microscopy of rice kernels during cooking

The recorded time required for cooking in the grain softening experiment was cross verified by light microscopy using a Zeiss Axio microscope (Oberkochen, Germany). Five grams rice sample was added in 15 mL of deionised water (rice: water ratio = 1:3) in a 50 mL glass beaker. The beaker was placed in a water bath at 95±1ºC. After every 5 min, about half gram of sample was taken out. Cross sections of rice kernel were studied under a light microscope to see the extent of gelatinization by observing the non-gelatinized white belly. Cooking was continued until there was no white belly observed in rice kernel cross-section.

4.3.6. Statistical analysis

The reported data was analyzed by analysis of variance (Completely Randomized Design) using Minitab R17 (Minitab® for Windows Release 17, Minitab Inc., Chicago) to determine significant differences. The data was then analyzed using Tukey's pair-wise comparison of different treatments, at 5 % level of significance.

4.4. Results and discussion

4.4.1. Comparison of grain softening and moisture uptake during hydration

The grain softening and moisture uptake curves of fresh TDK8 soaked at various temperatures (22±1ºC, 40±1ºC, and 50±1ºC) are shown in Fig. 4.4a and 4.4b, respectively. As expected the uptake of moisture caused the softening of the grain which was measured by the displacement of the compression probe in the TMCT device attached to a texture analyser (Fig. 4.1). Results revealed that grains absorbed moisture quickly and became softer with an increase in soaking temperature. The rate of water absorption and grain softening was higher in the initial 20 min of soaking. The probe displacement due to grain softening and moisture uptake results corresponded very well (Fig. 4.4b).

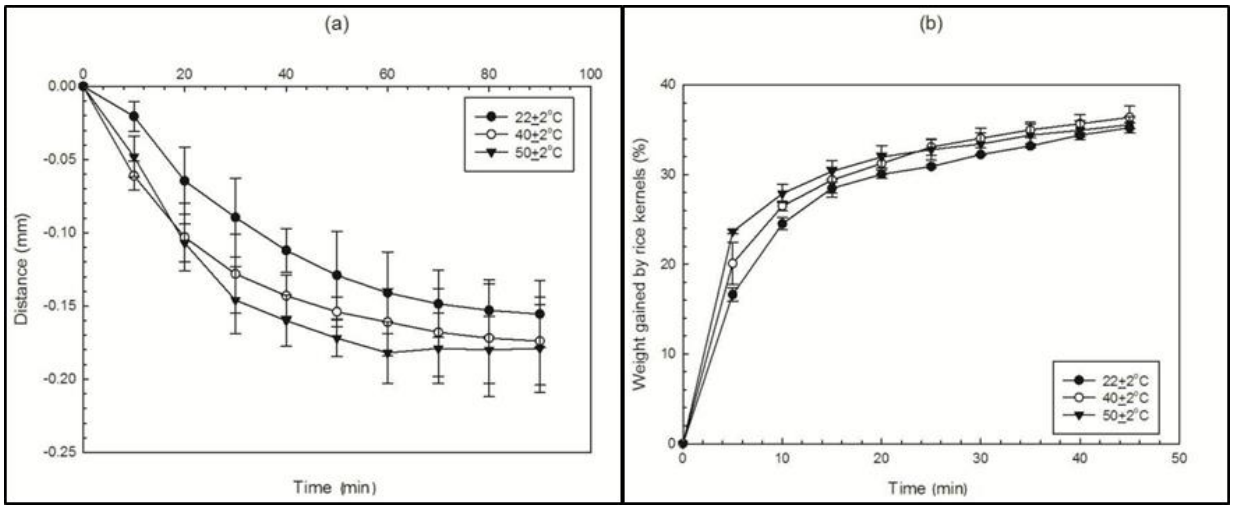

Figure 4.4 Comparison of (a) grain softening measured as probe displacement by using TMCT and (b) moisture uptake during hydration of fresh TDK8 at different soaking temperatures (22, 40 and 50ºC)

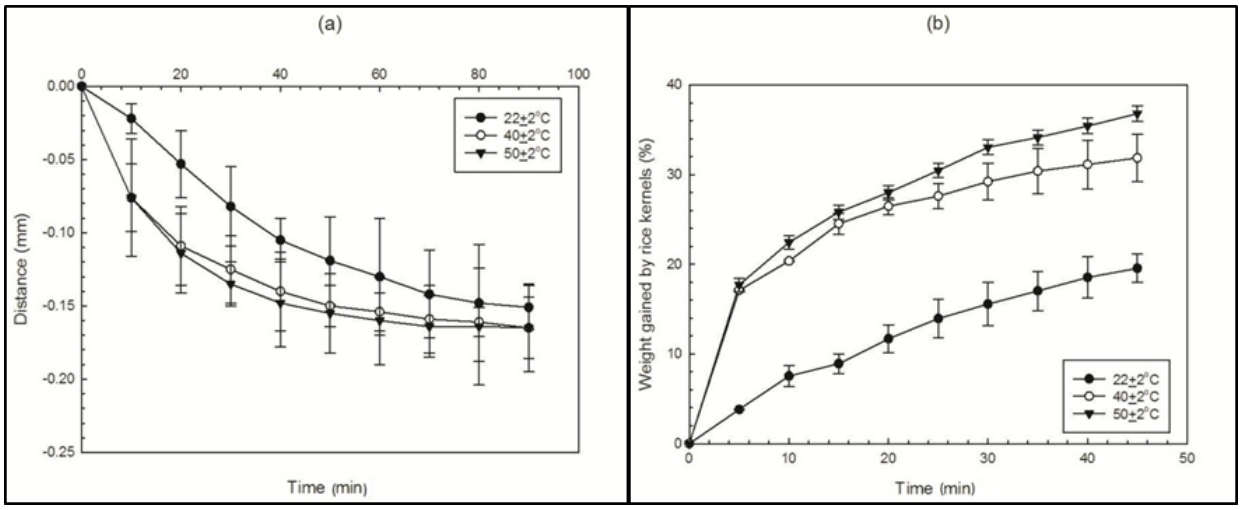

Figure 4.5 Comparison of (a) grain softening measured by the displacement of the probe by using TMCT and (b) moisture uptake during hydration of aged TDK8 at different soaking temperatures (22, 40 and 50ºC)

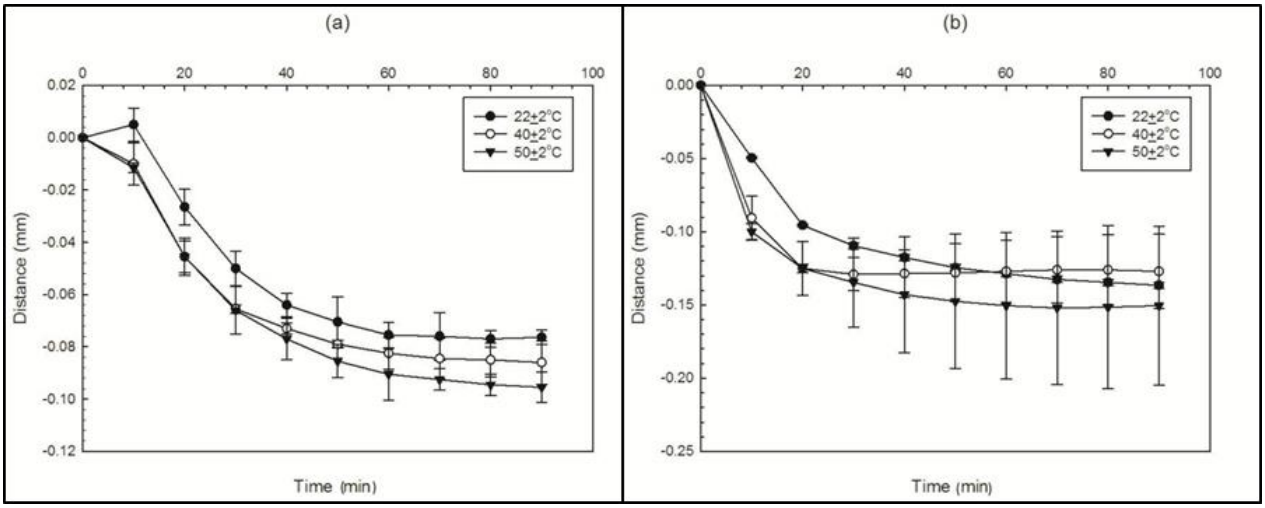

Figure 4.6 Grain softening measured by probe displacement using TMCT for Doongara (a) and TDK11 (b) rice varieties during hydration at different soaking temperatures (22, 40 and 50ºC)

The results showed that most of the water was taken up in 45 min and showed a slow rate of moisture adsorption and also probe displacement beyond this time (Fig. 4.4a) might be due to moisture distribution change within a grain. A similar experiment was undertaken for the aged (6 months) of the same variety (TDK 8) and other varieties of rice. The results also showed the similar trend for aged (Fig. 4.5) and other rice varieties; Doongara and TDK11 (Fig. 4.6a and 4.6b, respectively). The rate of infusion of water into the kernels was increased by increasing the soaking

temperature, resulting in faster softening of grains. The higher water diffusivity at higher soaking temperature is well understood (Miah et al. 2002b; Kashaninejad et al. 2009), and this can also be reflected by the softening of the grain using *in situ* measurement by TMCT device. To note that the variability of the results was evident in both methods. Therefore, the statistical significance ($P<0.05$) only existed between 22 and 50ºC temperature of rehydration. However, the average trend of probe displacement or moisture adsorption as a function of temperature and time was very consistent at all three hydration temperatures. The large error seen in the results are probably originated by the loss of solids during rehydration and variation while blotting the moisture before weighing. This type of variability has been reported in the previous studies with rice (Bello et al. 2010). It should also be noted that the water adsorption rate of aged rice samples was more affected by the temperature of rehydration than the fresh sample. The slow rate of water adsorption by aged rice is well known (Butt et al. 2008).

4.4.2. The *in situ* TMCT analysis of cooking properties

The cooking curves of various rice varieties (fresh and aged TDK8, TDK11, and Doongara) are shown in Fig. 4.7. Results revealed that the fresh and aged TDK8, TDK11 and Doongara grains took 31.5, 53.8, 26.2 and 19.8 min, respectively to absorb all added moisture and completely gelatinize (cooked). It was observed that the rice grain was fully gelatinized and the later part of probe displacement (Fig. 4.7) curve was distorted possibly due to evaporation of moisture from cooked grains as no free water was visible on the sample holder aluminum block when this distortion was observed.

In aged rice, the rate of water uptake and gelatinization were slower than the fresh grains possibly due to age-induced physicochemical changes. Therefore, probe displaced at a much slower rate after 40 min and distortion started at 53.8 min. This assumption was validated by using excess water (rice: water ratio = 1:10) by cooking half a gram of grains in 5 mL of deionised water. Grains were covered with water even after the gelatinization was complete. An indication of completion was done during preliminary trials; samples were taken out of sample block at different time intervals to check the ungelatinized white belly. It was observed that when the samples were completely gelatinised and there was no free water available, “probe distortion” started. So, the start of probe distortion was the cooking time of respective samples. No probe distortion was observed in excess water as depicted in Fig. 4.8.

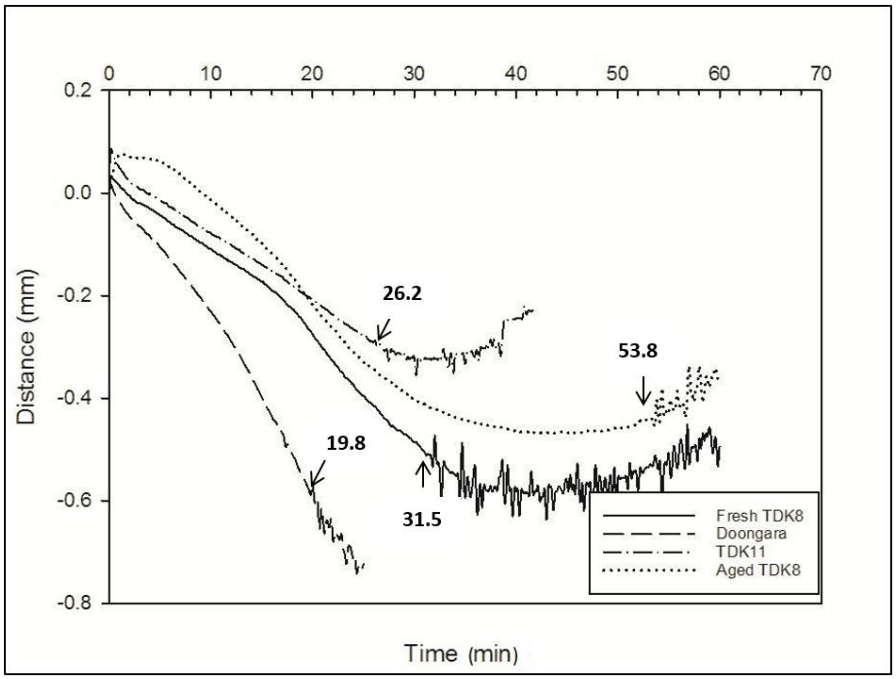

Figure 4.7 The *in situ* TMCT cooking curves for various rice varieties (Fresh TDK8, Doongara, TDK11 and aged TDK8) cooked at 95±1ºC using rice to water ratios of 1:3. Black arrows and numbers correspond to the cooking time

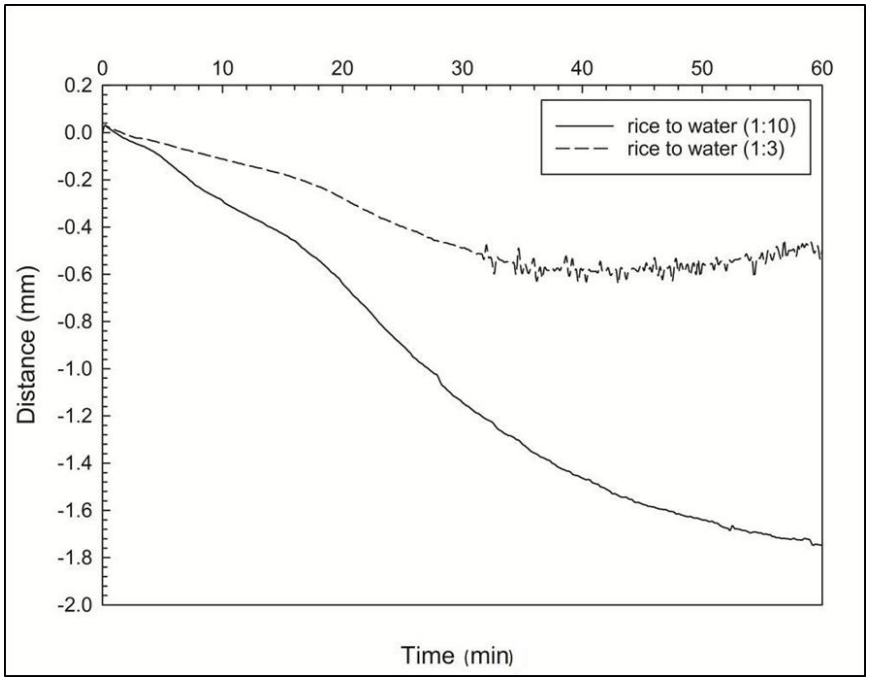

Figure 4.8 The *in situ* TMCT cooking of rice at 95±1ºC with two different rice to water ratios (1:10 and 1:3)

4.4.3. Estimation of the rate of cooking by using the *in situ* TMCT cooking method

The cooking proceeds from outside the grain to the centre. This process is time dependent. In general, the initial 20 min of *in situ* TMCT cooking curves were linear in all rice samples used in the study. Therefore, only initial cooking curves were used to establish the rate of cooking. The rate of cooking will thus signify the rate of softening of the grain. In general, it was observed that higher the cooking rate lower would be the cooking time except for TDK11 (Table 4.1).

Table 4.1 The rate of cooking indicated by the rate of probe displacement during *in situ* cooking of selected rice varieties (fresh and aged TDK8, TDK11, and Doongara). Higher rate signifies faster cooking rate*

Rice varieties	**The rate of cooking$_{20\ min}$ (mm/sec)**	**Cooking time (min)**
Fresh TDK8	-0.018±0.002^{a}	31.5±1.1^{b}
Aged TDK8	-0.012±0.003^{a}	53.8±2.5^{c}
TDK11	-0.013±0.003^{a}	26.2±2.02^{b}
Doongara	-0.029±0.006^{b}	19.8±2.47^{a}

* Means ± SD. Within a column, means with different superscripts are significantly different at 5 % probability level.

4.4.4. Pasting properties

The peak time (PT) estimated by RVA can indicate the cooking characteristics of rice (Champagne et al. 1999; Zambrano et al. 2016). The RVA viscographs of all rice varieties (fresh and aged TDK8, TDK11, and Doongara) are shown in Fig. 4.9a, 4.9b, 4.9c, and 4.9d, respectively. The time required by rice flours to reach the peak viscosity (V_p) is known as peak time (PT). It is the indication of complete gelatinization of starch. Results showed that the fresh and aged TDK8 rice flours took 4.6 and 3.9 min to reach V_p (Fig. 4.9a and 4.9b), while TDK11 and Doongara took 3.5 and 6.0 min, respectively (Fig. 4.6c and 4.6d). The PTs of all rice flours used in this study were significantly ($P<0.05$) less than the cooking time estimated by the *in situ* TMCT cooking method. The grains are ground to mm size before RVA analysis. The reduction in particle size enhances the gelatinization due to increased heat and mass transfer rate (Zhu et al. 2013). Therefore, PT of ground rice flour is not the same as the cooking time of whole grains.

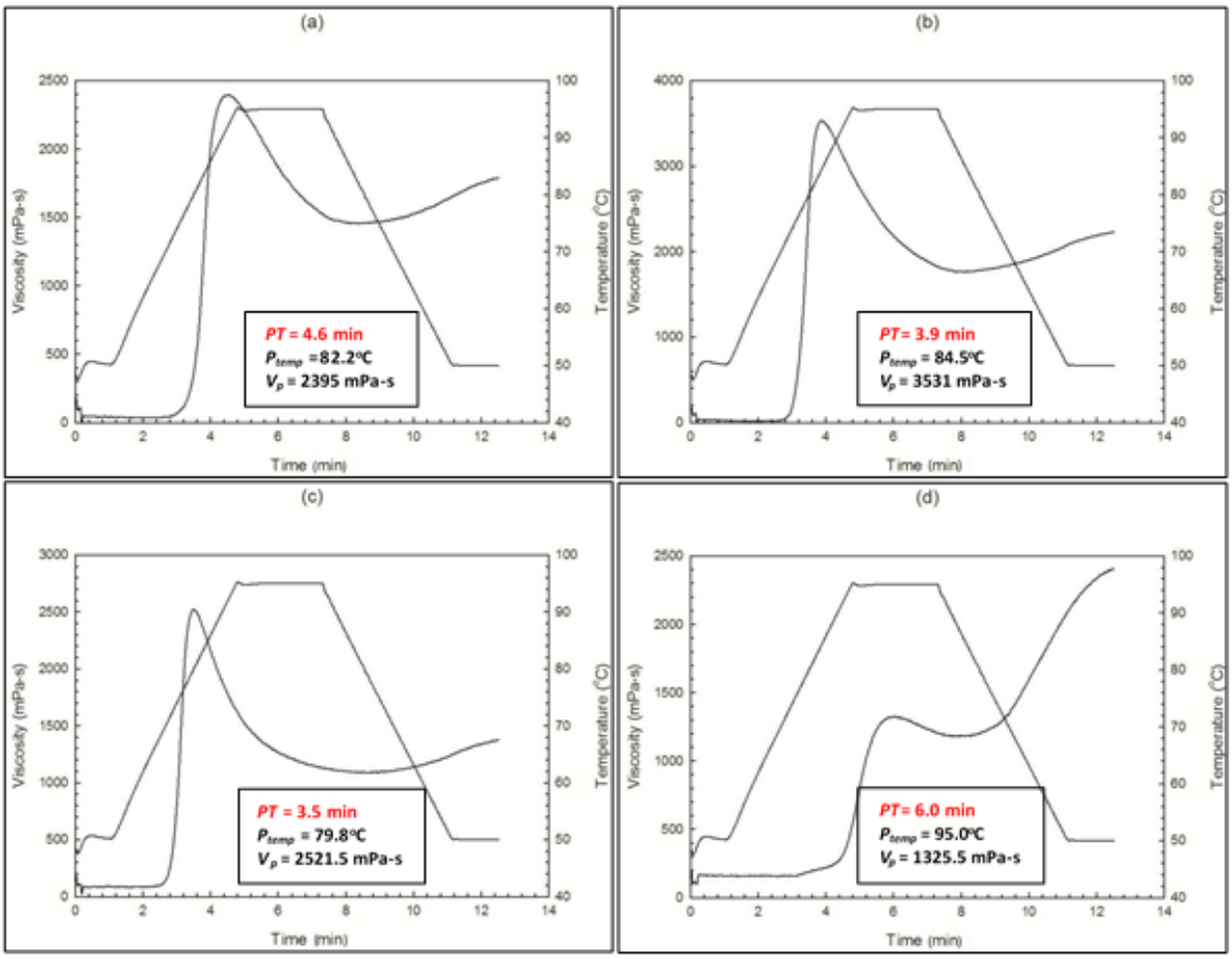

Figure 4.9 The RVA viscographs of various rice varieties, (a) Fresh TDK8, (b) Aged TDK8, (c) TDK11, and (d) Doongara

4.4.5. Estimation of cooking time by using light microscopy

The extent of gelatinization of starch while cooking of fresh and aged TDK8, TDK11 and Doongara rice samples were observed over the time by using light microscopy. The non-gelatinized starch was observed by the presence of white belly in the cooking grain (Fig. 4.10). Fresh TDK8 took 30 min at 95±1ºC of cooking temperature to gelatinize the starch to the core of the kernel completely. Moreover, aged TDK8, TDK11, and Doongara took 50, 25 and 20 min respectively to be completely cooked. This method gives actual cooking time (Faruq et al. 2015), but it is tedious to draw the sample periodically and does not provide the quantitative value.

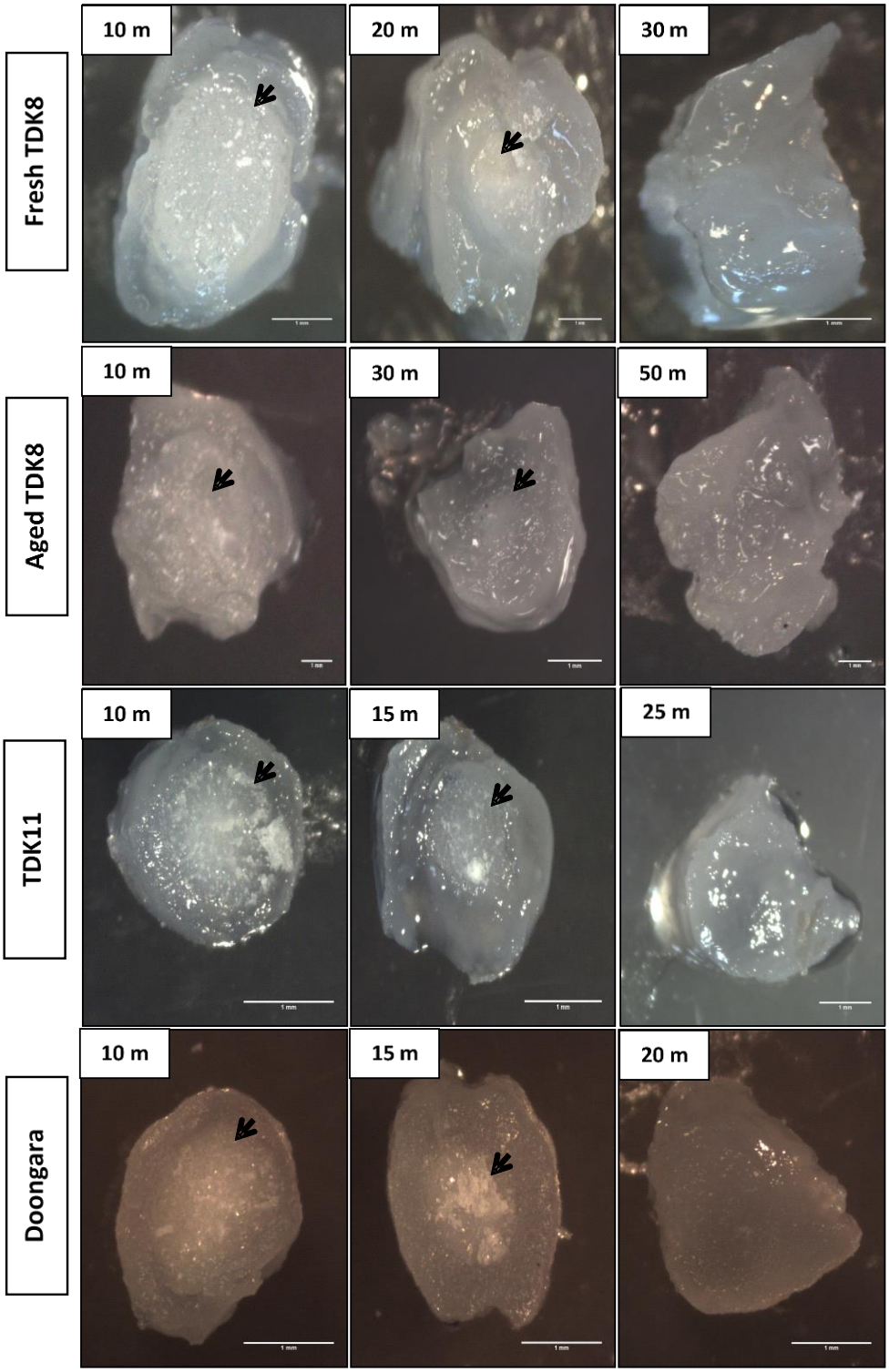

Figure 4.10 The light microscopy of fresh and aged TDK8, TDK11 and Doongara rice kernels during cooking at 95±1ºC (Black arrows indicate the non-gelatinized white belly areas)

4.4.6. Comparison of *in situ* TMCT cooking, RVA and light microscopy

It was interesting to note that the *in situ* TMCT cooking has a possible correspondence to peak viscosity (V_p) of RVA findings. The *in situ* TMCT cooking time decreased with decreasing V_p in all rice varieties used except TDK11. Such as V_p of aged TDK8 is significantly ($P<0.05$) higher than fresh TDK8 (Fig. 4.9a and 4.9b). A similar trend was found in *in situ* TMCT analysis. The cooking time of aged grains was significantly ($P<0.05$) higher than fresh grains (Fig. 4.7). Doongara had the lowest *in situ* TMCT cooking time and V_p among all the rice varieties used in the study. So, the RVA results indicated the swelling and integrity of swollen particles but did not indicate the actual cooking time due to the limitation of flour usage instead of whole grains. The findings of *in situ* TMCT cooking analysis correlated well with microscopic observation. The *in situ* TMCT cooking analysis was well correlated with the conventional microscopic analysis of the cooking process with the minor variation of 4 to 7 % in all selected rice samples. The *in situ* TMCT results showed that aged TDK8 gelatinised at a very slow rate after 30 min (Fig. 4.7). A similar trend was observed in the microscopic observation; around 70 % area of the kernel was gelatinised in first 30 min while the remaining 30 % took 20 min (Fig. 4.10). Microscopic observations were found to be very tedious, time-consuming, requiring large sample size (at least 3 to 5 g) and providing only subjective observations. The *in situ* TMCT cooking is very feasible and easy method and provides detailed information about the rate of water absorption during soaking and gelatinization or cooking. This technique can be used for sample size as small as 0.50 g. The only limitation of this method is the large standard deviation possibly due to the variation in multiple kernel dimensions of the sample taken for the analysis. Further work will be undertaken to relate the *in situ* TMCT cooking data with the sensory properties of the rice.

4.5. Conclusions

Knowledge of water uptake and cooking behavior of rice is a key indicator in predicting the quality of rice. In the present study, three different cooking methods (*in situ* TMCT cooking, RVA and light microscopy) were used to analyse the cooking behavior of selected rice (fresh and aged TDK8, TDK11, and Doongara) varieties. Results showed that the new *in situ* TMCT cooking method could provide information on the softening of the grain due to water uptake during soaking at various rehydration temperatures. The rate of cooking can also be calculated from the slope of *in situ* TMCT cooking curve. This work represents the starting point of a new and rapid approach

for evaluating rice-cooking behavior. Current work focused on representative glutinous and non-glutinous rice varieties and the set-up of the test conditions. A wide range of rice verities showing different cooking behavior needs to be further tested to validate the method. This provides a new opportunity for analysis of rice cooking quality.

Chapter 5 X-ray photoelectron spectroscopic analysis of rice kernels and flours: Measurement of surface chemical composition

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5.1. Abstract

The objectives of this study were to evaluate the ability of x-ray photoelectron spectroscopy (XPS) to differentiate rice macromolecules and to calculate the surface composition of rice kernels and flours. The uncooked kernels and flours surface composition of the two selected rice varieties, Thadokkham-11 (TDK11) and Doongara (DG) demonstrated an over-expression of lipids and proteins and an under-expression of starch compared to the bulk composition. The results of the study showed that XPS was able to differentiate between rice polysaccharides (mainly starch), proteins and lipids in uncooked rice kernels and flours. Nevertheless, it was unable to distinguish components in cooked rice samples possibly due to complex interactions between gelatinised starch, denatured proteins, and lipids. High-resolution imaging methods (Scanning Electron Microscopy and Confocal Laser Scanning Microscopy) were employed to obtain complementary information about the properties and location of starch, proteins, and lipids in rice kernels and flours.

5.2. Introduction

Rice kernels and flour are used to produce a large variety of cereal-based foods, including semolina, gluten-free bread, noodles, and biscuits. The functional properties (e.g., water absorption, pasting properties, etc.) and biochemical composition of rice affect the overall quality of the processed foods (Matos & Rosell 2013). Rice can be classified into waxy and non-waxy varieties based on the native starch type present in the endosperm. Waxy rice contains branched amylopectin and becomes very sticky after cooking. However, non-waxy rice contains straight-chain amylose and is less sticky (Nawaz et al. 2016a).

A scientific understanding has been established that the functional properties of complex biological materials are greatly dependent on the surface characteristics (Rouxhet et al. 2008). Therefore, the spatial distribution of components is a key to understanding complex biological systems (Saad et al. 2009). Recent studies carried out on biological powders have shown that the surface chemical composition of particles is significantly different from their bulk composition (Shrestha et al. 2007a; Baer & Engelhard 2010; Zhao et al. 2015).

X-ray photoelectron spectroscopy (XPS) has become a well-established technique to study the nature of many different types of surfaces (Gaiani et al. 2011). XPS has been extensively used to

investigate the surface composition of biological powders (mainly milk powders) obtained by spray or freeze drying of complex biological solutions (Kim et al. 2002; Zhao et al. 2011). On the other hand, there has been very limited research focused on investigating the application of XPS for natural biological powders obtained by the milling of agricultural produce (Russel et al. 1987; Saad et al. 2009; Saad et al. 2011; Zhao et al. 2015). The surface composition of rice flour and/or kernels has received relatively little research attention, despite the importance of these factors in providing a better understanding of some of the functional properties of cooked rice. The inter-particulate interactions (such as stickiness in the case of rice) and exposure to an external environment that may cause chemical changes (such as oxidation of fat/oil) are depended on the surface composition and properties of these surface materials.

The determination of the surface composition of a material by XPS is first considered at an elemental level. XPS provides the relative atomic elemental composition of approximately 5-10 nm of the surface layer (Rensmo & Siegbahin 2015). The elemental composition of the biological material is defined by considering only the three main elements, carbon, oxygen, and nitrogen. Usually, minor elements (such as phosphorus, sulphur, silicon, boron, manganese or other minerals) are ignored, they account for as little as 1 % the bulk composition (Gaiani et al. 2006; Nijdam & Langrish 2006; Rouxhet et al. 2008; Rensmo & Siegbahin 2015). The relative elemental composition (carbon, oxygen, and nitrogen) is used to identify components such as proteins, lipids, and polysaccharides. For milk powders, the XPS apparent atomic stoichiometry has been found to have reasonable agreement with theoretical stoichiometry based calculations (Nikolova et al. 2015). In addition, the C_{1s}, N_{1s}, and O_{1s} peaks obtained from the XPS survey scans can be decomposed at specific binding energies into various sub-peaks and assigned to well-identified chemical functions (e.g., C-C(H), C-O, C-N, C=O, O-C=O, etc.) that are typical for specific components, such as lipids, sugar derivatives, glucose polymers, and poly-amino acids (Rouxhet & Genet 2011).

The surface composition of biological materials can be estimated by using the relative elemental composition of isolated components. Fäldt and co-workers (1993) developed a method by quantifying the relative atomic concentrations of carbon, oxygen, and nitrogen, and by using a matrix formula related to the surface content of the different compounds (i.e., polysaccharides, proteins, and lipids) that make up the sample. These calculations have been found to be reliable

only when significant differences between C, O, and N are present in the various components (Saad et al. 2011). XPS has its limitations when it comes to differentiation of the multiple functional groups that have similar percentages of atoms. Therefore, isolated components should be significantly different in elemental composition to be able to be differentiated by XPS. As an example, it appears not to be possible to differentiate whey proteins and caseins in milk from the C, O, and N signatures, as these two protein categories are present in the same atomic percentages (Gaiani et al. 2011).

The application of XPS to biopolymers appears to be very reliable, and versatile with a wide range of applicability (Kelly et al. 2015). However, natural surface contamination during the experiment can complicate XPS analysis by overexpression of carbon, because most abundant surface contaminants on air-exposed samples consist of carbonated particles (McArthur et al. 2014). Moreover, some biological specimens (such as wood pulps) have shown instability during XPS-scanning due to X-ray induced irradiation damage and adsorption or desorption of volatiles in ultrahigh vacuum conditions during the experimentation. This damage can distort the data and further complicate the data interpretation (Zhou et al. 2006).

As noted above, over the past ten years XPS has been extensively used to evaluate the surface composition of dairy powders. However, only a limited number of studies have been undertaken to investigate natural agricultural products, with most research to date focused on wheat flours (Rouxhet et al. 2008; Saad et al. 2009; Saad et al. 2011). The objective of the present study was to evaluate the ability of XPS to identify the surface composition of rice kernels and the flour of waxy and non-waxy rice varieties. First, XPS survey scans of pure rice components (starch, proteins, and lipids) were obtained. Then an assessment was made of the surface composition of rice kernels and rice flours. To quantify the surface composition, only macro-nutrients (starch, protein, and lipid) were taken into account.

5.3. Materials and methods

Two rice varieties, Thadokkham-11 (TDK11) (glutinous) and Doongara (DG) (non-glutinous) were used in this study. The rice grains were provided by Rice Research Australia Pty Ltd. (RRAPL). Powdered rice starch (Sigma S7260, Castle Hill, NSW, Australia), commercially available rice protein (Bulk Nutrients, TAS, Australia) and pure rice bran oil (Coles, QLD,

Australia) were used to estimate the relative elemental composition of pure rice components. The flow chart of the experimental design is presented in Appendix 1.

5.3.1. Milling of paddy

The effect of the degree of milling (DOM) on the surface composition of rice kernels was analysed for TDK11 only. Paddy rice was milled to brown rice by using rice husker (Satake, Japan). The brown rice was milled to white rice using an abrasive polisher (Satake, Japan). Three different DOMs, 0 % or brown rice, 9 %, and 16 %, were used in the study. DOM was calculated using the following equation (5.1) as described by Marshall (1992);

$$DOM\ (\%) = \ [1 - (WWR/WBR)] \times 100 \qquad \text{Eq. 5.1}$$

WWR and *WBR* are the weight of white rice and brown rice in grams, respectively.

5.3.2. Grinding of rice kernels

The milled white TDK11 and DG rice grains were ground to flour using a hammer mill (Good Friends of the Guangzhou Machinery Co. Ltd., Guangzhou, China) equipped with a plate of 500 µm size.

5.3.3. Chemical analysis of milled white rice

The starch content of the milled rice flours was determined according to the AACC 76-13.01 method (AACC 1999). Total nitrogen content (TN) was determined by the Kjeldahl method, and crude protein content was calculated as TN X 5.95. Lipid content was determined by using the Soxhlet extraction method according to AACC 30-25.01 (AACC 1999). The apparent amylose content (AAC) was determined by the iodine colorimetric method (Hoover & Ratnayake 2005).

5.3.4. Sample preparation of defatted rice kernels and flours

The milled rice kernels and flour of TDK11 and DG were defatted using solvent extraction. Kernels/flour (10±1 g) samples were taken in cellulose thimbles and treated with petroleum spirit at 70ºC for 2 hrs. After 2 hrs of reflux, the petroleum spirit was separated from the sample using rotary evaporator (RV 10, IKA® Werke GmbH & Co. KG, Germany). The defatted samples were left in a fume hood overnight to evaporate the petroleum spirit fully.

5.3.5. Sample preparation of cooked rice kernels

To five gram samples of milled rice kernels, 15 mL of deionised water (rice to water ratio 1:3) was added in a glass beaker. The samples were then cooked at 95±1ºC in a water bath, after which they were held overnight in a freezer. The frozen cooked rice samples were then freeze-dried using an Alpha 1-4 LSC Freeze Dryer (John Morris Scientific, Australia). The moisture content of the samples was reduced to 10 % to ensure sample stability in ultrahigh vacuum conditions (base pressure as low as 1.33×10^{-7} to 1.33×10^{-6}) during XPS imaging.

5.3.6. Surface chemical analysis

The surface chemical analysis of pure rice components (starch, proteins, and lipids), rice kernels with three different DOM (0 %, 9 %, and 16 %) (TDK11 only), uncooked rice kernels (control and defatted), freeze-dried, cooked rice kernels, and rice flour (control and defatted) were analyzed by using a Kratos AXIS Ultra Kratos Analytical (Manchester, UK) spectrometer with a monochromatic Al X-ray source at 150 W. Prior to analysis, the samples were outgassed under vacuum for 24 hrs. The analysis started with a survey scan from 0 to 1200 eV with a dwell time of 100 ms, pass energy of 160 eV at steps of 1 eV, with a single sweep. For the high-resolution analysis, the number of sweeps was increased, the pass energy was lowered to 20 eV, at steps of 50 meV, and the dwell time was increased to 250 ms. Data was acquired using a Kratos Axis ULTRA X-ray photoelectron spectrometer, incorporating a 165 m hemispherical electron energy analyser. The incident radiation was Monochromatic Al X-rays (1486.6 eV) at 225 W (15 kV, 15 ma). Survey (wide) scans were taken at analyser pass energy of 160 eV, and multiplex (narrow) higher resolution scans at 80 eV. Base pressure in the analysis chamber was 1.33×10^{-7} Pa and, during sample analysis, 1.33×10^{-6} Pa. XPS was applied to measure the relative atomic concentrations of carbon, nitrogen, and oxygen in the layer of the samples to a maximum thickness of 10 nm. Typical XPS survey and high-resolution spectra of rice sample are shown in Appendix 2.

5.3.7. Matrix formula used in the research

The relative elemental composition of pure rice components was used in a set of linear relations in a matrix formula according to a method proposed by Fäldt and co-workers (1993). Adjustments were made in the matrix formula in accordance with the components/macromolecules to be

analyzed (Fäldt 1995). In the present study, the calculations were made by using a matrix, as presented in equations 5.2, 5.3, and 5.4 for the three rice components.

$$I^{C}{}_{sample} = I^{C}{}_{starch}\gamma_{starch} + I^{C}{}_{proteins}\gamma_{proteins} + I^{C}{}_{lipids}\gamma_{lipids} \quad \text{Eq. 5.2}$$

$$I^{N}{}_{sample} = I^{N}{}_{starch}\gamma_{starch} + I^{N}{}_{proteins}\gamma_{proteins} + I^{N}{}_{lipids}\gamma_{lipids} \quad \text{Eq. 5.3}$$

$$I^{O}{}_{sample} = I^{O}{}_{starch}\gamma_{starch} + I^{O}{}_{proteins}\gamma_{proteins} + I^{O}{}_{lipids}\gamma_{lipids} \quad \text{Eq. 5.4}$$

Where $I^{C}_{strach,}$ I^{N}_{strach}, I^{O}_{starch}, $I^{C}_{proteins}$, $I^{N}_{proteins}$, $I^{O}_{proteins}$, I^{C}_{lipids}, I^{N}_{lipids}, and I^{O}_{lipids} are the relative contents of atomic elements (C, N, and O) measured on the surface of the pure rice components (Table 5.2). I^{C}_{sample}, I^{N}_{sample}, and I^{O}_{sample} are the relative contents of elements found by XPS for the sample. The parameters γ_{starch}, $\gamma_{proteins}$, and γ_{lipids} are unknown values corresponding to approximately 100 % component/macromolecules surface contents (starch, proteins, and lipids).

5.3.8. Confocal analysis of rice kernels and flours

Rice kernels and flours were dyed using a mixture (1:1) of 0.01 % (w/v in water) Rhodamine B (Sigma R6626, Castle Hill, NSW, Australia) and 0.02 % (w/v in poly (ethylene glycol) 200 (Fluka 81150, Castle Hill, NSW, Australia)) Nile Red (Sigma 72485, Castle Hill, NSW, Australia) for labelling proteins and lipids, respectively. The samples were treated with dyes in the dark with intermittent shaking. After 10 min dye-labelled samples were washed with deionised water until the supernatant became clear. The microstructure of rice kernels and flours was observed by using an LSM 700 confocal laser scanning microscope (CLSM, Zeiss, Germany).

5.3.9. Scanning electron microscopy of surface and cross-section of uncooked rice kernels

Whole grains and cross sections of milled rice kernels of TDK11 and DG were mounted onto SEM stubs by placing them on a double-sided carbon adhesive tape. Biological materials suffer from extensive charge build-up under the electron beam; hence they need to be coated with conductive material. Samples were iridium-coated for 3 min (~ 15 nm thick). The samples were examined using a Philips XL30 Scanning Electron Microscope operating at 10 kV accelerated voltage.

5.3.10. Statistical analysis

All measurements presented in this paper are based on two independent samples. The data were analysed by analysis of variance (Completely Randomized Design) using KyPlot software version

2.0 to determine significant differences. The data was then analysed using the Tukey's pair-wise comparison of different treatments, at 5 % level of significance.

5.4. Results and discussion

5.4.1. Chemical composition

The bulk chemical composition of the two rice varieties at 9 % DOM is presented in Table 5.1. Starch (main rice polysaccharide) constituted over 90 % (w/w) of the milled rice in both varieties, followed by crude protein (6 – 8 %, w/w). Lipid contents were only 0.8 – 1 % (w/w) of the bulk composition of milled white rice samples. TDK11 had a significantly ($P<0.05$) higher starch and lower protein content than DG. Apparent amylose content (AAC) showed that TDK11 has mainly branched starch (amylopectin) and is classified as a glutinous rice variety. DG has intermediate amylose content and is classified as a non-glutinous rice variety (Nawaz et al. 2016a).

Table 5.1 Bulk chemical composition of rice varieties TDK11 and Doongara (DG) at 9 % degree of milling*

Bulk chemical composition content (g/100 g dry matter)	**TDK11**	**DG**
Starch	92.63 ± 0.22^{a}	90.44 ± 0.14^{b}
Apparent amylose content (AAC)	3.72 ± 0.05^{a}	19.71 ± 0.42^{b}
Protein	6.55 ± 0.15^{a}	8.52 ± 0.11^{b}
Lipid	0.82 ± 0.07^{a}	1.05 ± 0.03^{a}

*Means ± SE. Within a row, means with different superscripts are significantly different at 5 % probability level.

5.4.2. Pure rice components analysed by XPS to construct the matrix

To test the usefulness of XPS matrix, pure rice components (starch, proteins, and lipids) were first individually analysed by XPS. Rice endosperm is comprised of two closely related polysaccharides, primarily starch and traces of arabinoxylans. XPS analysis has limitations in distinguishing starch and arabinoxylans, as their atomic percentages are similar (Saad et al. 2011). Therefore, only starch was considered to be the only polysaccharide in the rice endosperm to overcome the complexity of analysis. Three atoms elements (C, O, and N) were detected in pure rice starch and proteins (Table 5.2), and only two (C and O) for lipids. The survey scans obtained for pure starch (Table 5.2) gave major signals at 286 and 533 binding energy (eV) corresponding

to C (59.47±0.07 %) and O (40.22±0.49 %), respectively and a very low signal at 400 eV corresponding to N (0.63±0.10 %). The XPS survey of pure rice proteins (Table 5.2) gave a major signal corresponding to C (77.60±0.01 %), a relatively lower signal corresponding to O (16.10±0.01 %), and a significant signal corresponding to N (6.39±0.01 %). However, the XPS survey spectra for rice bran oil gave the main signal corresponding to C (91.81±0.01 %), and a lower signal is corresponding to O (8.20±0.01 %). No N_{1s} peak was detected (Table 5.2).

Table 5.2 Relative elemental compositions of pure rice components measured by XPS*

Binding energy (eV)	**Functions**	**Atomic abundance (%) of elements at the surface of pure rice components**		
		Starch	**Protein**	**Lipid**
286	C	59.47±0.07[a]	77.60±0.01[b]	91.81±0.01[c]
400	N	0.63±0.10[a]	6.39±0.01[b]	-
533	O	40.22±0.49[a]	16.10±0.01[b]	8.20±0.01[c]
Stoichiometry		**Starch**	**Protein**	**Lipid**
C/O		1.47	4.76	11.11
C/N		94.39	12.50	-

*Means ± SE. Within a row, means with different superscripts are significantly different at 5 % probability level.

The C/O stoichiometry for pure starch was found to be 1.47 (Table 5.2), which is relatively higher than the theoretical value for anhydroglucose (1.20) when the theoretical chemical composition $(C_6H_{10}O_5)_n$ for starch was taken into consideration. Similar findings have been reported for pure wheat starch by Saad and co-workers (2011). There is a likelihood that the surface components of the granules other than glucose polymers may be present at a lower level. Moreover, traces of nitrogen were also found on the starch granules at C/N stoichiometry of only 94.39, suggesting the possible presence of proteins (mostly protein-based enzymes) on the starch granule surface.

Almost all known cereal proteins have the basic molecular formula $C_{3.3}H_{5.9}O_{1.06}N_1S_{0.033}$ (C/O = 3.12 and C/N = 3.33) (Torabizadeh 2011; Saad et al. 2011). A lower content of both O and N were found in pure rice protein samples during XPS analysis with calculated values of C/O and C/N stoichiometry as 4.76 and 12.5, respectively (Table 5.2). These results indicate the presence of lipid traces within the surface layers of protein particles (Rouxhet et al. 2008). Similar findings have been reported by Saad and co-workers (2011) for wheat proteins. Also, no traces of sulphur

were detected, which suggests the absence of sulphur containing amino acids (cysteine, cysteine, and methionine) on the surface of the rice protein samples.

The rice lipids C/O stoichiometry (11.11) (Table 5.2) was found to be very similar to that of soya oil (10) reported by Jones et al. (2013). However, rice lipids differed from wheat lipids where amide-linked N was detected due to the presence of protein residues (Saad et al. 2011).

5.4.3. Effect of degree of milling (DOM) on the surface composition of waxy rice TDK11

The effect of the degree of milling on surface properties was investigated for TDK11. The relative elemental and estimated surface composition of TDK11 kernels with different DOM (0 % or brown rice, 9 %, and 16 %) are shown in Table 5.3. Four peaks, corresponding to Si, C, N, and O, were detected during XPS survey scanning. The presence of Si on the surface of grains may be due to contamination of the husk (Park et al. 2004). The main peak at a binding energy of 286 eV, corresponding to C, was detected in all three DOMs. The relative C composition was 85.31±0.82 %, 86.54±0.69 % and 82.14±0.86 % for 0 %, 9 %, and 16 % DOM, respectively. Relatively weaker signals at binding energy 533 eV were attributed to relative O composition. A significant ($P<0.05$) increase in O composition was found in 16 % DOM when compared with 0 % and 9 % DOMs. Very weak signals corresponding to N were also detected at 400 eV binding energy. The relative N composition in selected DOMs ranged between 2.27±0.02 % to 3.30±0.72 %.

Only C, N, and O relative composition were considered and used in matrix formula for the calculation of the surface composition of selected DOMs, while Si composition was ignored (Table 5.3). The results show that the calculated surface composition of the upper 10 nm layer of brown TDK11 was primarily composed of lipids (52.57±9.79 %) and proteins (47.44±9.79 %). Brown rice endosperm is covered by two distinct layers, the cuticle, and aleurone (Bagchi et al. 2016). These layers have high contents of proteins and lipids. The average thickness of these layers usually ranges from 30 to 50 µm (Wu et al. 2016a). The 9 % DOM TDK11 surface was composed of lipids (63.84±1.51 %), proteins (35.17±0.51 %) with a low content of starch (0.79±0.79 %). A further increase in the DOM resulted in increased surface starch and proteins and reduced lipids. Similar results were reported by Gangidi and co-workers (2002), who found a reduction in surface lipids in response to an increase in DOM (0-40 %) in long and medium-grain rice varieties, estimated by Diffuse Reflectance Fourier Transform Infrared Spectroscopy (DRIFTS). The 16 %

DOM TDK11 surface was composed of proteins (51±11.41 %), lipids (42.66±9.12 %), and starch (5.72±2.26 %). It should be noted that the surface composition of milled kernels, even with 16 % DOM, had a higher content of lipids and proteins than the bulk composition of rice kernels. This result showed that a thin layer of protein particles and bran oil covering the rice kernels, despite their overall very low content in the kernel.

5.4.4. Surface composition and microstructure of rice kernels

5.4.4.1. Uncooked rice kernels

The relative elemental composition and estimated surface composition of rice kernels of TDK11 and DG are shown in Table 5.3. XPS spectra showed that the surface composition of rice kernels of both rice varieties in this study mainly consisted of C, N, and O, with traces of Si. Again, the traces of Si may reflect husk or bran particles contamination on the surface. The surface composition of milled TDK11 and DG was found significantly different from bulk chemical composition (Table 5.1). The surface of TDK11 had high a protein concentration (49.69±8.26 %) followed by lipids (36.21±6.78 %). However, the surface of variety DG had more lipids (58.94±0.64 %) than proteins (29.32±0.79 %); possibly due to a higher level of intact bran residues on the surface of DG, which resulted in more fat being found in bulk composition analysis. It should be noted that an extremely thin layer of bran oil spread on the surface of the kernel or particle will increase the surface lipids composition. XPS measures the composition of a 5-10 nm layer. The surface starch content ranged between 11.77±0.08 % and 13.42±1.51 %. To further validate the XPS matrix and estimated surface composition, the kernels were defatted using petroleum spirit and analysed again. A significant increase in surface proteins (75.20±2.09 and 87.14±3.45 %) and starch (24.81±2.09 and 12.87±3.45 %) was found in the defatted TDK11 and DG rice kernels, respectively. Lipids were absent in the defatted kernel.

The XPS surface analysis was well correlated with the microstructure of rice kernels estimated by confocal laser scanning microscopy (CLSM). The micrographs of control and defatted rice labelled with Rhodamine B and Nile Red showed that the surface of both rice kernels (TDK11 and DG) was covered by a very thin layer of lipids (labelled in red) followed by proteins (labelled in green) (Fig. 5.1a and 5.1b). However, in defatted rice kernels, only proteins were found on the surface. In these images, only the surface was visible, as the dye was not able to penetrate the interior of the kernel.

To get greater clarification, scanning electron microscopy (SEM) was performed for whole grains and cross sections of TDK11 and DG (Fig. 5.2). The surface of both rice samples was found to be covered with particles (which may be bran residue) as shown in Fig. 5.2a and 5.2c, resulting in more proteins and lipids than starch. Also, more lipid layers were observed in DG than TDK11, as was also found in the XPS analysis. Cross sections micrographs (Fig. 5.2b and 5.2d) showed that the outer surface layer of over 10 µm thickness had no polygonal structure of starch. Possibly, the outer surface layer was composed of only round protein bodies, lipid droplets, and cell walls. As the XPS method is unable to differentiate between starch and cell walls (arabinoxylans), more proteins and lipids were observed on the upper 10 nm surface layer. Around 10-14 % starch calculated using the matrix formula, may be contributed by cellulose (Saad et al. 2011).

5.4.4.2. Cooked rice kernels

The relative elemental composition of cooked rice kernels of TDK11 and DG is shown in Table 5.4. Four peaks corresponding to C (50.03±1.46 %), N (6.82±1.22 %), O (38.68±1.78 %) and Mn (4.48±0.54 %) were detected in TDK11. However, the XPS survey of cooked DG was significantly different and five peaks, corresponding to Si (1.09±0.15 %), B (0.29±0.03 %), C (74.94±0.38 %), N (2.81±0.44 %), and O (20.89±0.70 %), were detected. Cooked DG had a significantly ($P<0.05$) higher percentage of C and lower O than cooked TDK11. It was not possible to calculate the surface composition of cooked rice kernels using the matrix formula developed for dry samples, as the relative elemental composition of C (50.03±1.46 %) in cooked TDK11 was found to be lower than the carbon in pure components, while the N level (6.82±1.22 %) was higher than the N found in pure components. Therefore, high-resolution XPS scanning was conducted for the C_{1s}, N_{1s}, and O_{1s} peaks of cooked TDK11 and DG. The deconvolution of C_{1s} showed six distinct sub-peaks (C-C, C-COOH, C-N, C-O, C=O, and O-C=O) in both TDK11 and DG, corresponding to polysaccharides, protein or lipid side chains, etc. Cooking of rice kernels possibly resulted in complex interactions of gelatinised starch with denatured proteins and lipids. Although there were no significant ($P>0.05$) differences found between cooked TDK11 and DG, the deconvolution of the C_{1s} peak provided good information about the surface composition of cooked rice.

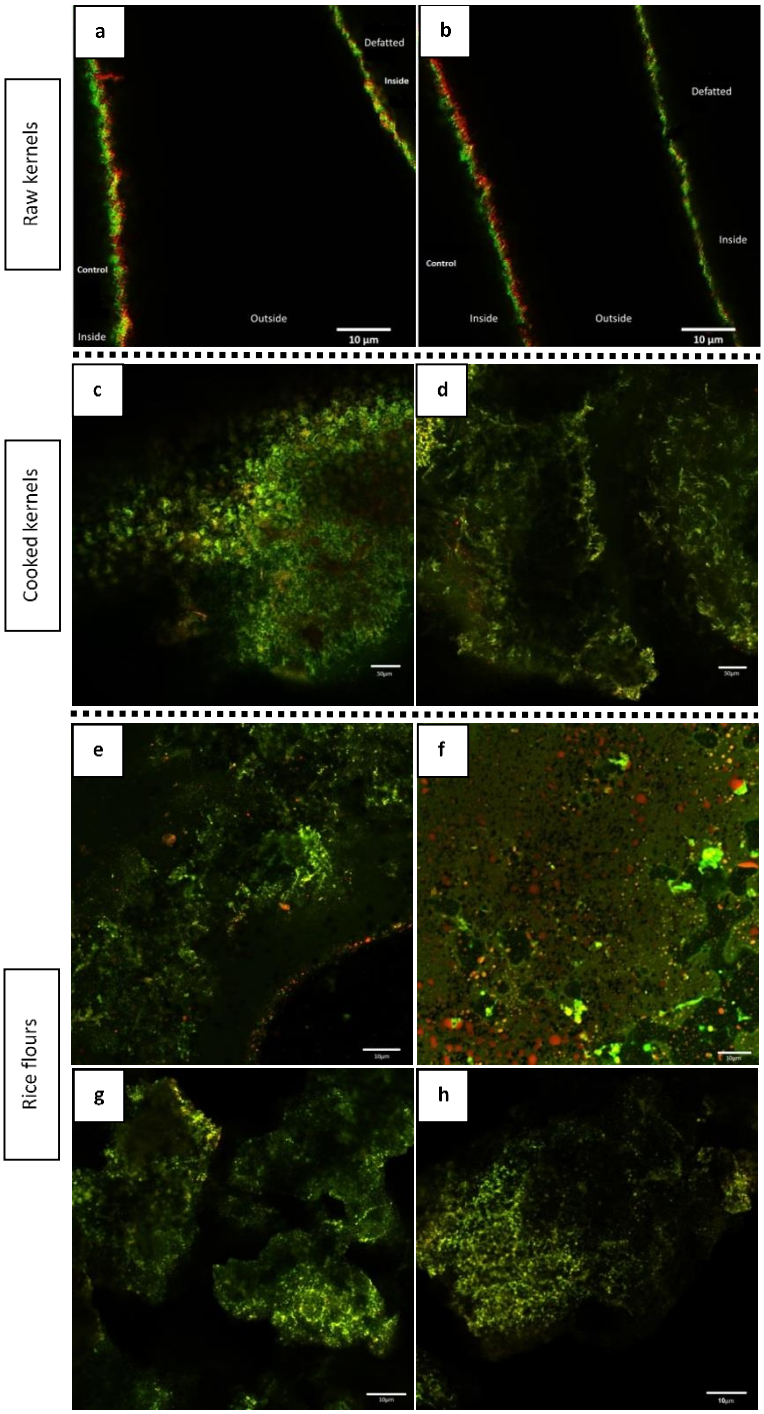

Figure 5.1 Confocal laser scanning micrographs (CLSM) of control and defatted uncooked rice kernels of TDK11 (a) and Doongara (DG) (b), cooked rice kernels of TDK11 (c) and Doongara (DG) (d), rice flours of control TDK11 (e), control Doongara (DG) (f), defatted TDK11 (g), and defatted Doongara (DG) (h). Lipids and proteins are labelled in red and green respectively

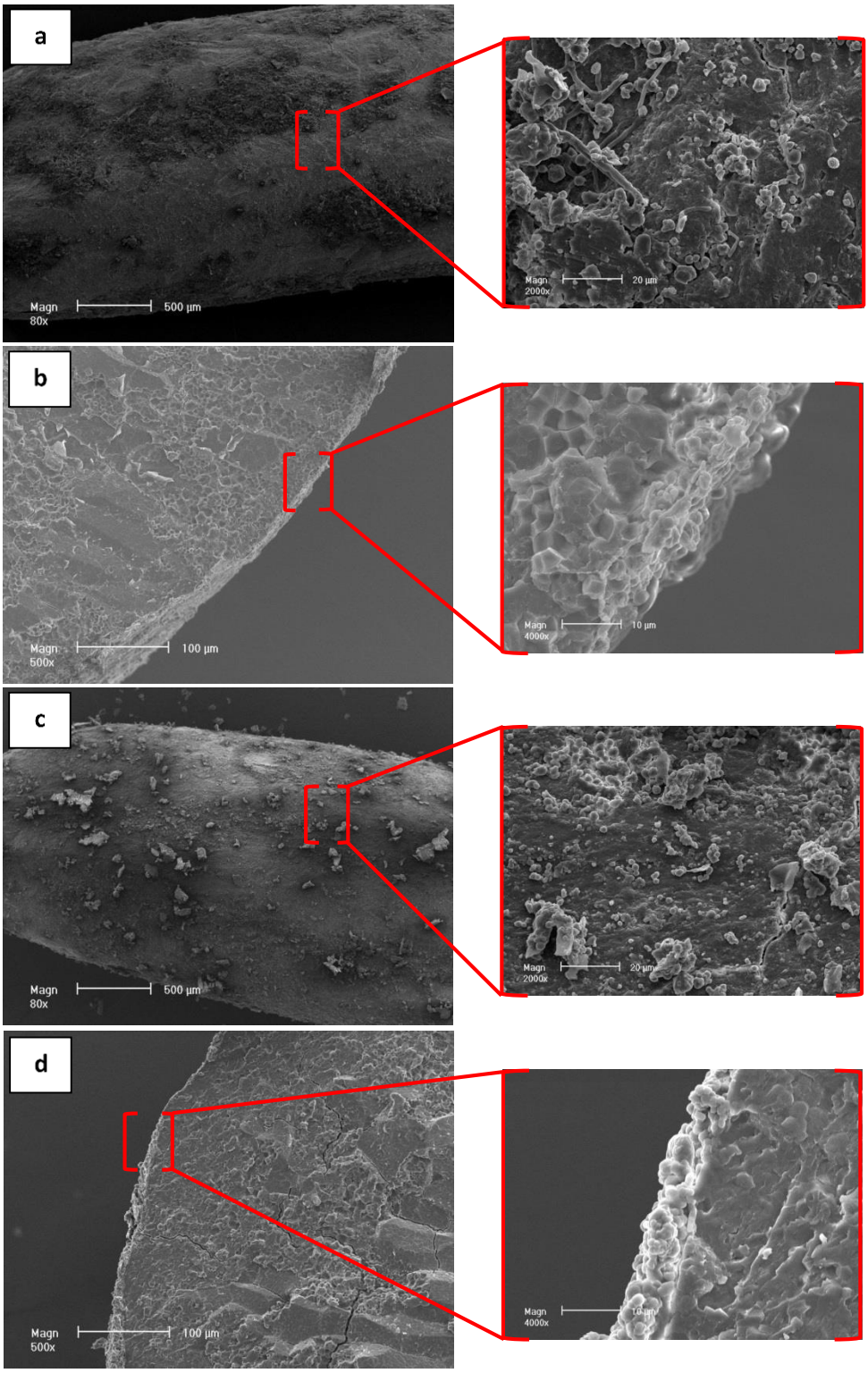

Figure 5.2 Scanning electron micrographs of uncooked rice kernels of TDK11 and Doongara (DG); (a) surface of milled TDK11 kernel, (b) cross-section of TDk11 kernel, (c) surface of milled Doongara (DG) kernel, and (d) cross section of Doongara (DG) kernel

Table 5.3 Relative elemental and calculated surface composition (%) of TDK11 with different DOM (%), control and defatted rice kernels, and control and defatted rice flours of TDK11 and Doongara (DG)*

		The degree of milling (DOM)			Rice kernels				Rice flour			
					Control kernels		Defatted kernels		Control flour		Defatted flour	
		0 %	9 %	16 %	TDK11	DG	TDK11	DG	TDK11	DG	TDK11	DG
Elemental atomic percentage (%)	Si	0.59±0.16^{a}	0.45±0.08^{a}	0.55±0.04^{a}	0.58±0.03^{ab}	-	0.76±0.15^{a}	0.11±0.11^{b}	-	-	-	-
	C	85.31±0.82^{a}	86.54±0.69^{a}	82.14±0.86^{a}	79.78±0.76^{a}	83.86±0.02^{b}	65.55±0.07^{c}	71.37±0.07^{d}	74.13±0.47^{a}	79.17±0.18^{b}	66.37±0.02^{c}	68.66±0.31^{d}
	N	3.10±0.69^{a}	2.27±0.02^{a}	3.30±0.72^{a}	3.29±0.52^{a}	1.97±0.05^{a}	6.79±0.35^{b}	6.38±0.43^{b}	4.43±0.74^{ab}	2.67±0.52^{b}	7.04±0.47^{a}	6.11±0.20^{a}
	O	11.00±0.29^{a}	10.75±0.75^{a}	14.01±0.18^{b}	16.37±0.17^{a}	14.29±0.05^{b}	26.91±0.14^{c}	21.46±0.66^{d}	21.44±0.28^{a}	18.16±0.34^{b}	26.60±0.48^{c}	25.24±0.12^{c}
Surface composition (%) after using matrix formula	Starch	-	0.79±0.79^{a}	5.72±2.26^{a}	13.42±1.51^{ab}	11.77±0.08^{a}	24.81±2.09^{b}	12.87±3.45^{ab}	24.89±3.83^{a}	21.35±3.15^{a}	-	24.76±0.87^{a}
	Protein	47.44±9.79^{a}	35.17±0.51^{a}	51.00±11.41^{a}	49.69±8.26^{a}	29.32±0.79^{b}	75.20±2.09^{c}	87.14±3.45^{c}	66.88±11.96^{a}	39.68±8.45^{b}	100±0.00^{a}	75.25±0.87^{a}
	Lipid	52.57±9.79^{a}	63.84±1.51^{a}	42.66±9.12^{a}	36.21±6.78^{a}	58.94±0.64^{b}	-	-	8.12±8.12^{a}	38.87±5.30^{b}	-	-

*Means ± SE. For DOM, Rice kernels and Rice flour, in each respective section, the means within a row with different superscripts differ significantly at 5 % probability level.

The calculated values for C/O and C/N stoichiometry for cooked TDK11 were found to be 1.29 and 7.14, respectively (Table 5.4). The results reflected that the cooked TDK11 (waxy rice) kernel surface was mainly covered by gelatinised amylopectin (branched starch) with high levels of proteins. More starch on the kernel surface may induce the adhesive properties of sticky rice (Keawpeng & Venkatachalam 2015). However, the cooked DG kernels had C/O and C/N stoichiometry values of 3.57 and 25, respectively (Table 5.4). Therefore, the kernel surface was mainly covered by fat with some proteins. Fat and proteins are responsible for the hardness and cohesiveness of cooked non-waxy rice kernels (Choi et al. 2015). These findings need to be the subject of further investigation.

The results were validated by using CLSM. The dye labelled microstructures of both cooked TDK11 and DG are shown in Fig. 5.1c and 5.1d. The results show lipids and protein networks fluoresced in red and green, respectively in cooked DG (Fig. 5.1d). The rice proteins exist in the form of round protein bodies in the endosperm (Saito et al. 2012), but form a continuous honeycomb-like structure during cooking, due to heat denaturation (Likitwattanasade & Hongsprabhas 2010). However, only gelatinised starch (not in granular shape) was visible in TDK11 (Fig. 5.1c). This may be due to variations in the functional properties of glutinous (TDK11) and non-glutinous (DG) rice varieties. The glutinous rice starch consists almost entirely of amylopectin (Zeng et al. 2016). When cooked, the grain usually loses its original shape and becomes very sticky (Nawaz et al. 2016a). On the other hand, non-glutinous rice starch contains amylose as well as amylopectin, and the cooked grain tends to retain its shape and is less sticky (Nawaz et al. 2016a; Li et al. 2016). It is understood that starch is stickier than protein (Hamaker et al. 1991). More starch and proteins were found on the surface of cooked TDK11 kernel (sticky rice variety) and fat on the surface of cooked DG kernel (non-sticky rice variety). This suggests the role of protein in the stickiness of the cooked rice.

5.4.5. Surface composition and microstructure of rice flours

The relative elemental composition of rice flours (TDK11 and DG) is shown in Table 5.3. In both rice samples, three peaks were detected during the XPS survey scans. The relative C composition of TDK11 and DG was found to be 74.13±0.47 % and 79.17±0.18 %, respectively. Relatively weaker signals corresponding to O were detected in both rice flours. The calculated O composition of TDK11 and DG was found to be 21.44±0.28 % and 18.16±0.34 %, respectively, while the

calculated N composition of TDK11 and DG was estimated to be 4.43±0.74 % and 2.67±0.52 %, respectively.

The surface composition of both rice flours was also found to be significantly different from the bulk composition (Table 5.1). The calculated surface composition of TDK11 flour consisted of proteins (66.88±11.96 %), starch (24.89±3.83 %), and lipids (8.11±8.12 %). The DG flour surface consisted of proteins (39.68±8.45 %), starch (21.35±3.15 %), and lipids (38.87±5.30 %).

Table 5.4 Relative elemental and calculated surface composition (%) of cooked rice kernels of TDK11 and Doongara (DG)*

Elements	**Functions**	**TDK11**	**DG**
Si		-	1.09±0.15
B		-	0.29±0.03
C		50.03±1.46[a]	74.94±0.38[b]
	C-C	52.49±0.10[a]	56.54±2.09[a]
	C-N	9.78±6.36[a]	12.69±0.41[a]
	C=O	8.68±1.05[a]	6.46±0.36[a]
	O-C=O	3.84±0.17[a]	3.41±0.02[a]
	C-COOH	3.84±0.17[a]	3.42±0.02[a]
	C-O	21.38±4.88[a]	17.51±2.11[a]
N		6.82±1.22[a]	2.81±0.44[a]
O		38.68±1.78[a]	20.89±0.70[b]
Mn		4.48±0.54	-
Stoichiometry		**TDK11**	**DG**
C/O		1.29	3.57
C/N		7.14	25

*Means ± SE. Within a row, means with different superscripts are significantly different at 5 % probability level.

The surface composition of both rice flours had significant amounts of lipids and proteins. To validate the XPS matrix and estimated surface composition, the flours were defatted with petroleum spirit and analysed again. Significant changes in the relative elemental (C, N, and O) composition were recorded (Table 5.3). Significant increases in N and O and a decrease in C concentrations were found in both defatted rice flours. The surface composition of TDK11 showed

only proteins, with no lipids and starch. However, DG had 75.25±0.87 % proteins and 24.76±0.87 % starch. Similar results have also been reported by Saad and co-workers (2011) for wheat flours, in which an over-expression of proteins (54 %) and lipids (44 %) and an under-expression of starch only 2 %, were found when compared to the bulk composition. It was assumed that this reflected the heterogeneous distribution of components and molecular masking effects between the components. Also, the grinding procedures may result in the XPS analysis to give different results from the actual composition, due to the variations in exposed surfaces of the particles produced by grinding and spreading of lipids. Zhou et al. (2015) reviewed and illustrated the presence of protein bodies and nanometric fat droplets (100-500 nm) surrounding the endosperm cells. However, this work suggests that these free lipid droplets are in fact spread in a thin layer on the endosperm surface. The rice proteins may have been absorbed into the thin layer of lipids formed on the kernel surface due to rice proteins' hydrophobic properties. The over-expression of both protein and fat in the rice kernel and flour surfaces was an interesting result and needs to be further researched to investigate its implications for the functionality and chemical stability of these products.

The microstructures of control and defatted rice flours of TDK11 and DG were also analysed using CLSM (Fig. 5.1e, 5.1f, 5.1g and 5.1h). The results showed that control the flours of both rice varieties have lipids (labelled in red by Nile Red) and protein bodies (labelled in green by Rhodamine B) on the surface (Fig. 5.1e and 5.1f). However, only proteins were observed on the surface in defatted rice samples (Fig. 5.1g and 5.1h). Also, the surface lipids were found to be significantly ($P<0.05$) higher in DG flour than TDK11 during XPS scanning (Table 5.3). Similar findings were observed during CLSM analysis. DG flour (Fig. 5.1f) had bigger and more lipid bodies than TDK11 (Fig. 5.1e).

5.5. Conclusions

The results of the study demonstrated that XPS was able to be used for the evaluation of the surface of two different rice varieties. XPS imaging provided detailed information about the elemental composition of the upper 5-10 nm layer of rice kernels and flours. This relative elemental composition could be used in the calculation of macromolecules on the surface of rice kernels. Higher amounts of proteins and lipids were found on the surface of kernels and flours than in the bulk composition. The findings from XPS imaging were well correlated with CLSM microstructure analysis. These results had significance in relation the chemical stability and

functionality of rice grain. Further research needs to be done in future for optimising the XPS imaging and use of this technique for the analysis of aged rice samples and process related changes on the kernel surfaces which can potentially lead to a reduction in the functional quality of rice.

Chapter 6 Effect of alkali treatment on the milled grain surface protein and physicochemical properties of two contrasting rice varieties

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6.1. Abstract

A systematic study was conducted to explore the effect of grain surface proteins on the physicochemical properties (pasting, retrogradation, and textural quality) of rice. Milled rice grains of two selected glutinous (Thadokkham-8 (TDK8)) and non-glutinous (Doongara (DG)) varieties were treated with different concentrations (0 %, 0.004 %, 0.02 %, 0.04 %, and 0.2 % w/v) of NaOH solution for 1 h. After surface protein removal, the cooked rice grains showed a significant ($P<0.05$) increase in adhesiveness. Similarly, protein removal showed a significant ($P<0.05$) decrease in the final viscosity (V_f) of rice flours. Furthermore, NaOH treatment at a concentration of 0.04 % induced yellow color development in grains. The differential calorimetric study showed that alkali treatment resulted in increased onset (T_o), peak (T_p), conclusion (T_c) temperatures and enthalpy (ΔH) of both rice varieties. No significant ($P>0.05$) effect of alkali treatment was observed on the retrogradation thermal temperatures ($T_{o(r)}$, $T_{p(r)}$, and $T_{c(r)}$), but the amount of retrograded starch (as indicated by reduction in $\Delta H_{(r)}$) was decreased significantly ($P<0.05$) in both varieties. These findings suggest a good potential of applying alkali pre-treatments in the processing of rice to alter the hardness and stickiness properties of rice.

6.2. Introduction

An increasing trend in the consumption pattern of rice has been observed due to rising interest in gluten-free products. Rice can be broadly divided into two distinct types based on the native starch type present in the endosperm; glutinous rice cultivars are primarily containing branched amylopectin and non-glutinous rice cultivars containing linear chain amylose as well as amylopectin (Yu et al. 2015). The textural attributes of cooked glutinous and non-glutinous rice are quite different from each other due to this compositional difference. Good quality glutinous rice should be very sticky and vice versa for non-glutinous rice (Nawaz et al. 2016a). However, aging induces functional changes in the stored glutinous rice (Nawaz et al. 2017) making it less sticky. The mechanism of reduction in the cooked rice stickiness is still an area of research interest. The functional attributes of rice have long been ascribed to starch composition and property. Many studies to date have focused on the role of amylose content (Lu et al. 2013; Syahariza et al. 2013), fine structures of amylopectin (Syahariza et al. 2013), solubility of amylose (Fu et al. 2015), the gelatinization and melting temperatures of amorphous and crystalline regions of amylopectin (Zeng et al. 2014), and the amount of native structures remaining in starch granules after heating

(Klaovhanpong et al. 2015). Extensive consideration of investigation on only starch is not surprising considering that starch accounts for 92-95 % of the dry matter in milled rice grain. However, it has now been realised that starch may not be the only factor affecting the cooking/eating attributes of rice grains (Yadav et al. 2013).

Protein is the second most abundant macromolecule in rice endosperm after starch. Rice contains 6-8 % protein and does not fluctuate widely from this level (Yadav et al. 2013). Proteins in a rice kernel are present in the form of round discrete protein bodies (PBs). The estimated size of PB is usually around 4-5 µm. There are two types of PBs; Protein body I and protein body II (Han & Hamaker 2002). PBs in the subaleurone layer are not similar to those present in the endosperm (Baxter et al. 2004). Subaleurone PBs are rich in glutelin (alkali soluble) and albumin (water soluble). While endospermic PBs are rich in prolamin (alcohol soluble) (Baxter et al. 2004).

Various studies have been conducted in the past to find out the effect of protein (Yadav et al. 2013; Xie et al. 2008) and shown a weak correlation between the gross protein content and the texture of cooked rice, higher protein content rice is harder than low protein content rice (Baxter et al. 2004). Moreover, in a recent study, the surface analysis of rice kernels using X-ray Photoelectron Spectroscopy (XPS) and Confocal Laser Scanning Microscopy (CLSM) showed an over-expression of proteins and lipids and an under-expression of starch on the surface of rice endosperm compared to the bulk composition of endosperm (Nawaz et al. 2016b). Alkali extraction has been used in recent studies to extract protein from cereal flours, especially in rice (Souza et al. 2016). Alkaline treatment by agents such as lye or sodium hydroxide is widely used in the production of many value-added food products from cereals, including tortillas, waxy rice dumplings (Lai et al. 2002), and various extruded products such as instant noodles and yellow alkaline noodles (Nadiha et al. 2010). It is assumed that dilute alkali treatment to the whole rice grains may be a useful technique to remove surface protein residues, resulting in more starch on the surface. An increase in stickiness/adhesiveness in stored rice may be improved by removing surface proteins, as starch is stickier than protein (Hamaker et al. 1991). Alkali treatment may also wash surface lipids by saponification. However, alkali application to food products especially cereals should be employed carefully as steeping with higher concentration of alkali (such as 0.4 % NaOH) for longer time (7-14 days) can lead to structural changes in rice starches (Cai et al. 2014), resulting in changes in functional properties such as swelling power, water binding

capacity, gelatinization and pasting attributes (Karim et al. 2008; Wang & Copeland 2012). Our study has avoided the inappropriate alkali steeping by using lower NaOH concentration for a shorter period. The objective of the present study is to investigate if the removal of the protein bodies from the surface of the grain alters the stickiness of the cooked grain. For this, the milled rice grains of two contrasting rice varieties (waxy and non-waxy, respectively) were treated with various concentrations of sodium hydroxide solution to wash surface proteins and lipids. This washing was expected to lead to increasing in the stickiness of cooked rice grains which is one of the most important quality attributes of waxy rice.

6.3. Materials and methods

One *Oryza sativa* indica cultivar of glutinous rice from Lao PDR (Thadokkham-8 (TDK8) having 3.77 % apparent amylose contents (AAC)) and one *O. sativa* japonica non-glutinous rice from Australia (Doongara (DG), 19.71 % (AAC)) were used in this study. The milled TDK8 was provided by the National Agriculture and Forestry Research Institute (NAFRI), Lao PDR, while Doongara were provided by Rice Research Australia Pty Ltd (RRAPL), Mackay, QLD, Australia.

6.3.1. Alkali treatment

The milled rice grains of selected varieties were soaked in various concentrations of NaOH solution ($C_{c0} \simeq 0$ %, $C_{0.004} \simeq 0.004$ %, $C_{0.02} \simeq 0.02$ %, $C_{0.04} \simeq 0.04$ %, and $C_{0.2} \simeq 0.2$ %) at 40^{o}C for 1 hr with a rice to solution ratio of 1:8. After 1 hr the treated rice grains were washed with deionised water until completely neutralised (pH = 7.0 approx.). These concentrations corresponded to 7.0, 11.0, 11.7, 12.0 and 12.7 pH, respectively. The treated samples were spread on blotting paper and kept in a fume hood at room temperature (22±1^{o}C, RH~50 %) for 72 hrs to reduce the moisture contents to 14 %. One control (C_c) sample without any treatment was also kept for comparison.

6.3.2. Color estimation of alkali treated rice grains

A Konica Minolta Chroma Meter CR-400 (Tokyo, Japan) was used for all color measurements. Before color measurements, the color meter was calibrated with a white tile. Color measurements were made at least in three folds of samples placed in a clear petri dish. The color was measured in CIE*L*a*b** color space. *L** is a measurement of brightness from black (0) to white (100). Parameter *a** refers red-green color with positive *a**-values indicating redness and negative *a**-

values indicating greenness. Whereas, parameter *b** refers yellow-blue color with positive *b**-values indicating yellowness and negative *b**-values indicating blueness (Good 2002).

6.3.3. Confocal laser scanning microscopy (CLSM)

Alkali-treated rice grains were dyed with a mixture (1:1) of 0.01 % (w/v in water) Rhodamine B (Sigma R6626) and 0.02 % (w/v in poly (ethylene glycol) 200 (Fluka 81150)) Nile Red (Sigma 72485) for labelling protein and lipid, respectively. The samples were treated with dyes in the dark with intermittent shaking. After 10 min dye-labelled samples were washed with deionised water until the supernatant became clear. The microstructure of rice grains was observed by using an LSM 700 confocal laser scanning microscope (CLSM, Zeiss, Germany).

6.3.4. Crude protein analysis

Rice samples were ground to flour using a disc mill (Good Friends of the Guangzhou Machinery Co. Ltd., Guangzhou, China). The flour particles were sieved through 500 µm sieve to attain particle size $\leq$ 500 µm. Crude protein content in rice flour was determined by the semi-micro-Kjeldahl method using a Kjeltec 2300 Autoanalyser (Foss AB, Sweden). A nitrogen conversion factor of 5.95 was used to compute the protein value.

6.3.5. Textural profile analysis

Rice grains (5 g) were added in 15 mL of MilliQ water (rice: water ratio =1:3) in 50 mL glass beaker. The beaker was placed in a water bath at 95±1ºC. Cooking was continued until there was no ungelatinized white belly observed in rice kernel cross section (data not shown). Analysis of textural attributes was performed on a TA-XT*plus* Texture Analyzer (Stable Microsystems, UK) using 35-mm circular probe. Three cooked grains were placed on the flat stage, and the texture determined. The texture analyzer settings were as follows: pre-test speed, 2.00 mm/sec; post-test speed, 2.00 mm/sec; distance, 2.00 mm; time, 10.00 sec; (auto) trigger force, 0.05 N. From the force-time curve obtained, textural attributes of hardness (height of the force peak on cycle 1, N) and adhesiveness (negative force area of the first cycle, N.s) were computed using the EXPONENT Stable Micro Systems software supplied with instrument. The TPA values reported are the averages of 3 different determinations.

6.3.6. Pasting properties

Pasting properties of rice flour (particle size ≤ 500 µm) were determined according to the AACC International Method 61-02.01 using a Rapid Visco Analyzer (RVA-4 model Thermocline Windows Control and analysis software, Version 1.2 (New Port Scientific, Sydney, Australia)). Rice flour (3.01 g, 12.4 % moisture basis) was mixed with 25.0 g MilliQ water in the RVA canister. A programmed heating and cooling cycle were used, the samples were held at 50ºC for 1 min, heated to 95ºC in 3.45 min, held at 95ºC for 2.7 min before cooling to 50ºC in 3.91 min and holding at 50ºC for 1.24 min. Pasting temperature (P_{temp}), Peak viscosity (V_p), Trough viscosity (V_t), Breakdown (BD), Final viscosity (V_f) and Setback (SB) were recorded.

There was no peak viscosity found in DG viscographs. Therefore, Point of inflection (pi) was calculated using the 1st derivative for every 30 sec (F´ 30s) as shown in Appendix 3. Viscosity at the point of inflection (V_{pi}) was estimated by using pi.

6.3.7. Gelatinization and retrogradation properties

Differential Scanning Calorimeter (DSC) (Mettler Toledo, Schwerzenbach, Switzerland) with internal coolant and nitrogen/air purge gas was used to determine the gelatinization characteristics of rice flours. The DSC was calibrated for the heat flow using indium as standard. Rice flour (4 mg, dry weight basis) was accurately weighed into an aluminum pan and six µL MilliQ water was added. The pan was hermetically sealed and equilibrated at room temperature for 30 min, then scanned at the heating rate of 15ºC/min from 0 to 100ºC with the empty sealed pan as a reference. The onset (T_o), peak (T_p) and conclusion (T_c) temperatures, and enthalpy (ΔH) of gelatinization was determined by Star^e Software Version 9.1 (Mettler Toledo).

After cooling, the scanned samples pans were placed in a refrigerator at 4±1ºC for seven days. Retrogradation properties were measured by rescanning these samples at the rate of 15ºC/min from 0 to 100ºC. The onset ($T_{o(r)}$), peak ($T_{p(r)}$) and conclusion ($T_{c(r)}$) temperatures, and enthalpy of retrograded starch ($\Delta H_{(r)}$) were determined. The percentage of retrogradation (R %) was calculated as $\Delta H_{(r)}/\Delta H$ X 100.

6.3.8. Statistical analysis

All treatments were replicated three times to obtain mean values. The reported data for the CIE*L*a*b** color space, crude protein, textural profile analysis, pasting properties, and

gelatinization and retrogradation for each variety were analysed separately by analysis of variance (Completely Randomized Design) using Minitab R17 (Minitab® for Windows Release 17, Minitab Inc, Chicago) to determine significant differences. The data was then analysed using Tukey's pair-wise comparison, at 5 % level of significance, to compare the results between different treatments.

6.4. Results and discussion

6.4.1. Color estimation of alkali treated rice grains

The color parameters of the raw and cooked grains of control and alkali treated TDK8 and DG are shown in Table 6.1. Significant ($P<0.05$) decrease in the brightness (L^*) of raw TDK8 kernels was observed when treated with 0.2 % of NaOH. However, no change in L^* was observed in raw DG rice kernels. From 0.02 % to 0.2 % of NaOH concentration induced a significant ($P<0.05$) increase in greenness ($-a^*$) in raw TDK8 rice; whereas, only 0.2 % of NaOH induced a significant ($P<0.05$) greenness in raw DG rice grains. For raw TDK8 rice grains, yellowness (b^*) increased significantly with 0.2 % of NaOH. Raw DG rice grains were susceptible to alkali yellowness (b^*) and significant ($P<0.05$) increase in yellowness (b^*) was observed with an increase in alkali concentration. In cooked TDK8 rice kernels significant ($P<0.05$) decrease in the brightness (L^*) at 0.004 % of NaOH concentration was observed. However, no significant ($P>0.05$) decrease in L^* was observed with further increase in NaOH concentration. Moreover, NaOH concentration of 0.04 % induced significant ($P<0.05$) increase in L^* of cooked DG rice kernels. NaOH concentration of 0.02 % induced a significant ($P<0.05$) increase in greenness ($-a^*$) in cooked TDK8 rice. Further increase in NaOH concentration had no significant ($P>0.05$) effect on a^* of cooked TDK8 rice kernels. Whereas, NaOH concentration of 0.04 % induced a significant ($P<0.05$) increase in greenness ($-a^*$) in cooked DG rice kernels. From 0.02 % to 0.2 % of NaOH concentration induced a significant ($P<0.05$) increase in yellowness (b^*) in both cooked TDK8 and DG rice kernels. Previous studies on the effect of alkali treatment on cereal products also reported the induced yellowness in sodium hydroxide treated grains and flour (Lai et al. 2004; Nadiha et al. 2010). The induced yellow color in alkali treated rice grains may be attributable to the naturally occurring flavonoids (such as apigenin-*C*-diglycosides) present in cereal aleurone and sub-aleurone layers. These compounds are colorless at acidic or neutral pH but turn yellow at

basic pH (Asenstorfer et al. 2006). The scanned images showing color differences of raw and cooked grains of control and alkali treated TDK8 and DG are presented in Appendix 4.

6.4.2. Confocal laser scanning microscopy (CLSM)

The CLSM images of control and alkali treated rice kernels are shown in Fig. 6.1. The surface of C_c and C_{c0} in both rice varieties (TDK8 and DG) showed a layer of lipids (labelled as red with Nile red) and protein (labelled as green with Rhodamine B). Reduction of surface proteins and lipids was observed with increase in NaOH concentration from 0.004 to 0.2 %, showing washing of surface proteins (possibly mostly glutelin) and lipids. Also, besides surface proteins, some of the proteins located internally might have been removed. Although CLSM was unable to detect this, as the dyes (Rhodamine B and Nile red) were unable to penetrate the interior of the kernel.

Table 6.1 CIE*L*a*b** color space of control and alkali treated rice grains of Thadokkham-8 (TDK8) and Doongara (DG)*

Rice variety	**Treatment**	**CIE*L*a*b** color space**		
		*L**	*a**	*b**
Raw TDK8 rice grains	C_c	97.4±0.18^{ab}	-0.41±0.04^{a}	7.56±0.09^{a}
	C_{c0}	97.6±0.01^{a}	-0.38±0.05^{a}	7.99±0.12^{a}
	$C_{0.004}$	97.2±0.41^{ab}	-0.41±0.02^{a}	7.82±0.28^{a}
	$C_{0.02}$	97.7±0.04^{a}	-0.59±0.04^{b}	9.00±0.29^{a}
	$C_{0.04}$	97.6±0.52^{a}	-0.86±0.04^{c}	9.27±0.95^{a}
	$C_{0.2}$	96.4±0.09^{b}	-1.88±0.03^{d}	13.21±0.20^{b}
Cooked TDK8 rice grains	C_c	96.8±0.35^{ab}	-0.96±0.02^{a}	0.55±0.01^{a}
	C_{c0}	97.7±0.37^{a}	-0.68±0.32^{a}	0.37±0.01^{a}
	$C_{0.004}$	95.6±0.21^{c}	-0.84±0.05^{a}	0.65±0.04^{a}
	$C_{0.02}$	95.4±0.57^{cd}	-1.69±0.02^{b}	2.54±0.06^{b}
	$C_{0.04}$	94.8±0.14^{cd}	-1.79±0.01^{b}	3.55±0.10^{c}
	$C_{0.2}$	94.2±0.05^{d}	-1.85±0.04^{b}	4.35±0.32^{d}
Raw DG rice grains	C_c	94.1±0.23^{a}	0.43±0.04^{a}	8.87±0.08^{a}
	C_{c0}	94.6±0.71^{a}	0.38±0.02^{a}	9.69±0.03^{b}
	$C_{0.004}$	93.9±1.38^{a}	0.34±0.04^{a}	9.97±0.18^{bc}
	$C_{0.02}$	93.9±0.60^{a}	0.35±0.01^{a}	10.34±0.18^{d}
	$C_{0.04}$	94.3±0.55^{a}	0.39±0.02^{a}	10.30±0.03^{bc}
	$C_{0.2}$	94.1±0.18^{a}	-0.55±0.10^{b}	13.10±0.05^{e}
Cooked DG rice grains	C_c	93.1±0.26^{a}	1.20±0.28^{a}	7.25±0.35^{ab}
	C_{c0}	94.3±0.35^{ab}	0.73±0.04^{ab}	6.45±0.35^{a}
	$C_{0.004}$	93.4±0.21^{a}	0.65±0.04^{b}	7.55±0.21^{ab}
	$C_{0.02}$	93.8±0.57^{a}	0.39±0.01^{b}	8.55±0.21^{b}
	$C_{0.04}$	95.2±0.07^{bc}	-0.13±0.04^{c}	10.34±0.09^{c}
	$C_{0.2}$	95.5±0.19^{c}	-0.35±0.07^{c}	14.10±0.71^{d}

*Means ± SD (n = 3). For a particular rice variety, means with different letters in the same column denote significant difference at 5 % probability level within each rice variety.

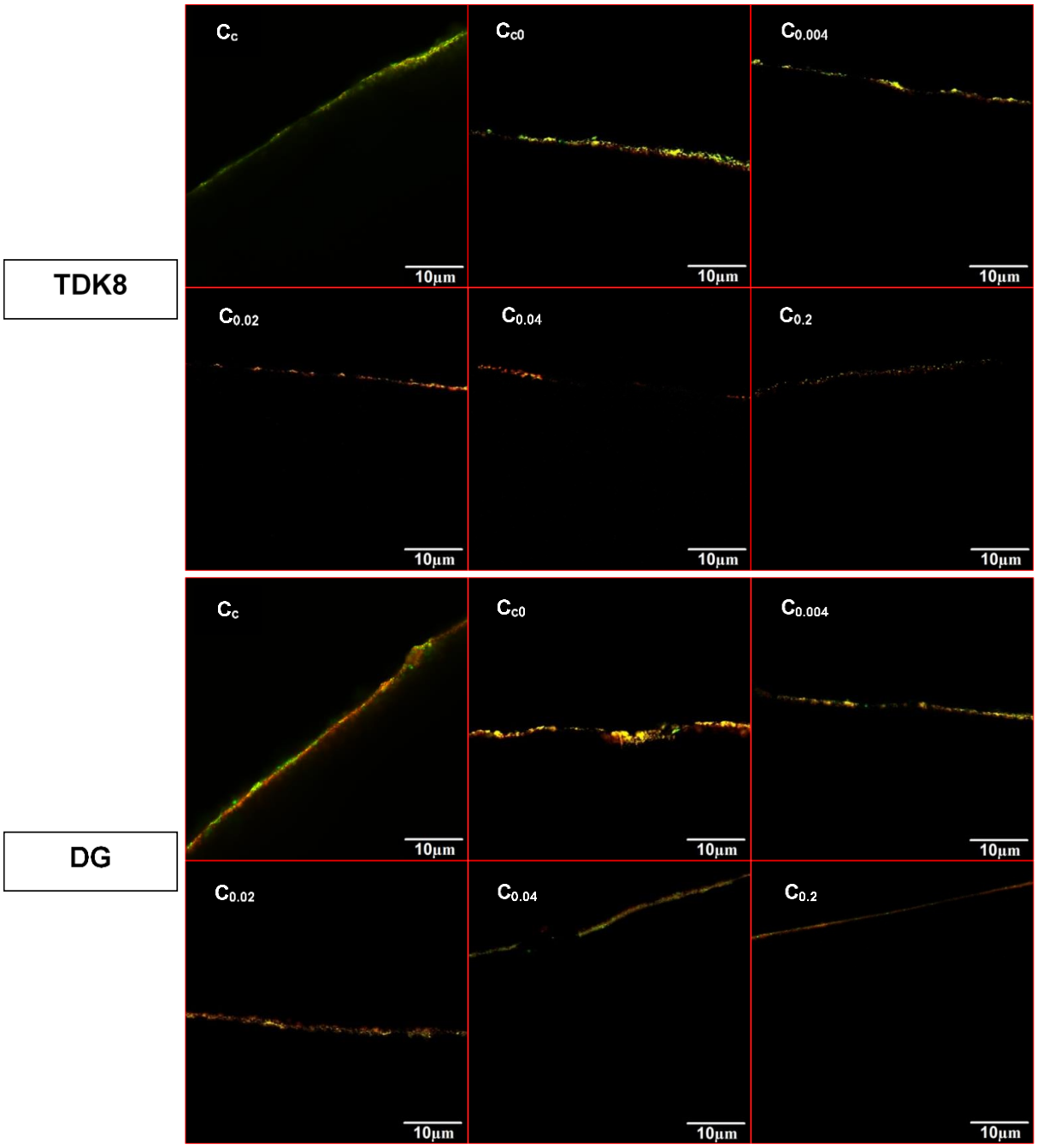

Figure 6.1 Confocal laser scanning micrographs of control and alkali treated rice grains of Thadokkham-8 (TDK8) and Doongara (DG). Lipids and proteins are labelled in red and green, respectively

6.4.3. Mass loss during alkali treatment

Less than 3 - 4.5 % of mass loss was recorded in the water, and maximum alkali (0.2 % NaOH w/v) treated samples (data not shown). Soaking of milled rice kernels with alkali solution not only

washed surface proteins and saponify surface lipids but also removed intact dust and bran residues. No doubt there might be a loss of water-soluble components from grains which were not analysed in the present study and needed further investigation.

6.4.4. Crude protein of control and alkali treated rice samples

The crude protein content of control and alkali treated TDK8 and DG are presented in Fig. 6.2a and 6.2b, respectively. Results showed that DG might have more alkali-soluble protein (such as glutelin) content than TDK8. There was no significant ($P>0.05$) reduction of the total crude protein content of TDK8 up to 0.04 % of NaOH when compared to C_c (control). However, significant ($P<0.05$) reduction of total protein content was found in DG even at 0.004 % of NaOH.

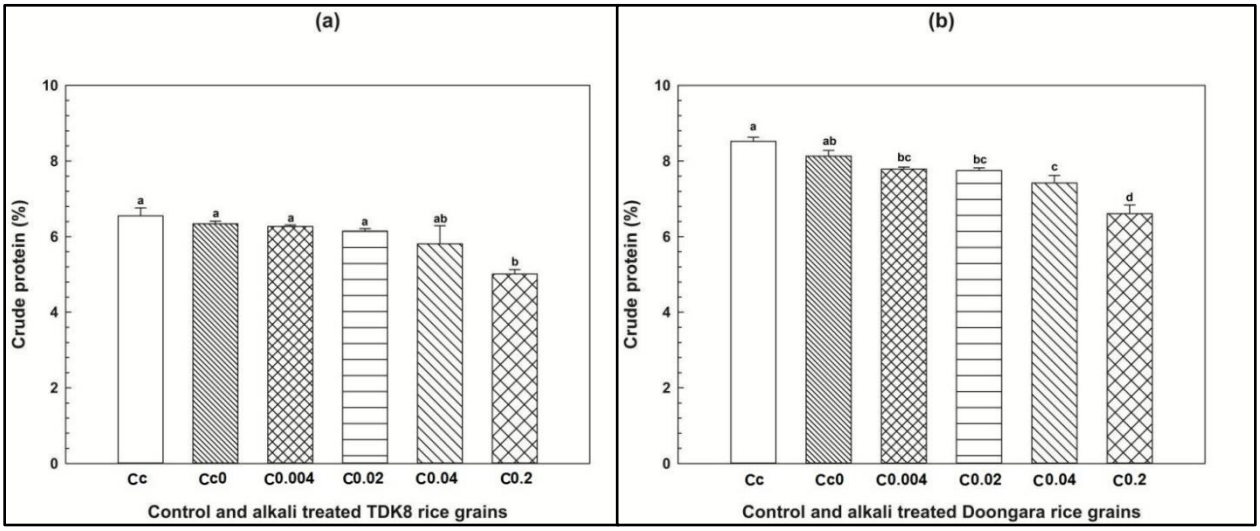

Figure 6.2 Crude protein content of control and alkali treated rice grains; (a) Thadokkham-8 (TDK8), and (b) Doongara (DG)*

*Means ± SD (n = 3). Within figure, different letters denote significant difference at 5 % probability level.

6.4.5. Textural profile analysis

Textural profiles of cooked control and alkali treated TDK8, and DG rice grains are shown in Fig. 6.3a and 6.3b, respectively. Cooked TDK8 rice hardness values ranged from 2.12±0.004 N (C_{c0}) to 7.84±0.011 N ($C_{0.2}$) and from 8.03±0.058 N (C_{c0}) to 18.56±0.157 N ($C_{0.2}$) for cooked DG rice. The rice samples treated with 0 % water showed the least hardness in both rice varieties. Results showed a significant ($P<0.05$) increase in cooked rice hardness with an increase in NaOH concentration from $C_{0.004}$ to $C_{0.2}$ in both rice varieties. Baxter and co-workers (2004) reported that

removal of prolamin by 100 % propan-2-ol resulted in significant (P<0.05) increase in hardness which is similar to our results where we removed the water-soluble proteins by treating with alkaline conditions.

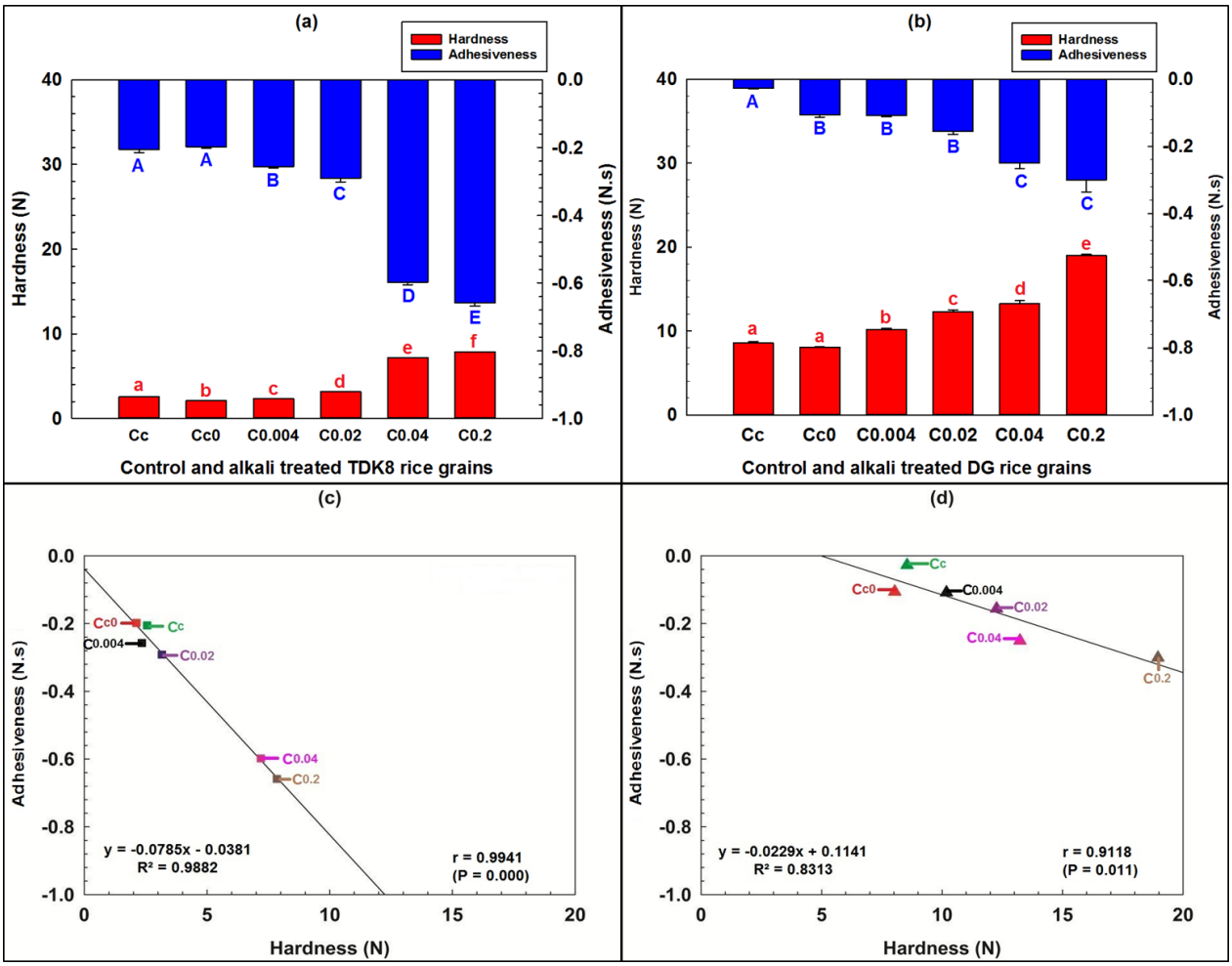

Figure 6.3 Textural profile analysis of control and alkali treated rice grains (a) Thadokkham-8 (TDK8), and (b) Doongara (DG).* Correlation (r) between hardness and adhesiveness of control and alkali treated rice grains; (c) Thadokkham-8 (TDK8), and (d) Doongara (DG)+

*Means ± SD (n = 3). Within figure, significant differences are denoted by lowercase letters for hardness and uppercase letters for adhesiveness at 5 % probability level.

+The negative sign associated with adhesiveness was ignored while calculating the correlation (r) due to more adhesiveness associated with a greater negative value.

The effect of protein removal through alkali (NaOH) treatment on the adhesiveness/stickiness of cooked rice grains of selected rice varieties TDK8 and DG is shown in Fig. 6.3a and 6.3b, respectively. Cooked rice stickiness increased significantly (P<0.05) with a decrease in grain

surface protein contents, as indicated in CLSM images of control and alkali treated rice grains of selected varieties (Fig. 6.1). Similar to the current findings, Chrastil (1990b) also reported that the adhesiveness/stickiness of rice could be increased with the reduced amount of glutelin it contained. Also, there was significant ($P<0.05$) correlation found between hardness and adhesiveness of alkali treated rice grains TDK8 and DG as shown in Fig. 6.3b and 6.3c, respectively. Cooking is a complex process due to several physicochemical changes taking place simultaneously. The surface layers, cells and granules disintegrate, and components leach out from the cells (Tamura et al. 2014). This disintegration allows starch granules to interact with protein bodies, mostly amylopectin with glutelin (Chrastil 1990b; Baxter et al. 2014). This starch-protein interaction affects the overall stickiness of the cooked rice, greater the interaction higher the stickiness will be. Previous studies reported the reduced starch-protein interaction in aged rice resulted in decreased stickiness (Chrastil 1990b; Derycke et al. 2005b; Baxter et al. 2014), probably due to a thin layer of non-interacted protein (glutelin) bodies on the rice endosperm surface. This explanation supports the hypothesis and findings of the present study; washing of surface proteins led to the increased stickiness of cooked rice.

6.4.6. Pasting properties

The pasting properties of control and alkali treated TDK8, and DG rice flours are shown in Table 6.2. Results showed the diverse behavior of samples due to alkali treatment. Increase in pasting temperature (P_{temp}) with an increase in surface proteins removal was observed for TDK8. However, a slight decrease in P_{temp} was found in DG with an increase in surface proteins removal. Increase in surface proteins removal restricted the swelling of starch granules in TDK8 flour, resulting in significant ($P<0.05$) decrease in peak viscosity (V_p). However, protein removal did not affect viscosity at the point of inflection (V_{pi}) in the case of DG. Probably, sodium hydroxide (NaOH) treatment had masked the effect of protein on V_p. It was also found that an increase in alkali concentration also significantly ($P<0.05$) reduced the V_p. Interestingly, these results are not in agreement with the findings of Lai et al. (2004), who reported significant ($P<0.05$) increase in V_p of native cereal starches in sodium carbonate (Na_2CO_3) and NaOH solutions. However, similar results of restricted V_p of alkali treated sago starch were reported by Karim et al. (2008). The possible reason for the variation in the pasting behaviors may be the difference in the sample preparation. In this study and the study carried out by Karim et al. (2008), cereal samples were

treated with alkali and subsequently washed with deionised water for 15 min to neutralise the pH before examining the pasting behavior. However, Lai et al. (2004) carried out pasting studies on starch samples suspended in alkali solutions such as 1 % Na_2CO_3 and 1 % NaOH. As the sample preparation in the present study is similar to that of Karim et al. (2008), the reduced V_p could be due to the disrupted amorphous regions and weakened granular rigidity of alkali treated starch samples.

In general, protein removal resulted in decreased Breakdown (BD) and Setback (SB) of TDK8 flour and Final viscosity (V_f) of both TDK8 and DG flours. Although there was no BD and Trough viscosity (V_t) observed in DG, increase in alkali concentration reduced the rate of increase in viscosity during holding at 95ºC as shown in Appendix 5. The results are on par with the findings of Baxter and co-workers (2004), who studied the effect of prolamin on the pasting properties of rice flour. They reported that extraction of approx. 95 % of total prolamin fractions in rice flours resulted in significant ($P<0.05$) reduction in BD and V_f and a slight reduction in SB. However, this study did not investigate the effect of surface protein removal from the rice flour or kernels.

6.4.7. Gelatinization and retrogradation properties by DSC

The gelatinization and retrogradation properties of control and alkali treated TDK8, and DG rice flours are shown in Table 6.3. Protein removal via alkali treatment resulted in increased onset (T_o), peak (T_p), conclusion (T_c) temperatures and enthalpy (ΔH) during gelatinization of both rice varieties. The rise in gelatinization transition temperatures and enthalpy with an increase in NaOH concentration may be attributed to the starch granule stability, possibly through electrostatic interactions between hydroxyl groups of starch and Na^+ ions. Starch exhibits Donnan-potential in the presence of water due to its weak acidic ion-exchanging behavior (Oosten 1990). The starch particles have a negative charge; therefore, penetration of Na^+ into the amorphous regions of starch granules is promoted. Moreover, under alkaline conditions, hydroxyl groups of starch might have a greater tendency to ionise and create even more binding sites for cations. It is hypothesised by Oosten (1990) that anions might tend to destabilise starch granules by breaking hydrogen bonds. However, such destabilising effects of anions might be much weaker than the stabilising effects of cations. Several researchers have reported similar stabilising effects of sodium salts such as sodium chloride (Abd Ghani et al. 1999; Evans & Haisman 1982), sodium acetate (Evans & Haisman 1982), sodium sulphate (Evans & Haisman 1982), and sodium carbonate (Lai et al. 2002).

On the other hand, no significant ($P>0.05$) effect of protein removal was observed on the retrogradation thermal temperatures ($T_{o(r)}$, $T_{p(r)}$, and $T_{c(r)}$), but enthalpy of retrograded starch ($\Delta H_{(r)}$) was decreased significantly ($P<0.05$) in the flour samples of both varieties. This indicates that a higher concentration of alkali treatment on starch restricts retrogradation. This was the highest amount of decrease in retrogradation at the highest concentration (0.2 % w/v) of alkali treatment. In fact, $\Delta H_{(r)}$ provides an overall measure of the energy requirement for melting or uncoiling of double helices of recrystallised amylopectin (Russell 1987). Significant ($P<0.05$) decrease in $\Delta H_{(r)}$ with an increase in alkali treatment may be indicative of structural changes in amylopectin, possibly alkali-induced depolymerisation. This will need further investigation.

6.5. Conclusions

This study showed that the protein surface layer from rice kernel or rice flour could be washed by using very dilute (as low as 0.004 %) alkali (NaOH) solutions. It was found that NaOH treatment at a concentration of 0.04 % induced yellow color development in grains. Moreover, it was also observed that textural, pasting, thermal attributes and retrogradation properties were also affected by alkali solution washing. The stickiness of cooked glutinous and non-glutinous (TDK8 and DG, respectively) rice could be significantly increased by washing with dilute NaOH solutions. The contrasting effects of washing of surface proteins and NaOH concentration mean that it might be promising to manipulate the textural properties of glutinous and non-glutinous rice kernels to achieve desirable sensory outcomes by varying the proportions of the surface proteins in milled rice kernels.

Table 6.2 Pasting properties of control and alkali treated flour of Thadokkham-8 (TDK8) and Doongara (DG)*

Rice variety	Treatment	Pasting properties					
		P_{temp} (°C)	V_p (mPa-s)	V_t (mPa-s)	BD (mPa-s)	V_f (mPa-s)	SB (mPa-s)
TDK8 flour	C_c	74.7±0.67^a	2417±27.58^a	2121±46.67^a	296±74.25^{ab}	2527±43.13^a	406±3.54^a
	C_{c0}	73.3±0.78^a	2784±108.19^b	2183±61.52^a	601±169.71^a	2584±45.96^a	401±15.56^a
	$C_{0.004}$	73.9±0.49^a	2692±25.46^b	2063±41.01^{ab}	629±15.59^a	2473±17.68^a	410±23.33^a
	$C_{0.02}$	73.8±0.64^a	2357±70.00^a	1966±7.78^b	391±77.78^{ab}	2244±22.63^b	279±14.85^b
	$C_{0.04}$	74.1±0.28^a	2184±62.23^a	1722±12.73^c	462±74.95^a	2029±52.33^c	307±39.60^b
	$C_{0.2}$	79.7±0.46^b	1535±14.14^c	1481±16.26^d	255±2.12^b	1751±14.14^d	271±2.12^b
Rice variety	**Treatment**	**P_{temp} (°C)**	**V_{pi} (mPa-s)**	**V_f (mPa-s)**			
DG flour	C_c	83.3±0.60^a	304±44.55^a	3469±151.32^a			
	C_{c0}	82.5±1.73^a	297±87.68^a	3014±177.48^b			
	$C_{0.004}$	82.1±0.78^a	341±50.91^a	3037±62.93^b			
	$C_{0.02}$	80.8±0.49^a	363±87.68^a	2848±75.66^{bc}			
	$C_{0.04}$	80.7±0.18^a	316±3.54^a	2466±3.54^c			
	$C_{0.2}$	79.9±0.35^a	225±31.82^a	2594±11.31^c			

*Means ± SD (n = 3). For a particular rice variety, means with different letters in the same column denote significant difference at 5 % probability level within each variety.

Table 6.3 Gelatinization and retrogradation properties of control and alkali treated flour of Thadokkham-8 (TDK8) and Doongara (DG)*

Rice variety	Treatment	Gelatinization				Retrogradation				
		T_o (°C)	T_p (°C)	T_c (°C)	ΔH (Jg^{-1})	$T_{o(r)}$ (°C)	$T_{p(r)}$ (°C)	$T_{c(r)}$ (°C)	$\Delta H_{(r)}$ (Jg^{-1})	R (%)
TDK8 flour	C_c	64.4±0.40^a	71.6±0.01^a	84.6±0.47^a	10.4±0.23^{ab}	41.1±0.18^a	51.9±0.33^a	60.0±0.45^a	4.4±0.26^{ab}	42.2±3.44^{ab}
	C_{c0}	64.9±0.45^a	72.5±0.16^b	87.8±0.81^b	9.3±0.78^a	41.4±1.22^a	52.2±1.01^a	60.3±0.23^a	4.5±0.14^a	48.2±2.53^a
	$C_{0.004}$	65.1±0.75^a	72.5±0.22^b	86.3±0.18^{ab}	9.3±0.19^a	41.7±1.28^a	52.2±0.34^a	59.8±0.16^a	3.5±0.06^c	38.0±0.10^b
	$C_{0.02}$	64.1±0.71^a	72.5±0.19^b	87.8±0.27^{ab}	10.6±0.21^{ab}	41.5±1.97^a	52.3±0.86^a	60.3±0.18^a	3.8±0.09^c	35.9±1.56^b
	$C_{0.04}$	64.8±0.16^a	72.9±0.43^b	88.7±0.13^b	11.0±0.08^b	42.0±0.23^a	52.7±0.34^a	60.2±0.25^a	3.9±0.02^{bc}	35.5±0.47^b
	$C_{0.2}$	68.2±1.24^b	75.1±0.03^c	92.6±1.74^c	11.1±0.02^b	41.8±0.02^a	52.7±0.33^a	60.5±0.04^a	3.9±0.04^{bc}	35.0±0.39^b
DG flour	C_c	70.3±0.45^a	76.1±0.49^a	83.3±0.73^a	6.9±0.35^{ab}	39.2±2.17^a	53.4±0.01^a	62.4±0.11^a	4.1±0.12^{ab}	58.6±1.26^b
	C_{c0}	70.7±0.08^{ab}	76.3±0.43^a	83.8±0.34^a	6.0±0.12^a	40.2±0.54^a	53.7±1.00^a	62.7±0.30^a	4.2±0.06^b	70.2±2.47^a
	$C_{0.004}$	70.9±0.16^{ab}	76.6±0.23^a	84.1±0.17^a	6.2±0.17^a	41.3±0.52^a	53.6±0.54^a	63.1±0.07^a	3.5±0.02^{bc}	56.6±1.21^b
	$C_{0.02}$	70.9±0.02^{ab}	76.5±0.25^a	83.7±0.62^a	6.2±0.06^a	42.0±1.10^a	54.1±0.66^a	62.8±0.37^a	3.2±0.05^c	50.9±1.26^{bc}
	$C_{0.04}$	70.6±0.16^{ab}	76.3±0.22^a	83.1±0.04^a	7.4±0.40^b	42.2±0.68^a	54.6±0.01^a	62.3±0.13^a	3.3±0.07^c	45.1±3.36^c
	$C_{0.2}$	71.5±0.47^b	78.2±0.15^b	88.3±1.67^b	8.4±0.09^c	42.3±0.62^a	54.8±0.52^a	62.8±1.46^a	2.4±0.36^d	29.0±3.97^d

*Means ± SD (n = 3). For a particular rice variety, means with different letters in the same column denote significant difference at 5 % probability level within each variety.

Chapter 7 Effect of starch modification in the whole white rice grains on physicochemical properties of two contrasting rice varieties

This chapter has been published in the Journal of Cereal Science;

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7.1. Abstract

The effect of acetylation of milled rice of selected rice varieties viz. TDK8 and DG on their physicochemical properties were investigated at different acetic anhydride concentrations (1 – 7 g per 100 g of milled rice samples in 225 mL of water). Results showed that the intact starch of milled grains of both selected verities could be acetylated (Acetyl % for TDK8 = 2.18 and DG = 0.89) even with 1 g of acetic anhydride. X-ray diffraction patterns showed that acetylation resulted in reduced crystallinity. Acetylation resulted in reduced peak and final viscosities and gel strength, particularly in glutinous (TDK 8) and non-glutinous (DG) rice. The thermal study showed acetylation resulted in reduced thermal transition temperatures and enthalpy of both varieties. Although the increase in retrogradation thermal temperatures was observed, the amount of retrograded starch was decreased in both varieties. Furthermore, the texture of cooked acetylated grains was less hard and more adhesive. *In vitro* digestion showed a significant decrease in GI possibly due to structural changes in the native starch during acetylation. These findings suggest a good potential of applying acetic anhydride pre-treatments in rice processing, especially glutinous varieties to control the hardness and maintain the stickiness properties of rice.

7.2. Introduction

Rice (*Oryza sativa* L.) together with wheat and maize provides the major portion of daily food calories to more than 4.5 billion people in developing countries around the globe (Shiferaw et al. 2011). Rice grain quality is mostly assessed on four main aspects; milling yield, grain appearance, nutritional value and cooking/eating attributes. Among these quality aspects, the cooking and eating attributes are the most important traits affecting consumer acceptability of rice. Starch, is the major component of rice, is considered as the main contributor influencing the physicochemical properties such as endosperm physical appearance, water absorption and swelling, gelatinization and retrogradation behavior (Pan et al. 2017).

Among all factors, the amylose-amylopectin ratio is the most important parameter affecting physicochemical properties of starch pastes/gels and characteristics of cereals (Jiamjariyatam et al. 2015). Amylose contents (AC) of the rice endosperm are widely used to classify the rice varieties such as glutinous rice (< 5 % AC), very low amylose rice (5-10 % AC), low amylose rice (10-19 % AC), intermediate amylose rice (20-25 % AC), and high amylose rice (> 25 % AC) (Gayin et al. 2015; Nawaz et al. 2016a). Glutinous rice, also known as sticky or waxy rice primarily

contains branched amylopectin or very low amylose content, has a white and opaque endosperm because of the air spaces between the starch granules. When cooked, the grain loses its original shape and becomes very sticky. Non-glutinous or non-waxy rice has a translucent appearance and contains amylose as well as amylopectin. The cooked grain tends to retain its shape and is less sticky (Zhu et al. 2017).

Rice quality is usually assessed by functional properties such as water absorption, pasting properties and thermal properties (Pang et al. 2016; Nawaz et al. 2016b). Fresh, good quality glutinous rice usually swells to a greater extent, and cooked grains have high adhesiveness than their non-glutinous counterparts. However, storage at ambient temperature can lead to decrease in water absorption, longer cooking time and reduced stickiness of cooked glutinous rice, which is usually unacceptable attribute by the consumers (Thanathomvarakul et al. 2016). Therefore, controlled storage is usually recommended for glutinous rice to slow down the age-related changes in functional properties of glutinous rice. The current controlled storage techniques to maintain the stickiness of rice are mainly chilling temperature and vacuum storage which increase the retail price of the milled grains due to high infrastructure and operational cost. Pre-processing of milled grains such as alkali and acid treatment can be a potential option to slow down the storage deterioration of glutinous rice (Nawaz et al. 2016b; Hatae et al. 1995).

It has been reported that partial starch modification can be a useful processing technique to improve the glossiness, stickiness, and softness of cooked starches (Bao et al. 2003). This can be achieved by acetic anhydride reaction with starch by esterification to produce acetylated starch. In this reaction, the hydroxyl groups on anhydrous-glucose units are substituted with acetyl groups of acetic acid (Ačkar et al., 2015). Shon & Yoo (2006) performed the rheological analysis of acetylated rice starch pastes with different acetyl substitution degrees and reported that the acetylation could increase the swelling power and solubility of starch pastes. They also reported that the increase in the degree of acetylation resulted in high shear-thinning fluids with high magnitudes of yield stresses.

The nutritional quality of food is also assessed by its glycemic index (GI). Glycemic response of dietary starch is directly related to the rate of digestion. Starchy foods such as rice and rice flour products have a high GI. With the new advances in human nutrition and dietetics, products with a low GI are preferable (Han & BeMiller 2007). It has been reported that modification of isolated

starches resulted in reduced production of postprandial hyperglycemic and hyperinsulinemic spikes associated with rapidly digestible starch (RDS) fractions (Hung et al. 2016). To our understanding, the effect of acetylation of intact starch in rice grain endosperm to improve physiochemical properties and nutritional quality of whole rice grains is least studied.

Therefore, the objective of current study is to treat uncooked milled rice grains of varieties with varying amylose contents with dilute acetic anhydride solution (1 – 7 g per 100 g of milled rice grains in 225 mL of water) to partially acetylate the starch prior to cooking and investigate the changes in the physicochemical properties of rice. Acetic anhydride may induce structural changes in proteins and fat, but these changes are negligible as protein and fat contents are less than 6 and 1 %, respectively of the bulk composition. This partial or limited acetylation was expected to increase the stickiness of cooked rice grains which is one of the most important quality attributes of low amylose-containing glutinous rice.

7.3. Materials and methods

One *Oryza sativa* indica cultivar of glutinous rice Thadokkham-8 (TDK8) having 3.77 % apparent amylose contents (AAC) and one *O. sativa* japonica non-glutinous rice Doongara (DG), 19.71 % (AAC) were used in this study. Both rice paddies were provided by the Department of Primary Industries (DPI), Yanco, NSW, Australia.

7.3.1. Paddy milling

Paddy was milled to brown rice using rice husker (Satake, Japan). The brown rice was milled to white rice using an abrasive polisher (Satake, Japan). To avoid the effect of the degree of milling (DOM), brown rice of all selected rice varieties were kept at 9 % DOM.

7.3.2. Grinding of rice kernels

For the analysis of pasting and thermal properties, the milled white rice grains were ground to flour using a disc mill (Good Friends of the Guangzhou Machinery Co. Ltd., Guangzhou, China) equipped with a plate of 500 µm size.

7.3.3. Acetylation of milled rice

Acetylation of starch in intact rice grain followed the method mentioned by Sodhi & Singh (2005) for rice flour with some modifications. Milled rice grains (100 g) were dispersed in 225 mL of

MilliQ water and kept in shaking water bath at 25ºC for 60 min. pH of the mixture was adjusted at 8.0 using 3.0 % (w/v) NaOH solution. Acetic anhydride (AA_1 ~ 1 g, AA_3 ~ 3 g, AA_5 ~ 5 g, and AA_7 ~ 7 g per 100 g of milled rice samples in above mixture) was added dropwise to the mixture while maintaining pH within 8.0 – 8.4 using 3.0 % (w/v) NaOH solution. Acetylation reaction was allowed to proceed for 10 min after the addition of acetic anhydride. The mixture was then adjusted to pH 4.5 with 0.5 N HCl. Grains were then washed free of acid twice with deionised water and once with 95 % ethanol and then dried at 40ºC. One set of the sample (AA_0 ~ 0 g acetic anhydride) was also prepared to see the effect of soaking. The treated samples were reweighed to estimate the mass loss of water-soluble components during acetic anhydride treatment. Moreover, one set of control (C) sample without any treatment was also kept for comparison.

7.3.4. Acetyl (%) and degree of substitution

The degree of substitution is defined as the average number of hydroxyl groups per glucose unit substituted by acetyl group (Whistler & Daniel 1995). Acetyl (%) and the degree of substitution (DS) were determined by the titration method. Flour of acetylated grains (1.0 g) was placed in a 250 mL conical flask, and 50 mL of 75 % (v/v) of ethanol in water was added. The flasks were loosely stoppered and put in shaking water bath at 50ºC for 30 min, cooled to room temperature (22±1ºC), and 40 mL of 0.5 N KOH was added (Colussi et al. 2014). The excess alkali was then back-titrated with 0.5 N HCl using phenolphthalein as an indicator. The solution was kept for 2 hrs in a fume hood, and any additional alkali, which might have leached out from the sample was titrated. A blank, using the flour of control (C) sample was also used. Acetyl (%) and degree of substitution (DS) were calculated using equation 7.1 and 7.2 (Garg & Jana 2011).

$$Acetyl\ (\%) = \frac{[(blank - sample) \times normality\ of\ the\ HCl + m \times 100]}{sample\ weight} \qquad \text{Eq. 7.1}$$

$$DS = (162 \times acetyl\ \%)/((M \times 100) - [(M - 1) \times acetyl\ \%]) \qquad \text{Eq. 7.2}$$

Where *m* is the decimal molecular weight of substituent and *M* is molecular weight of substituent.

7.3.5. X-ray diffraction

X-ray diffraction pattern measurement of rice flour of control and acetylated samples (particle size ≤ 125 μm) was analysed by using Bruker Advance D8 X-Ray diffractometer equipped with a LynxEye detector and Cu-kα (1.54 Å) radiation. The accelerating voltage and current of 30 kV

and 30 mA, respectively, in combination with scan rate 2°/min, were used. The diffractograms were recorded in a 2θ ranged from 4° to 35° with sampling width of 0.02°. Traces were analysed using the Diffract*plus* Evaluation Package Release V3.1, PDF-2 Release 2014.

The crystallinity percentage was calculated with normalised values of the intensities at each diffraction angle, using the method of Htoon & coworkers (2009). The ratio of the upper diffraction peak area taken as the crystalline portion, to total diffraction area (two-phase model), represented the percentage of crystallinity. The diffractograms were smoothed by 13 points using Traces version 3.01 software (Diffraction Technology Pty LTD, Mitchell, ACT, Australia) before calculating the percentage of crystallinity.

7.3.6. Pasting properties

Pasting properties of rice flour of control and acetylated samples (particle size < 500 µm) were determined according to the AACC International Method 61-02.01 using a Rapid Visco Analyser (RVA-4 model Thermocline Windows Control and analysis software, Version 1.2 (New Port Scientific, Sydney, Australia). Rice flour (3.01 g, 12.4 % moisture basis) was mixed with 25.0 g MilliQ water in the RVA canister. A programmed heating and cooling cycle were used, the samples were held at 50ºC for 1 min, heated to 95ºC in 3.45 min, held at 95ºC for 2.7 min before cooling to 50ºC in 3.91 min and holding at 50ºC for 1.24 min. Pasting temperature (P_{temp}), Peak viscosity (V_p), Trough viscosity (V_t), Breakdown (BD), Final viscosity (V_f) and Setback (SB) were recorded.

7.3.7. Gel strength

The RVA canisters after the estimation of pasting properties of acetylated and untreated samples were sealed with parafilm tape and stored at refrigeration temperature (4±1ºC) for 24 h. A similar method was used by Saartrat & co-workers (2005). The refrigerated RVA canisters were left at room temperature (23±1ºC) for 10 min before analysis. The gel was compressed at a speed of pre-test 2.0 mm/sec, test 0.2 mm/sec, and post-test 0.2 mm/sec, to a distance of 5.0 mm with 2.5-mm cylindrical probe under the texture profile analysis (TPA) test mode. The peak force of the first penetration was termed gel strength. The gel strength values reported are the averages of 3 different determinations.

7.3.8. Gelatinization and retrogradation properties

Differential Scanning Calorimeter (DSC) (Mettler Toledo, Schwerzenbach, Switzerland) with internal coolant and nitrogen/air purge gas was used to determine the gelatinization characteristics of rice flours. The DSC was calibrated for the heat flow using indium as standard. Rice flour (approximately 4 mg, dry weight basis) was accurately weighed into a pan and 6 µL MilliQ water was added. The pan was hermetically sealed and equilibrated at room temperature for 30 min, then scanned at the heating rate of 15ºC/min from 0 to 100ºC with the empty sealed pan as a reference. The onset (T_o), peak (T_p) and conclusion (T_c) temperatures, and enthalpy (ΔH) of gelatinization were determined by Stare Software Version 9.1 (Mettler Toledo).

After cooling, the scanned samples pans were placed in a refrigerator at 4±1ºC for 7 days. Retrogradation properties were measured by rescanning these samples at the rate of 15ºC/min from 0 to 100ºC. The onset ($T_{o(r)}$), peak ($T_{p(r)}$) and conclusion ($T_{c(r)}$) temperatures, and enthalpy of retrograded starch ($\Delta H_{(r)}$) were determined (Nawaz et al. 2016c). The percentage of retrogradation (R %) was calculated as $\Delta H_{(r)}/\Delta H$ X 100.

7.3.9. Textural profile analysis

Rice grains (5 g) were added in 15 mL of Milli-Q water (rice: water ratio =1:3) in 50 mL glass beaker. The beaker was placed in a water bath at 95±1ºC. Cooking was continued until there was no ungelatinized white belly observed in rice kernel cross section (data not shown). Analysis of textural attributes was performed on a TA-XT*plus* Texture Analyzer (Stable Microsystems, UK) using 35-mm circular probe. Three cooked grains were on the flat stage, and the texture determined. The texture analyzer settings were as follows: pre-test speed, 2.00 mm/sec; post-test speed, 2.00 mm/sec; distance, 2.00 mm; time, 10.00 sec; (auto) trigger force, 0.05 N. From the force-time curve obtained, textural attributes of hardness (height of the force peak on cycle 1, N) and adhesiveness (negative force area of the first cycle, N.s) were computed using the EXPONENT Stable Micro Systems software supplied with instrument. The TPA values reported are the averages of 3 different determinations.

7.3.10. Starch hydrolysis and GI estimation

Starch hydrolysis was done by using the in vitro digestion method proposed by Goñi et al. (1997). Starch hydrolysis rate was estimated at 30, 90 and 180 min. Glucose concentration was estimated

with a glucose oxidase-peroxidase kit (Sigma 510-A), and GI values for each treatment were estimated using the non-linear first-order kinetic model.

7.3.11. Statistical analysis

All treatments were replicated three times to obtain mean values. The reported data of acetylation and degree of substitution, x-ray diffraction patterns, pasting properties, gel strength, gelatinization and retrogradation properties, textural profile analysis and starch hydrolysis for each variety was analyzed separately by analysis of variance (Completely Randomized Design) using Minitab R17 (Minitab® for Windows Release 17, Minitab Inc, Chicago) in order to determine significant differences. The data was then analysed using Tukey's pair-wise comparison, at 5 % level of significance, to compare the means between different treatments.

7.4. Results and discussion

7.4.1. Effect of acetic anhydride concentration on acetyl (%) and degree of substitution (DS) of intact starch in the rice grains

The acetyl (%) and degree of substitution were increased by increasing the quantity of acetic anhydride as shown in Table 7.1. In general, TDK8 acetylated starch showed high acetyl (%) and degree of substitution than DG. This might be attributed to the difference in amylose contents and the difference in the intra-granule packing of rice starch in the endosperm. Moreover, the chemical substitution reaction in the glucose units of starch macromolecules may be affected by the pattern in which the amylose chains are packed in amorphous regions and the arrangement of amylopectin and amylose chains (Sodhi & Singh 2005). The addition-elimination mechanism takes place during starch acetylation and all the free hydroxyl groups of starch viz. C_6OH, C_2, and C_3 have different reactivity (Garg & Jana 2011). It has been reported by Colussi et al. (2014) that C_6OH (primary hydroxyl group) is more reactive than C_2 and C_3 (secondary hydroxyl groups) due to steric hindrance and can be readily acetylated. These findings can justify the higher degree of substitution in TDK8 (glutinous) than that of DG (non-glutinous), and further work will be conducted to analyze the structural changes due to acetylation in the starch fine structures of glutinous and non-glutinous rice varieties. Food products with acetyl (%) more than 2.5 % are not generally recognized as safe (GRAS) by Food and Drug Administration of USA (FDA 2012). Results showed that only AA_1 for TDK8 and AA_1 as well as AA_3 for DG have acetyl (%) lower than 2.5 % and can be considered as GRAS. Further study should be conducted by using lower

concentrations (< 1 g acetic anhydride per 100 gram of rice in 225 mL of water) of acetic anhydride to achieve intact starch esterification in the whole grain less than the FDA standard.

The outer surface of grain might have higher acetylation than the grain core due to more exposure to the acetic anhydride. However, this assumption could not be verified in the present study due to technical difficulties. Soaking and subsequent drying resulted in increased internal fissures making it impossible to separate the outer layers without damaging (and therefore mixing) the grain core while milling. Further studies will be conducted in the future to overcome this challenge.

Table 7.1 Acetylation and degree of substitution of control and acetic anhydride (1-7 g acetic anhydride per 100 g of milled rice in 225 mL of water) treated flour of Thadokkham-8 (TDK8) and Doongara (DG)*

Rice variety	Treatment	Acetyl (%)	Degree of Substitution
TDK8	C	-	-
	AA_0	-	-
	AA_1	2.18 ± 0.13^{a}	0.08 ± 0.01^{a}
	AA_3	3.01 ± 0.12^{b}	0.12 ± 0.00^{b}
	AA_5	3.81 ± 0.21^{c}	0.15 ± 0.01^{c}
	AA_7	4.86 ± 0.02^{d}	0.19 ± 0.00^{d}
DG	C	-	-
	AA_0	-	-
	AA_1	0.89 ± 0.11^{a}	0.03 ± 0.00^{a}
	AA_3	2.18 ± 0.15^{b}	0.08 ± 0.01^{b}
	AA_5	3.69 ± 0.26^{c}	0.14 ± 0.01^{c}
	AA_7	5.88 ± 0.06^{d}	0.24 ± 0.00^{d}

*Means ± SD (n =3). Within each variety, means with different letters in the same column denote significant difference at 5 % probability level.

7.4.2. Mass loss during acetic anhydride treatment

In general, less than 3-4.5 % of solid mass loss was recorded in water, and acetic acid treated samples of all rice varieties (data not shown). There might be a loss of water-soluble components from grains which were not analysed in the present study and needed further investigation. We assume that such a loss will be negligible affecting the results.

7.4.3. X-ray diffraction

X-ray diffraction analysis was performed to check if acetylation altered the diffraction and the crystallinity of rice flour. The x-ray diffraction spectra of control and acetylated rice flours of all varieties are presented in Fig. 7.1. As expected, the x-ray diffraction pattern of control samples of selected rice varieties exhibited A-type diffraction (XRD) pattern detected with main peaks at 14.9º, 16.9º, and 22.8º. The crystallinity for TDK8 and DG was recorded as 25.87±0.57 % and 18.73±0.36 %, respectively. It is now a well-established concept that the crystallinity depends upon the amylose/amylopectin ratio. The results confirm the earlier finding that the lower the ratio of amylose/amylopectin, the higher the degree of crystallinity (Park et al. 2007).

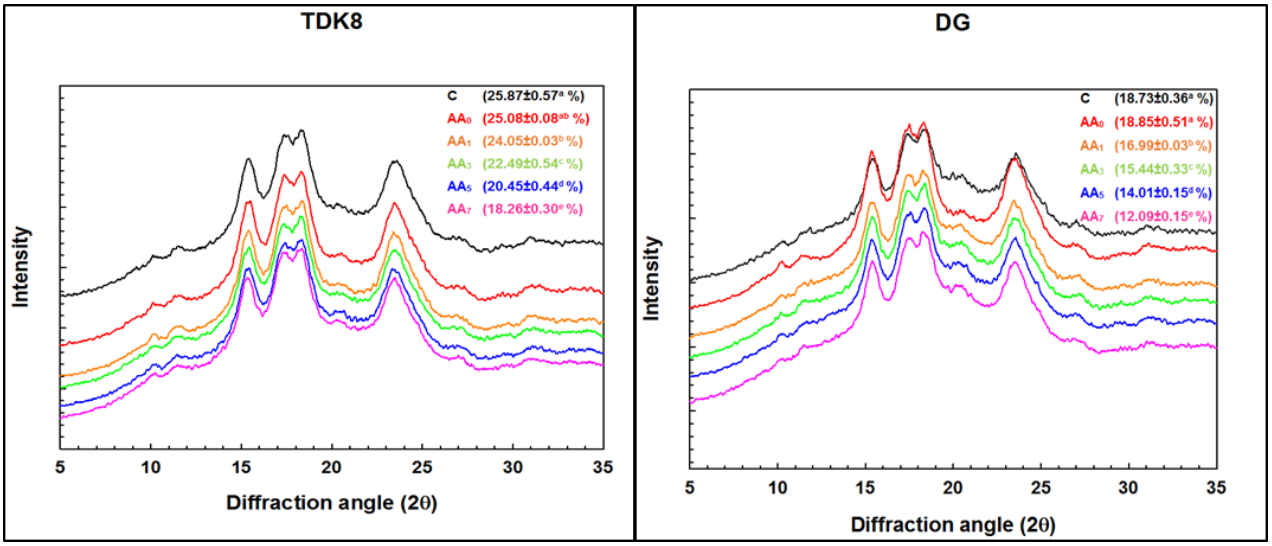

Figure 7.1 X-ray diffraction and calculated crystallinity (%) of control and acetic anhydride (1-7 g acetic anhydride per 100 g of milled rice in 225 mL of water) treated rice flour of Thadokkham-8 (TDK8) and Doongara (DG) as shown on the right side of the figures*

*Means ± SD (n = 3). Within each variety, means with different letters in the same figure denote significant difference at 5% probability level.

Native starch modification of rice showed that the samples treated with a higher acetic acid concentration depicted significant ($P<0.05$) decrease in crystallinity, possibly because of more starch acetylation and subsequently reduced reordering. Highly ordered crystalline structures in starch are due to the intra- and intermolecular hydrogen bonds. Acetylation damaged the ordered crystalline structure by reducing the formation of intermolecular hydrogen bonds (Luo & Shi 2012).

7.4.4. Pasting properties and gel strength

The pasting properties of control and acetic anhydride treated rice flours are shown in Table 7.2. Results showed the diverse pasting behavior of samples due to acetylation. The decrease in pasting temperature (P_{temp}) with an increase in acetylation was observed. Increase in starch acetylation restricted the swelling of starch granules especially in glutinous (TDK 8) rice, resulting in significant ($P<0.05$) decrease in peak viscosity (V_p). Starch swelling may be predominantly controlled by the amylose and/or amylopectin amorphous regions close to the flour particle surface (Wu et al. 2010).

Table 7.2 Pasting properties and gel strength of control and acetic anhydride (1-7 g acetic anhydride per 100 g of milled rice in 225 mL of water) treated flour of Thadokkham-8 (TDK8) and Doongara (DG)*

Rice variety	Treatment	P_{temp} (°C)	V_p (mPa-s)	V_t (mPa-s)	BD (mPa-s)	V_f (mPa-s)	SB (mPa-s)	Gel strength (N)
TDK8	C	71.5±0.53^{a}	3370.5±17.7^{b}	2218.5±21.9^{c}	1152.0±39.6^{c}	2757.5±14.8^{b}	539.0±7.1^{ab}	0.48±0.006^{a}
	AA_0	71.3±0.33^{a}	4191.0±2.8^{a}	2633.5±29.0^{a}	1557.5±26.2^{a}	3147.0±26.9^{a}	513.5±55.9^{ab}	0.44±0.004^{b}
	AA_1	70.1±0.04^{b}	4164.5±17.7^{a}	2515.5±16.3^{b}	1649.0±1.4^{a}	3120.0±22.6^{a}	604.5±6.4^{a}	0.41±0.011^{c}
	AA_3	69.2±0.11^{bc}	3096.0±24.0^{c}	2163.5±13.4^{c}	932.5±37.5^{c}	2634.5±33.2^{c}	471.0±19.8^{b}	0.36±0.005^{d}
	AA_5	68.6±0.21^{cd}	2776.0±26.9^{d}	2015.5±9.2^{d}	760.5±17.7^{d}	2546.0±11.3^{c}	530.5±20.5^{ab}	0.32±0.004^{e}
	AA_7	67.4±0.26^{d}	2590.5±20.5^{e}	1914.0±15.6^{e}	676.5±36.1^{d}	2387.0±22.6^{d}	473.0±7.1^{b}	0.29±0.004^{f}
DG	C	78.2±0.10^{a}	1771.5±21.9^{c}	1473.5±17.7^{a}	298.0±4.2^{d}	4877.5±14.8^{a}	3404.0±2.8^{a}	0.62±0.004^{a}
	AA_0	77.1±0.14^{b}	2086.5±13.4^{a}	1470.0±8.5^{a}	616.5±21.9^{a}	3522.5±16.3^{b}	2052.5±7.8^{b}	0.58±0.004^{ab}
	AA_1	76.3±0.14^{c}	1949.5±14.8^{b}	1468.0±14.1^{a}	481.5±0.7^{c}	3473.0±11.3^{b}	2006.0±2.8^{b}	0.58±0.003^{ab}
	AA_3	75.7±0.19^{c}	1928.5±12.0^{b}	1427.0±8.5^{ab}	501.5±20.5^{bc}	3326.0±18.4^{c}	1899.0±26.9^{c}	0.58±0.004^{ab}
	AA_5	74.6±0.18^{d}	1830.0±21.2^{c}	1376.0±14.1^{b}	454.0±35.4^{c}	3169.0±11.3^{d}	1793.0±2.8^{d}	0.55±0.040^{bc}
	AA_7	73.8±0.13^{e}	1824.0±8.5^{c}	1255.0±17.0^{c}	569.0±8.5^{ab}	2925.5±12.0^{e}	1670.5±29.0^{e}	0.49±0.002^{c}

*Means ± SD (n = 3). Within each variety, means with different letters in the same column denote significant difference at 5% probability level.

Increase in starch acetylation may degrade these amorphous regions, resulting in reduced water absorption and starch swelling in glutinous and low amylose varieties. In general, increased starch acetylation resulted in decreased breakdown (BD), setback (SB) and final (V_f) viscosity values of rice. The starch substitution with acetyl group of acetic anhydride may restrict the recrystallisation of amylose and/or amylopectin, resulting in reduced V_f and weak gelation, therefore, significant ($P<0.05$) reduction in the gel strength was recorded.

7.4.5. Gelatinization and retrogradation properties

The thermal (gelatinization and retrogradation) properties of control and acetic anhydride treated rice flours are presented in Table 7.3. Results showed reduced thermal transition temperatures viz. onset (T_o), peak (T_p), and conclusion (T_c), and enthalpy (ΔH) of gelatinization with an increase in acetic acid pre-treatment. This shift to lower thermal transition temperatures and enthalpy of gelatinization indicated that the greater extent of starch acetylation might accelerate the starch gelatinization at a lower temperature with less energy requirement (Ohishi et al. 2007). The DSC findings are in agreement with pasting properties results, where a slight reduction in pasting temperature (P_{temp}) was observed with increase in acetic anhydride concentration during pre-treatment.

On the other hand, increase in retrogradation temperatures viz. onset ($T_{o(r)}$), peak ($T_{p(r)}$), and conclusion ($T_{c(r)}$) was observed, but the amount of retrograded starch (as indicated by reduction in enthalpy ($\Delta H_{(r)}$) of retrograded starch) was decreased with increase in acetic anhydride concentration during pre-treatment. It was hypothesised that greater extent of starch acetylation might restrict the retrogradation possibly due to structural changes in the amylose and/or amylopectin fine structures. Modified starch molecular structures may be unable to recrystallise during storage. Interestingly, rice varieties with a higher amount of amylopectin showed a more significant decrease in retrogradation (R %) after acetic anhydride pre-treatment than the one with the higher amount of amylose. These findings revealed that branched amylopectin might be acetylated to a greater degree than the straight chain amylose. This will need further investigation.

7.4.6. Textural profile analysis

The textural profiles of cooked control and acetic anhydride treated grains of different varieties are presented in Fig. 7.2. Results showed that acetic anhydride pre-treated cooked rice grain samples of all varieties were significantly ($P<0.05$) softer and showed significantly ($P<0.05$) higher stickiness than the control cooked samples. During cooking several physicochemical changes take place simultaneously such as the disintegration of surface layers, cells and granules, and leaching out of components from the cells (Tamura et al. 2014). This cellular disintegration and leaching of cell components result in softness and stickiness of cooked rice grains. On the other hand, the leached starch mainly amylose and amylopectin with smaller molecular weight and fewer branches can rearrange the double helical fine structures during storage after cooking. This

rearrangement or recrystallisation of fine structures is known as retrogradation, result in increased hardness and reduced adhesiveness of the cooked grains (Nawaz et al. 2016b). The starch modification caused by the addition of acetyl group during esterification of starch can retard the recoiling and rearrangement of starch fine structures during storage, resulting in softer and stickier cooked rice grains.

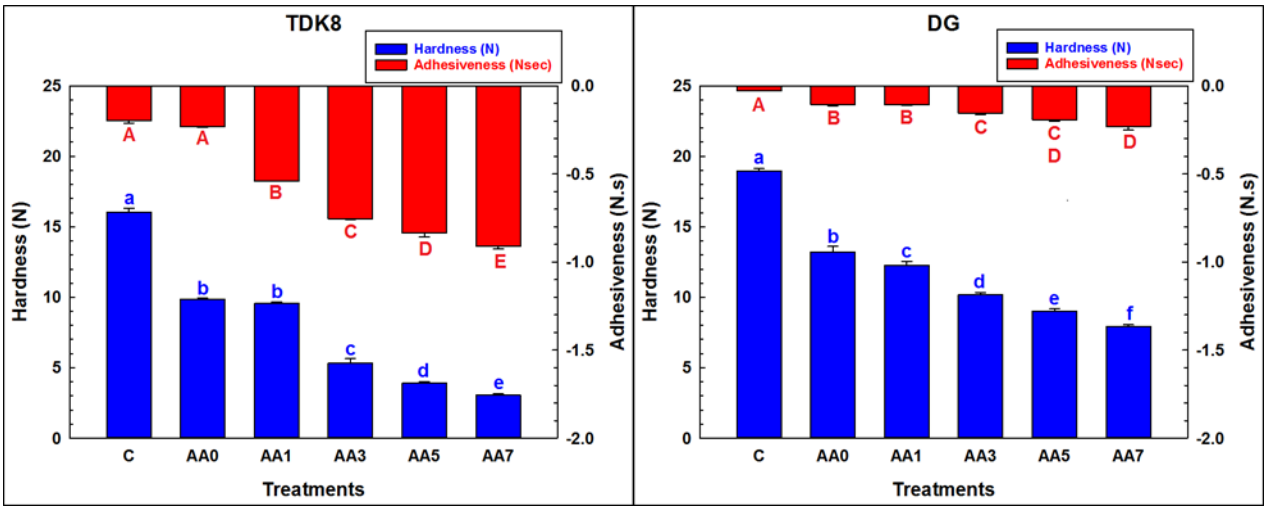

Figure 7.2 Textural profile analysis of control and acetic (1-7 g acetic anhydride per 100 g of milled rice in 225 mL of water) treated rice grains of Thadokkham-8 (TDK8) and Doongara (DG)*

*Means ± SD (n = 3). Within each variety, significant differences are denoted by lowercase letters for hardness and uppercase letters for adhesiveness at 5% probability level.

7.4.7. GI estimation

The results of estimated GI using *in vitro* digestion of 180 min are presented in Table 7.3. The GI value of TDK8 (glutinous rice) was relatively higher than that of DG (non-glutinous rice). The lower GI of DG might be due to the formation of complexes between amylose and lipids upon heating, resulting in reduced enzyme susceptibility (Frei et al. 2003). It was observed that the increase in acetyl (%) and DS of both selected verities resulted in significant ($P<0.05$) decrease in GI. These results demonstrated that the esterification of starch might have changed the structure of starch resulted in a reduced glycemic response. Reported observations are in agreement with the previous research conducted by Zhou et al. (2014).

Table 7.3 Experimental results of estimated GI, and gelatinization and retrogradation properties of control and acetic anhydride (1-7 g acetic anhydride per 100 g of milled rice in 225 mL of water) treated flour of Thadokkham-8 (TDK8) and Doongara (DG)*

Rice variety	Treatment	Estimated GI	Gelatinization				Retrogradation				
			T_o (ºC)	T_p (ºC)	T_c (ºC)	ΔH (Jg^{-1})	$T_{o(r)}$ (ºC)	$T_{p(r)}$ (ºC)	$T_{c(r)}$ (ºC)	$\Delta H_{(r)}$ (Jg^{-1})	R (%)
TDK8 flour	C	97.05±0.18^{a}	61.95±0.62^{a}	69.77±0.53^{a}	85.97±0.55^{a}	9.85±0.54^{a}	38.98±0.55^{d}	52.61±0.73^{ab}	61.29±1.27^{a}	1.59±0.03^{a}	16.17±0.61^{a}
	AA_0	93.77±0.24^{b}	61.24±0.18^{a}	68.45±0.01^{b}	80.85±0.20^{b}	8.50±0.40^{b}	41.09±0.44^{cd}	52.27±0.19^{b}	60.76±0.78^{a}	1.14±0.07^{b}	13.45±1.47^{a}
	AA_1	90.95±0.77^{c}	60.89±0.28^{ab}	67.94±0.08^{bc}	77.85±0.35^{c}	8.34±0.23^{b}	43.00±1.20^{bc}	53.07±0.15^{ab}	59.83±0.27^{a}	0.74±0.10^{c}	8.87±0.94^{b}
	AA_3	85.24±0.18^{d}	61.11±0.01^{a}	68.04±0.21^{bc}	77.93±0.82^{c}	8.23±0.09^{b}	46.20±1.67^{ab}	53.64±0.38^{ab}	61.28±1.61^{a}	0.31±0.04^{d}	3.77±0.56^{c}
	AA_5	73.11±0.41^{e}	60.94±0.01^{ab}	67.16±0.07^{cd}	76.99±0.05^{c}	7.97±0.04^{b}	47.91±0.04^{a}	53.53±0.20^{ab}	61.89±0.15^{a}	0.23±0.01^{d}	2.89±0.19^{c}
	AA_7	68.69±0.61^{f}	59.82±0.09^{b}	66.89±0.03^{d}	76.33±0.15^{c}	7.63±0.05^{b}	48.18±0.09^{a}	53.78±0.15^{a}	62.04±0.22^{a}	0.20±0.00^{d}	2.62±0.04^{c}
DG flour	C	78.24±0.02^{a}	61.09±0.16^{a}	67.48±0.09^{a}	79.15±0.15^{a}	11.31±0.14^{a}	35.70±0.38^{d}	48.67±0.50^{c}	58.81±0.11^{a}	4.47±0.36^{a}	39.46±2.70^{a}
	AA_0	77.64±0.43^{a}	60.65±0.18^{ab}	66.75±0.04^{b}	77.27±0.13^{b}	10.37±0.17^{b}	37.50±0.40^{c}	49.89±0.18^{b}	58.31±0.21^{b}	2.69±0.25^{b}	25.91±2.81^{b}
	AA_1	73.11±0.41^{b}	60.27±0.06^{bc}	66.39±0.04^{c}	76.33±0.07^{c}	9.42±0.09^{c}	38.17±0.13^{c}	49.89±0.17^{b}	58.32±0.04^{b}	1.89±0.13^{c}	20.06±1.17^{bc}
	AA_3	68.69±0.61^{c}	60.21±0.04^{bc}	66.31±0.03^{cd}	75.04±0.16^{d}	8.31±0.17^{d}	39.69±0.06^{b}	50.57±0.09^{ab}	58.02±0.10^{bc}	1.23±0.06^{cd}	14.81±0.98^{c}
	AA_5	64.07±0.61^{d}	60.01±0.12^{cd}	66.08±0.06^{d}	74.37±0.11^{e}	7.91±0.11^{de}	40.09±0.13^{ab}	50.90±0.05^{a}	57.68±0.09^{c}	1.09±0.04^{d}	13.72±0.26^{c}
	AA_7	62.92±1.42^{d}	59.70±0.11^{d}	65.78±0.10^{e}	73.90±0.17^{e}	7.48±0.06^{e}	40.82±0.09^{a}	51.12±0.06^{a}	57.18±0.08^{d}	1.02±0.01^{d}	13.65±0.07^{c}

*Means ± SD (n = 3). For a particular rice variety, means with different letters in the same column denote significant difference at 5 % probability level within each variety.

7.5. Conclusions

This study showed that the rice kernel could be acetylated by using very dilute acetic anhydride. It was observed that the textural, pasting, thermal attributes, retrogradation properties and glycemic index were affected by acetic anhydride treatment of native starch. The stickiness of cooked rice with varying apparent amylose contents (AAC) could be significantly increased by washing with acetic anhydride. The contrasting effects of limited acetylation mean that it might be promising to manipulate the textural properties of glutinous and non-glutinous rice kernels to achieve desirable sensory outcomes by modifying the native starch with varying extent of acetylation in milled rice kernels. The level of esterification and the effect of esterification of milled rice on the changes during storage will be another potential further study. Further study is needed on the level of acetylation across the grain.

Chapter 8 Effect of soaking medium on the physicochemical properties of parboiled Laotian glutinous rice

This chapter has been published in the International Journal of Food Properties;

Nawaz, MA, Fukai, S, Prakash, S & Bhandari, B 2018, 'Effect of soaking medium on the physicochemical properties of parboiled Laotian glutinous rice', *International Journal of Food Properties*, vol. 21, pp. 1896-1910.

8.1. Abstract

The effect of various soaking mediums viz. water (control), 3 % NaCl and 0.2 % acetic acid and without soaking on the physicochemical properties of parboiled selected glutinous (TDK8 and TDK11) and non-glutinous (Doongara) was investigated in the present study. Results showed that the chemistry of soaking had a significant effect on the head rice yield (HRY), grain hardness, crystallinity, color, pasting and thermal properties, textural attributes and glycemic index of these rice varieties. Soaking with NaCl and acetic acid significantly ($P<0.05$) increased the grain hardness and HRY than that of control and without soaking treatments. Acetic acid and NaCl soaking significantly ($P<0.05$) affected crystalline regions of starch, resulting in reduced crystallinity in X-ray diffraction analysis and thermal endotherms in DSC analysis. NaCl soaking induced swelling of starch granules, resulting in high peak and final viscosities. However, acetic acid restricted swelling, resulting in reduced peak and final viscosities. NaCl and acetic acid soakings also resulted in increased hardness and adhesiveness of cooked grains than normal water soaked and un-soaked parboiled rice samples. Interestingly, change in textural attributes was prominent in parboiled glutinous rice. The color difference values for fresh parboiled samples was significantly ($P<0.05$) lower for acetic acid soaked samples than NaCl soaked and un-soaked samples probably due to the bleaching effect of acetic acid. Moreover, parboiling also resulted in significant ($P<0.05$) reduction in glycemic index which shows nutritional benefits of parboiling of glutinous rice. These findings revealed the potential application of parboiling with modified soaking techniques to improve the grain quality.

8.2. Introduction

Parboiling is hydrothermal processing of paddy and/or brown rice to improve the head rice yield, nutritional and organoleptic properties by using a three-stage process viz. soaking, steaming and drying (Kar et al. 1999; Ballogou et al. 2015; Kwofie & Ngadi 2017). According to the recent statistics, 130 million tonnes of paddy is parboiled annually around the globe with about 3-4 million tonnes of high-value parboiled milled rice being moved in world trade (Bhattacharya 2013). Parboiling was initially originated and is largely practiced in India (Dutta & Mahanta 2014). It is also processed and consumed in other South Asian countries including Bangladesh (Hotz et al. 2015) and Sri Lanka (Gunaratne et al. 2013) as well as some Sub-Saharan African countries

including Ghana (Amissah et al. 2003), Benin (Demont et al. 2012), Senegal (Demont 2013), and Nigeria (Usman et al. 2014).

Parboiling results in some profound changes in the physicochemical and functional properties of rice (Haspari et al. 2016). Starch granules are swelled due to gelatinization, protein bodies are disrupted due to denaturation and disulfide cross-linking, and lipid-amylose complex formation takes place (Oli et al. 2014). Moreover, parboiling also results in the inward diffusion of water-soluble vitamins and minerals from bran to endosperm improving the nutritional quality (Patindol et al. 2017; Paiva et al. 2016). These changes in physicochemical properties of rice due to parboiling increase grain hardness and improve the milling yield (Buggenhout et al. 2013). Furthermore, physicochemical changes create more translucency and amber coloration in head rice (Lamberts et al. 2006). These changes also influence cooking properties such as the harder texture of cooked grains. Moreover, parboiling also results in increased resistant starch fraction and a low glycemic index (GI) which are health-promoting (Kale et al. 2017).

Traditionally to make parboiled rice, paddy is soaked in water for 48 hrs followed by steaming and drying in the rural rice producing communities (Kwofie & Ngadi 2017). As the global food index is taking a step forward from food security to food safety, innovations and improvements in the parboiling process are becoming a subject of research interest. Therefore, several types of research have been conducted in the past to improve the parboiling operation by varying the soaking temperature (Sareepuang et al. 2008), reduction in soaking process of paddy by using a tumbler (Hapsari et al. 2016), modifications in steaming process (Soponronnarit et al. 2006) and modifications in drying process (Swasdisevi et al. 2010). In recent years, dry-heat parboiling operations have also been introduced to improve parboiling efficiency (Dutta et al. 2015; Dutta et al. 2016).

In the past, scientists have considered on the soaking time and temperature on parboiled rice quality, but to our understanding, the effect of the modified soaking medium by the addition of organic acids and/or salt on the parboiled glutinous rice is still not well defined. Previous studies have established a weak correlation between water uptake by paddy during soaking and chemistry of soaking medium and reported that the rate of soaking could be altered by the addition of organic acids, alkalis, and salts (Bello et al. 2004). They reported that the incorporation of acids (hydrochloric acid, acetic acid, and phosphoric acid) into the soaking water reduced the water

absorption than control soaking. However, the addition of alkali (sodium hydroxide and sodium bicarbonate) significantly increased the water absorption than control soaking. Thammapat et al. (2015) reported that saline soaking of Thai glutinous rice (RD6) before parboiling significantly increased the amounts of total phenolic contents, phenolic acid, γ-oryzanol, saturated fatty acids and monosaturated fatty acids and decreased α-tocopherol and polyunsaturated fatty acids compared to controlled samples, resulting in improvement of bioactive compounds and cooking quality.

The researches reported on the parboiling of glutinous rice in the past focused only on head rice yield and nutrient diffusion, and less focus was given on the cooking quality and glycemic index (GI) of parboiled glutinous rice. Moreover, there is no data available on the parboiling of Laotian glutinous varieties such as TDK8 and TDK11. Therefore, in this study, the focus is given on the effect of soaking medium on the physicochemical properties of parboiled selected glutinous rice mainly focusing on cooking attributes and GI using *in vitro* digestion method.

8.3. Materials and methods

Fresh paddies (Feb. 2017) of two *Oryza sativa* indica cultivar of glutinous rice viz. Thadokhham-8 (TDK8) and Thadokhham-11 (TDK11) having 3.77 % and 3.72 % (w/w) apparent amylose contents (AAC), respectively were used in this study. Moreover, fresh paddy of one *O. sativa* japonica cultivar of non-glutinous rice viz. Doongara (DG) having 19.71 % (w/w) AAC was also studied for comparison.

8.3.1. Parboiling

8.3.1.1. Soaking

Paddies (250 grams each) were soaked in three different soaking mediums viz. Water, 0.2 % acetic acid and 3 % NaCl for 24 hrs at room temperature (22±1°C) with paddy to solvent ratio of 1:8. The concentrations of acetic acid and NaCl viz. 0.2 and 3 % (w/w), respectively were selected by doing preliminary soaking experiments and above-mentioned concentrations (>3 % NaCl and >0.2 % acetic acid) of soaking medium, no difference was observed on physicochemical properties of parboiled rice (data not shown). Fresh paddy without soaking was also analyzed for comparison.

8.3.1.2. Steaming

The overnight soaked paddies in various soaking mediums were drained and pressure cooked using super-heated steam at 50 kPa (gauge pressure) for 10 min in a pressure cooker (Breville, VIC, Australia). The actual time of steaming was 30 min as shown in time-pressure profile Appendix 6. Sample without soaking was also parboiled using same steaming conditions.

8.3.1.3. Drying

The parboiled paddy samples were spread on blotting paper and kept in a fume hood at room temperature (22±1^{o}C, RH~50 %) to dry for 72 hrs to the moisture content of 14 % (w/w).

8.3.2. Head rice yield (HRY)

The fresh and parboiled paddies (without soaking, water (control) soaking, 3 % NaCl soaking, and 0.2 % acetic acid soaking) were milled to brown rice using husker (Satake, Japan). The brown rice was then milled to white rice using an abrasive polisher (Satake, Japan). The degree of milling (DOM) was 9 % for all treatments, which was calculated by using equation 8.1.

$$DOM\ (\%) = [1 - (WMPR/WBPR)] \times 100 \qquad \text{Eq. 8.1}$$

WMPR and *WBPR* are the weight of milled parboiled rice and brown parboiled rice in grams, respectively.

The head rice yield (HRY %) was calculated as a percentage of whole milled grains with respect to the paddy rice.

8.3.3. Mechanical strength

The mechanical strength of individual sound milled grain (9 % DOM) of fresh and parboiled (without soaking, water (control) soaking, 3 % NaCl soaking, and 0.2 % acetic acid soaking) samples of three varieties were measured by using three-point bending test according to the method of Truong (2008). A special attachment including a cutting probe and a sample holder plate with grooves of five different sizes was used. The cutting probe was attached to the TA-XT*plus* Texture Analyzer (Stable Microsystems, UK) and grains were placed in the grooves of the sample holder. The bending test was performed in a compression test mode using pre-test, test, and post-test

speeds of 1 mm/sec, 2 mm/sec, and 10 mm/sec, respectively. The hardness (N), the maximum force required to break the grains was calculated.

8.3.4. Color estimation

The color measurements of samples for all treatments were done by using Konica Minolta Chroma Meter CR-400 (Tokyo, Japan). The color meter was calibrated with a white tile. Samples were packed in a clean petri dish. The color was measured in CIE$L^*a^*b^*$ color space. The color difference (ΔE^*_{ab}) between fresh and parboiled rice (without soaking, water (control) soaking, 3 % NaCl soaking, and 0.2 % acetic acid soaking) was also calculated using equation 8.2.

$$\Delta E^*_{ab} = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2} \qquad \text{Eq. 8.2}$$

Where, L^*_2 is the brightness of parboiled rice, L^*_1 is the brightness of fresh rice, a^*_2 refers to the red -green color of parboiled rice, a^*_1 refers to the red-green color of fresh rice, b^*_2 refers to the yellow-blue color of parboiled rice, and b^*_1 refers to the yellow-blue color of the fresh rice.

8.3.5. X-ray diffraction

Samples were ground to a flour (particle size ≤ 125 µm) using disc mill (Good Friends of the Guangzhou Machinery Co. Ltd., Guangzhou, China) equipped with a plate of 125 µm size. X-ray diffraction pattern measurement of rice flour was analysed by using Bruker Advance D8 X-Ray diffractometer equipped with a LynxEye detector and Cu-kα (1.54 Å) radiation. The accelerating voltage and current of 30 kV and 30 mA, respectively, in combination with scan rate 2°/min, were used. The diffractograms were recorded in a 2θ ranged from 4° to 35° with sampling width of 0.02°. Traces were analysed using the Diffract^{plus} Evaluation Package Release V3.1, PDF-2 Release 2014.

The crystallinity percentage was calculated with normalised values of the intensities at each diffraction angle, using the method of Htoon et al. (2009). The ratio of the upper diffraction peak area taken as the crystalline portion, to total diffraction area (two-phase model), represented the percentage of crystallinity. The diffractograms were smoothed by 13 points using Traces version 3.01 software (Diffraction Technology Pty LTD, Mitchell, ACT, Australia) before calculating the percentage of crystallinity.

8.3.6. Pasting properties

Samples were ground to a flour (particle size ≤ 500 µm) using disc mill (Good Friends of the Guangzhou Machinery Co. Ltd., Guangzhou, China). Pasting properties of rice flour samples were determined according to the AACC International Method 61-02.01 using a Rapid Visco Analyser (RVA-4 model Thermocline Windows Control and analysis software, Version 1.2 (New Port Scientific, Sydney, Australia)) (AACC, 1999). Rice flour (3.01 g, 12.4 % moisture basis) was mixed with 25.0 g MilliQ water in the RVA canister. A programmed heating and cooling cycle were used, the samples were held at 50°C for 1 min, heated to 95°C in 3.45 min, held at 95°C for 2.7 min before cooling to 50°C in 3.91 min and holding at 50°C for 1.24 min. Pasting temperature (P_{temp}), Peak viscosity (V_p), Trough viscosity (V_t), Breakdown (BD), Final viscosity (V_f) and Setback (SB) were recorded.

8.3.7. Thermal properties

Differential Scanning Calorimeter (DSC) (Mettler Toledo, Schwerzenbach, Switzerland) with internal coolant and nitrogen/air purge gas was used to determine the gelatinization characteristics of rice flours. The DSC was calibrated for the heat flow using indium as standard. Rice flour (approximately 4 mg, dry weight basis) was accurately weighed into pan and 6 µL MilliQ water was added. The pan was hermetically sealed and equilibrated at room temperature for 30 min, then scanned at the heating rate of 15°C/min from 0 to 100°C with the empty sealed pan as a reference. The onset (T_o), peak (T_p) and conclusion (T_c) temperatures, and enthalpy (ΔH) of gelatinization was determined by Star^e Software Version 9.1 (Mettler Toledo).

After cooling, the scanned samples pans were placed in a refrigerator at 4±1°C for 7 days. Retrogradation properties were measured by rescanning these samples at the rate of 15°C/min from 0 to 100°C. The onset ($T_{o(r)}$), peak ($T_{p(r)}$) and conclusion ($T_{c(r)}$) temperatures, and enthalpy of retrograded starch ($\Delta H_{(r)}$) were determined. The percentage of retrogradation (R %) was calculated as $\Delta H_{(r)}/\Delta H$ X 100.

8.3.8. Texture profile analysis

Rice grains (5 g) were added in 15 mL of MilliQ water (rice: water ratio =1:3) in 50 mL glass beaker. The beaker was placed in a water bath at 95±1°C and samples were cooked. Analysis of textural attributes was performed on a TA-XT*plus* Texture Analyzer (Stable Microsystems, UK)

using 35-mm circular probe. Three cooked grains were on the flat stage, and the texture determined. The texture analyzer settings were as follows: pre-test speed, 2.00 mm/sec; post-test speed, 2.00 mm/sec; distance, 2.00 mm; time, 10.00 sec; (auto) trigger force, 0.05 N. From the force-time curve obtained, textural attributes of hardness (height of the force peak on cycle 1, N) and adhesiveness (negative force area of the first cycle, N.s) were computed using the EXPONENT Stable Micro Systems software supplied with instrument.

8.3.9. Starch hydrolysis kinetics and GI prediction

8.3.9.1. Starch hydrolysis

The starch hydrolysis was conducted by adapting the method of Goñi et al. (1997). Rice samples (200 mg) were cooked in 4 mL of MilliQ water at 80^{o}C for 30 min. Then, 10 mL of HCl-KCl buffer (pH 1.5) was added to the cooked samples for protein digestion, and the mixture was smashed to make a paste. Solution (0.2 mL) containing 1.0 mg of pepsin in 10 mL of HCl-KCl buffer (pH 1.5) was added to samples. Samples were transferred to 50 mL beaker and incubated at 40^{o}C for 60 min in a shaking water bath (100 RPM). Then, 15 mL of Tris-maleate buffer (pH 6.9) to adjust the volume to 25 mL. For starch hydrolysis, 5 mL of Tris-maleate buffer containing 2.6 IU of porcine pancreatic α-amylase (A-3176, Sigma, Missouri, USA) was added and samples were incubated at 37^{o}C in a shaking water bath. Aliquots of 0.1 mL were taken in a test tube at 30, 90 and 180 min and boiled in a water bath (100^{o}C) for 5 min to inactivate the α-amylase. One mL of 0.4 M sodium acetate (pH 4.75) and 30 µL of amyloglucosidase from *Aspergillus niger* (A-1602, Sigma, Missouri, USA) was added to each tube followed by incubation at 60^{o}C for 40 min in a water bath to allow amyloglucosidase to decompose starch into glucose. The glucose released by *in vitro* digestion of rice was estimated by using glucose oxidase-peroxidase kit (GAGO-20, Sigma, Missouri, USA).

8.3.9.2. GI prediction

The kinetics of starch hydrolysis and Glycemic index (GI) were calculated by using the non-linear first order kinetic model as described by Rattanamechaiskul et al. (2014). Following equations (8.3, 8.4, 8.5, and 8.6) were used;

$$C = C_{\infty}(1 - e^{-kt}) \qquad \text{Eq. 8.3}$$

$$AUC = C_{\infty}(t_f - t_0) - \left({C_{\infty}}/{k}\right)\left(1 - exp\left(-k(t_f - t_0)\right)\right) \quad \text{Eq. 8.4}$$

$$HI = \left({AUC_{sample}}/{AUC_{white\ bread}}\right) \times 100 \quad \text{Eq. 8.5}$$

$$GI = 39.71 + 0.549(HI) \quad \text{Eq. 8.6}$$

Where, C is the percentage of starch hydrolyzed at time t, C_∞ is the equilibrium concentration of hydrolyzed starch, k is the kinetic constant (min^{-1}), t is the time (min), AUC is the area under the hydrolysis curve, t_f is the final time (180 min), t_0 is the initial time (0 min), and HI is the hydrolysis index, which is the percentage of area under hydrolysis curve of sample divided by the area under the hydrolysis curve white bread as a reference.

8.3.10. Statistical analysis

All treatments were replicated three times to obtain mean values. The reported data of head rice yield, CIE$L^*a^*b^*$ color space, pasting properties, thermal properties, texture profile analysis and GI prediction for each variety was analyzed separately by analysis of variance (Completely Randomized Design) using Minitab R17 (Minitab® for Windows Release 17, Minitab Inc, Chicago) in order to determine significant differences. The data was then analyzed using Tukey's pair-wise comparison, at 5 % level of significance, to compare the means between different treatments.

8.4. Results and discussion

8.4.1. The hardness of parboiled and milled kernels

The mechanical strength of milled grains of fresh and parboiled grain samples was expressed as grain hardness, which is the force (N) required to break the grains. Results showed that the parboiling and subsequent drying of paddy resulted in significant ($P<0.05$) increase in grain hardness as compared to the fresh non-parboiled grains (Fig. 8.1). These results are in agreement with the findings of previous researches, who reported that the increased grain hardness is due to less internal fissures in parboiled rice grain because of the gelatinization and fusion of starch granules during parboiling process (Buggenhout et al. 2013; Oli et al. 2014). Among various parboiling treatments, acetic acid treated grains were significantly ($P<0.05$) harder than NaCl treated followed by water (control), without soaking treatment and non-parboiled rice for all

varieties. It could be that the acetic acid and saline soakings might reduce internal fissures than in control soaking, resulting in harder grains.

8.4.2. Head rice yield (HRY)

The primary parameter used in the industry to quantify rice milling efficiency is the head rice yield (HRY) (Cnossen et al. 2003). The head rice yield (HRY) of fresh and parboiled samples of selected varieties using different soaking conditions are presented in Fig. 8.1. HRY was expressed as a percentage of whole milled grains (70 % of the full length) produced in the milling of paddy at 9 % DOM. Results showed that parboiling significantly ($P<0.05$) increased the HRY in all varieties, glutinous TDK8 (Fig. 8.1a), TDK11 (Fig. 8.1c) and non-glutinous DG (Fig. 8.1d). Moreover, it was observed that the chemistry of soaking medium also significantly ($P<0.05$) affected the HRY. Maximum yield was gained in the acetic acid soaked samples followed by NaCl soaked, water (control) and without soaking treatments in all selected varieties. As expected, there was a positive correlation between grain hardness and HRY (Fig. 8.1b, 8.1d, and 8.1f).

8.4.3. Color change in fresh and parboiled rice

CIE$L^*a^*b^*$ color space of fresh and parboiled milled rice and color difference (ΔE^*_{ab}) of parboiled grains (without, water (control), 3 % NaCl, and 0.2 % acetic acid soaking) with fresh samples of all selected varieties are presented in Table 8.1. Color represented in terms of L^*, a^*, and b^* values of parboiled samples are significantly ($P<0.05$) different from the fresh samples in all varieties. There was a significant ($P<0.05$) decrease in L^* value and significant ($P<0.05$) increase in b^* value due to parboiling in all varieties. However, a^* value significantly ($P<0.05$) increased in glutinous (TDK8 and TDK11) varieties and significantly ($P<0.05$) decreased in non-glutinous (DG) variety due to parboiling.

This shade conversion from milky white (fresh) to an amber color (parboiled) in glutinous rice is possibly due to the diffusion of husk color into the endosperm (Lamberts et al. 2006). The chemistry of soaking medium had a significant effect on the color of the parboiled rice. Without soaking, control soaking and NaCl soaking had significantly ($P<0.05$) higher ΔE^*_{ab} values than acetic acid soaking in glutinous (TDK8 and TDK11) varieties possibly due to the bleaching effect of acetic acid. Acetic acid acts as a weak inhibitor of browning due to its metal-chelating characteristics by lowering the pH and can also interact with phenols in rice (Son et al. 2001).

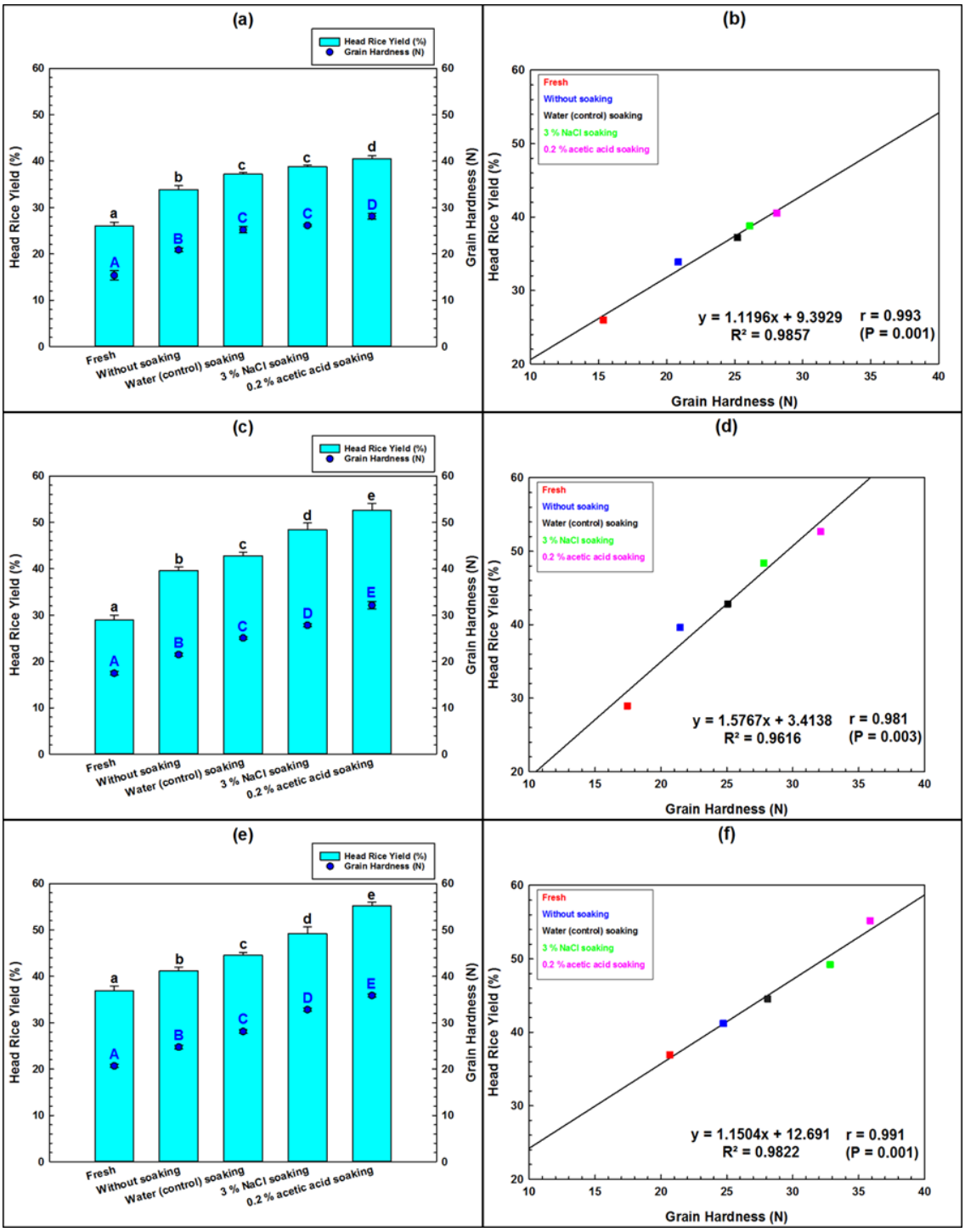

Figure 8.1 Head rice yield (%) and grain hardness (N) of fresh and parboiled rice grains; (a) Thadokkham-8 (TDK8), (c) Thadokkham-11 (TDK11), and (d) Doongara (DG).* Correlation (r) between head rice yield (%) and grain hardness (N) in; (b) TDK8, (d), TDK11, and (f) DG

*Means ± SD (n = 3). Within figure, significant differences are denoted by lowercase letters for head rice yield and uppercase letters for grain hardness at 5 % probability level.

Table 8.1 CIE*L*a*b** color space of fresh and parboiled rice grains of Thadokkham-8 (TDK8), Thadokkham-11 (TDK11), and Doongara (DG)*

Rice variety	Treatment	CIEL*a*b* color space			
		L^*	a^*	b^*	ΔE^*_{ab}
TDK8	Fresh	96.75±0.25^{a}	-0.96±0.02^{a}	0.55±0.01^{a}	-
	Without soaking	89.00±1.00^{bc}	-0.03±0.02^{b}	11.23±0.33^{c}	13.25±1.01^{bc}
	Water (control) soaking	86.50±0.50^{d}	0.47±0.03^{c}	11.85±0.45^{c}	15.32±0.84^{a}
	3 % NaCl soaking	87.50±0.35^{cd}	0.82±0.03^{d}	11.62±0.59^{c}	14.33±0.07^{ab}
	0.2 % acetic acid soaking	89.50±0.50^{b}	1.58±0.13^{e}	9.61±0.11^{b}	11.88±0.52^{c}
TDK11	Fresh	96.40±0.40^{a}	-0.97±0.02^{a}	0.56±0.02^{a}	-
	Without soaking	93.60±0.40^{b}	0.15±0.03^{b}	8.40±0.60^{b}	8.44±0.27^{c}
	Water (control) soaking	87.84±0.41^{d}	1.41±0.10^{d}	12.81±0.31^{d}	15.14±0.74^{a}
	3 % NaCl soaking	90.52±0.32^{c}	1.03±0.04^{c}	11.57±0.32^{c}	12.64±0.34^{b}
	0.2 % acetic acid soaking	90.75±0.30^{c}	0.89±0.09^{c}	10.65±0.33^{c}	11.71±0.36^{b}
DG	Fresh	93.19±0.19^{a}	1.20±0.20^{a}	7.25±0.25^{a}	-
	Without soaking	89.55±0.55^{c}	0.94±0.09^{ab}	7.90±0.30^{a}	3.73±0.44^{b}
	Water (control) soaking	91.50±0.50^{b}	0.16±0.02^{c}	10.19±0.39^{b}	3.56±0.62^{b}
	3 % NaCl soaking	88.50±0.27^{d}	0.85±0.14^{b}	11.94±0.40^{c}	6.68±0.12^{a}
	0.2 % acetic acid soaking	92.33±0.33^{ab}	0.20±0.02^{c}	13.12±0.32^{d}	6.02±0.08^{a}

*Means ± SD (n = 3). For a particular rice variety, means with different letters in the same column denote significant difference at 5 % probability level within each rice variety.

8.4.4. Pasting properties

The pasting profiles of fresh and parboiled rice flour of selected varieties are presented in Fig. 8.2. Results showed the diverse behavior of glutinous (TDK8 and TDK11) and non-glutinous (DG) samples due to parboiling. Parboiling significantly ($P<0.05$) reduced the pasting temperature in both glutinous flours except without soaking parboiling, where pasting temperature significantly ($P<0.05$) increased as compared to the fresh flour. However, pasting temperature of parboiled non-glutinous (DG) was increased as compared to fresh flour.

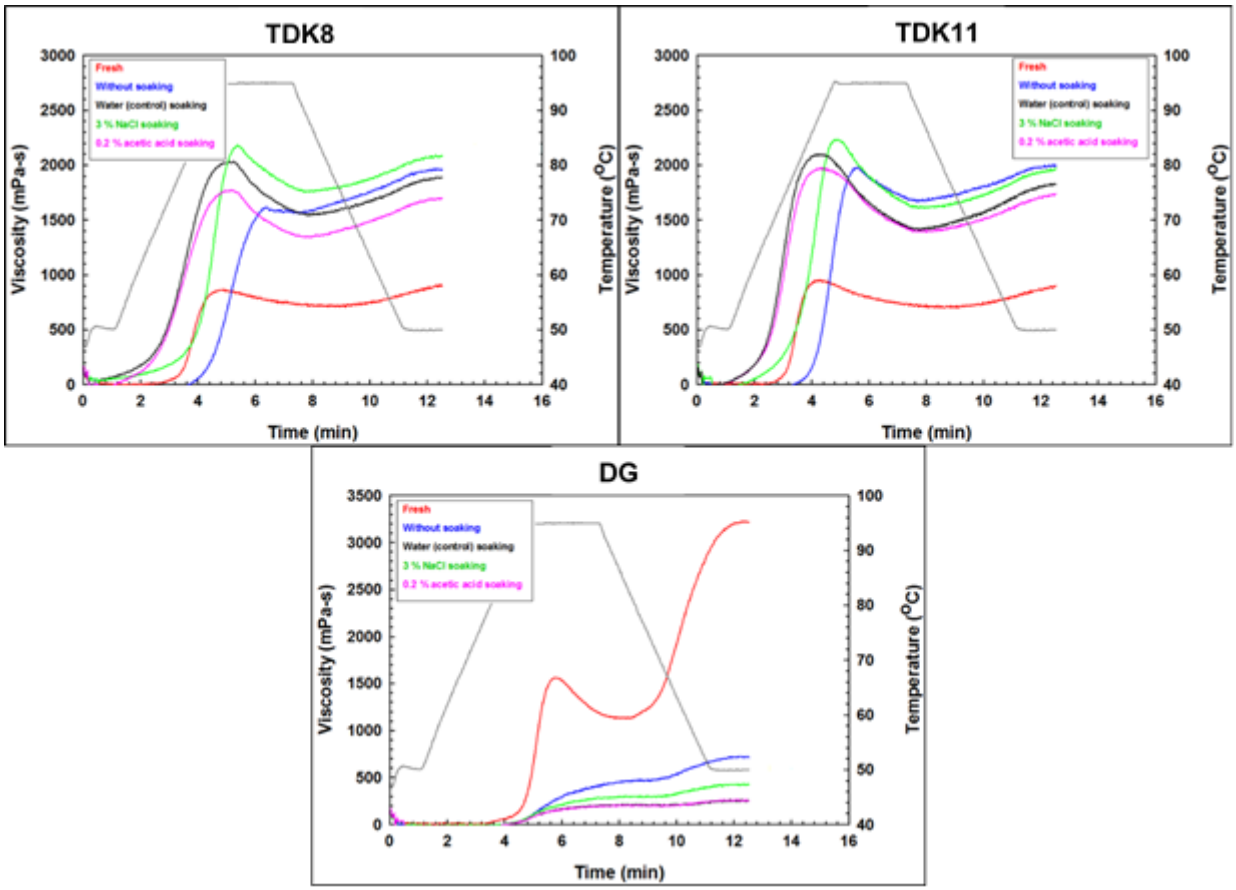

Figure 8.2 Pasting profiles of fresh and parboiled rice grains of Thadokkham-8 (TDK8), Thadokkham-11 (TDK11), and Doongara (DG)

Moreover, significant ($P<0.05$) increase in peak and final viscosities was recorded in parboiled glutinous flours which were significantly ($P<0.05$) decreased in non-glutinous flour when compared to fresh flours. This variation in the pasting profiles of glutinous and non-glutinous rice after parboiling may be due to increased starch damage owing to parboiling in DG than TDK8 and TDK11, resulting in resistance of starch granules to absorb water and swell during RVA analysis. Dutta and Mahanta (2012) reported that the parboiled rice varieties (Ranjit and Kola Chowkua) with higher amylose content showed reduced pasting profiles than glutinous rice varieties (Aghoni bora and Bhogali bora) possibly due to an extensive breakdown of straight chain amylose during parboiling (Mir & Bosco 2013). Among various parboiling treatments, saline soaking induced significant swelling of starch, resulting in high peak and final viscosities than water (control) in all samples. This might be due to the inter- or intra-molecular cross-linking of Na^+ ions with the amylopectin during parboiling (Samutsri and Suphantharika 2012), resulting in increased water absorption and viscous gels during RVA analysis. However, acetic acid soaking restricted the

swelling leading to reduced peak and final viscosities. Acetic acid soaking before parboiling may have degraded the amorphous regions (Ohishi et al. 2007), resulting in reduced water absorption and starch swelling.

8.4.5. Crystallinity change in starch

The X-ray diffraction spectra of fresh and parboiled rice flour of rice samples are shown in Fig. 8.3. The fresh flour samples of both glutinous (TDK8 and TDK11) and non-glutinous (DG) varieties displayed a typical A-type pattern with main crystalline peaks at 14.9°, 16.9°, 18° and 22.8° and crystallinity was recorded as 25.95, 22.62 and 18.79 %, respectively.

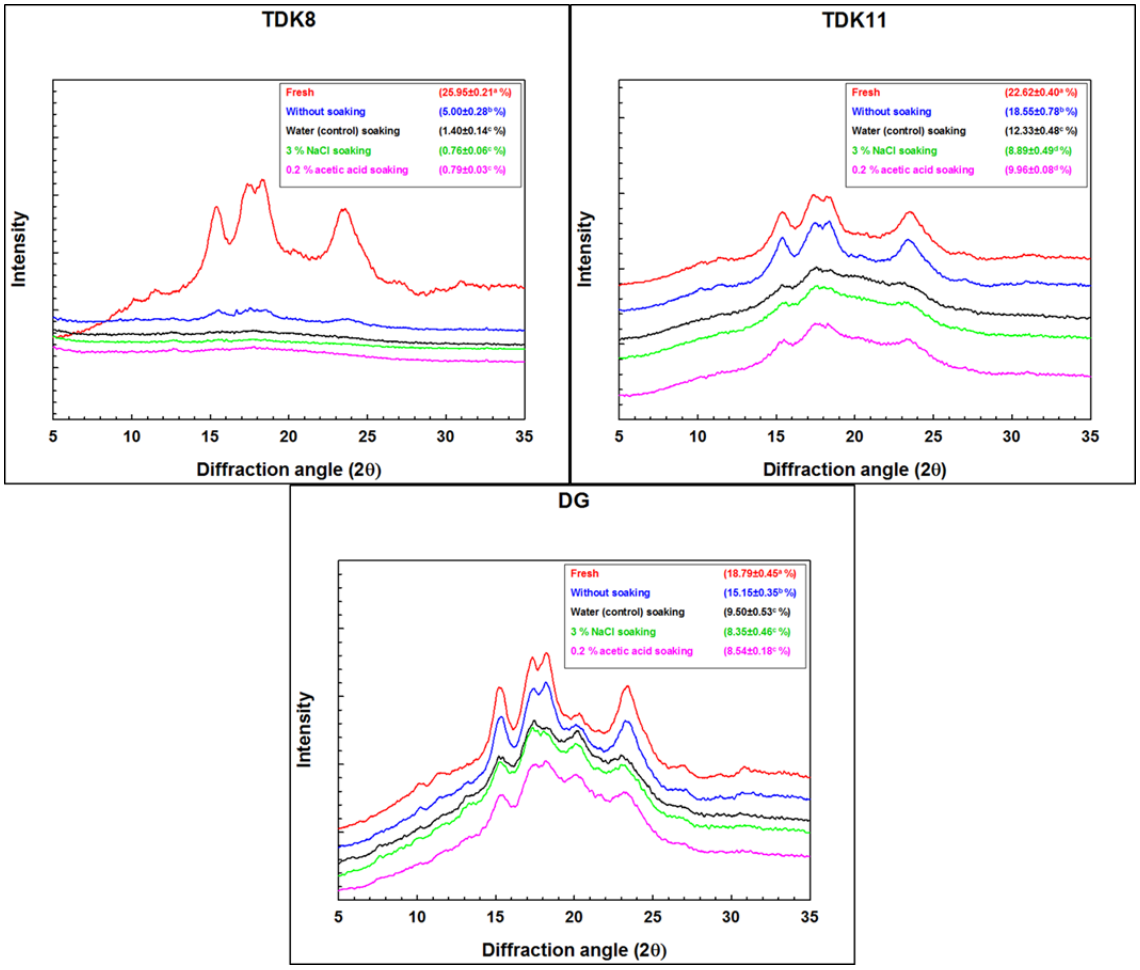

Figure 8.3 X-ray diffraction and calculated crystallinity (%) of fresh and parboiled rice grains of Thadokkham-8 (TDK8), Thadokkham-11 (TDK11), and Doongara (DG) as shown on the right side of the figures*

*Means ± SD (n = 3). Within each variety, means with different letters in the same figure denote significant difference at 5 % probability level.

Parboiled TDK8 in all soaking medium was almost completely amorphous, and no A-type pattern from residual starch was detected. However, a weak A-type pattern was noted in parboiled TDK11 and DG with significant ($P<0.05$) decrease in crystallinity as compared to untreated samples. The variation in the crystallinity of parboiled TDK8 and TDK11 is possibly due to the difference in the amylopectin chain lengths. It has been reported that external and inter-block chain lengths correlated with the retrogradation of amylopectin (Bertoft et al. 2016). Very short external chains (DP 6-8) prevent retrogradation (Vamadevan & Bertoft 2018), resulting in no crystalline region during XRD analysis. However, further studies on the amylopectin chain lengths of TDK8 and TDK11 should be done in the future to get a clearer picture.

The gelatinization temperature of starch of untreated rice flour samples was recorded as ~65°C for glutinous (TDK8 and TDK11) and ~75°C for non-glutinous (DG) (Fig. 8.2), therefore, almost complete gelatinization has occurred during steaming process of parboiling, resulting in complete disappearance of crystalline peaks in TDK8 and weak peaks in TDK11 and DG. The appearance of weak peaks could be attributed to the retrogradation of amylopectin in TDK11 and amylose in DG. Among various soaking medium before parboiling, acetic acid and saline soaking affected the residual starch more than water (control) and without soaking, resulting in significant ($P<0.05$) decrease in crystallinity.

8.4.6. Thermal properties

The thermal (gelatinization and retrogradation) properties of fresh and parboiled rice flour of rice samples are presented in Table 8.2. Parboiling resulted in significant ($P<0.05$) decrease in thermal transition temperatures (T_o, T_p, and T_c) and enthalpy (ΔH) of gelatinization than fresh flour in glutinous (TDK8 and TDK11) and non-glutinous (DG) flours. Moreover, the retrogradation thermal temperatures ($T_{o(r)}$, $T_{p(r)}$, and $T_{c(r)}$) and enthalpy ($\Delta H_{(r)}$) was also significantly ($P<0.05$) reduced in parboiled DG and without soaking parboiled TDK8 and TDK11. Interestingly, no retrogradation peaks were detected in water (control), saline and acetic acid soaking parboiled TDK8 and TDK11. However, significant ($P<0.05$) reduction in $\Delta H_{(r)}$ was recorded in without soaking parboiled samples led to significant ($P<0.05$) reduction in the percentage of retrogradation (R %). Results showed that the parboiled glutinous rice samples made without soaking might contain the residues of ungelatinized starch, resulting in the melting enthalpy of gelatinization and

retrogradation (Sittipod & Shi 2016). The gelatinization endotherms are in agreements with the XRD spectra (Fig. 8.3), where a significant reduction in the crystallinity was recorded.

8.4.7. Textural profile analysis

Textural profiles of cooked fresh and parboiled grains of glutinous (TDK8 and TDK11) and non-glutinous (DG) are shown in Fig. 8.4. Parboiling resulted in significant ($P<0.05$) increase in hardness (N) and adhesiveness (N.s) when compared to the freshly cooked samples for all varieties. Parboiling results in several physicochemical changes such as disruption of surface layers and leaching of components from cells. This cells disruption and leaching of cellar components possibly facilitate more damaged starch exposure on the surface, resulting in the increased stickiness of cooked parboiled glutinous rice (Ong & Blanshard 1995).

Among various parboiling treatments, saline soaking exhibited the hardest texture followed by acetic acid, water (control) and without soaking. Interesting, parboiling also resulted in significant ($P<0.05$) increase in adhesiveness (N.s) especially in glutinous (TDK8 and TDK11) varieties. Chemistry of soaking medium significantly ($P<0.05$) affected the adhesiveness in TDK8 as shown in Fig. 8.4, where adhesiveness of acetic acid parboiled rice was significantly ($P<0.05$) higher than saline, water (control) and without soaking. However, soaking medium had no significant ($P>0.05$) effect on the adhesiveness of TDK11 and DG. The TPA results are in agreement XRD analysis (Fig. 8.3), where no crystalline region was found in parboiled TDK8, and a weak A-type crystalline pattern was found in parboiled TDK11 and DG.

8.4.8. GI prediction

The results of estimated GI using *in vitro* digestion of 180 min is presented in Table 2. As expected, the predicted GI of glutinous rice varieties viz. TDK8 and TDK11 was higher than non-glutinous (DG) rice variety. Moreover, parboiling resulted in significant ($P<0.05$) decrease in GI in both TDK8/TDK11 and DG. Gelatinization and recrystallization are the major physicochemical changes that occur during parboiling (Zavareze et al. 2010). These changes may lead to higher levels of resistant starch, resulting in low glucose response and GI (Boers et al. 2015). The protein-starch interaction may have restricted the starch digestibility, resulting in reduced GI (Kaur et al. 2016). However, saline soaked parboiled glutinous rice showed significantly ($P<0.05$) higher GI than fresh. NaCl might have increased the postprandial plasma glucose by accelerating the

digestion of starch especially amylopectin by stimulating α-amylase activity (Thorburn et al. 1986).

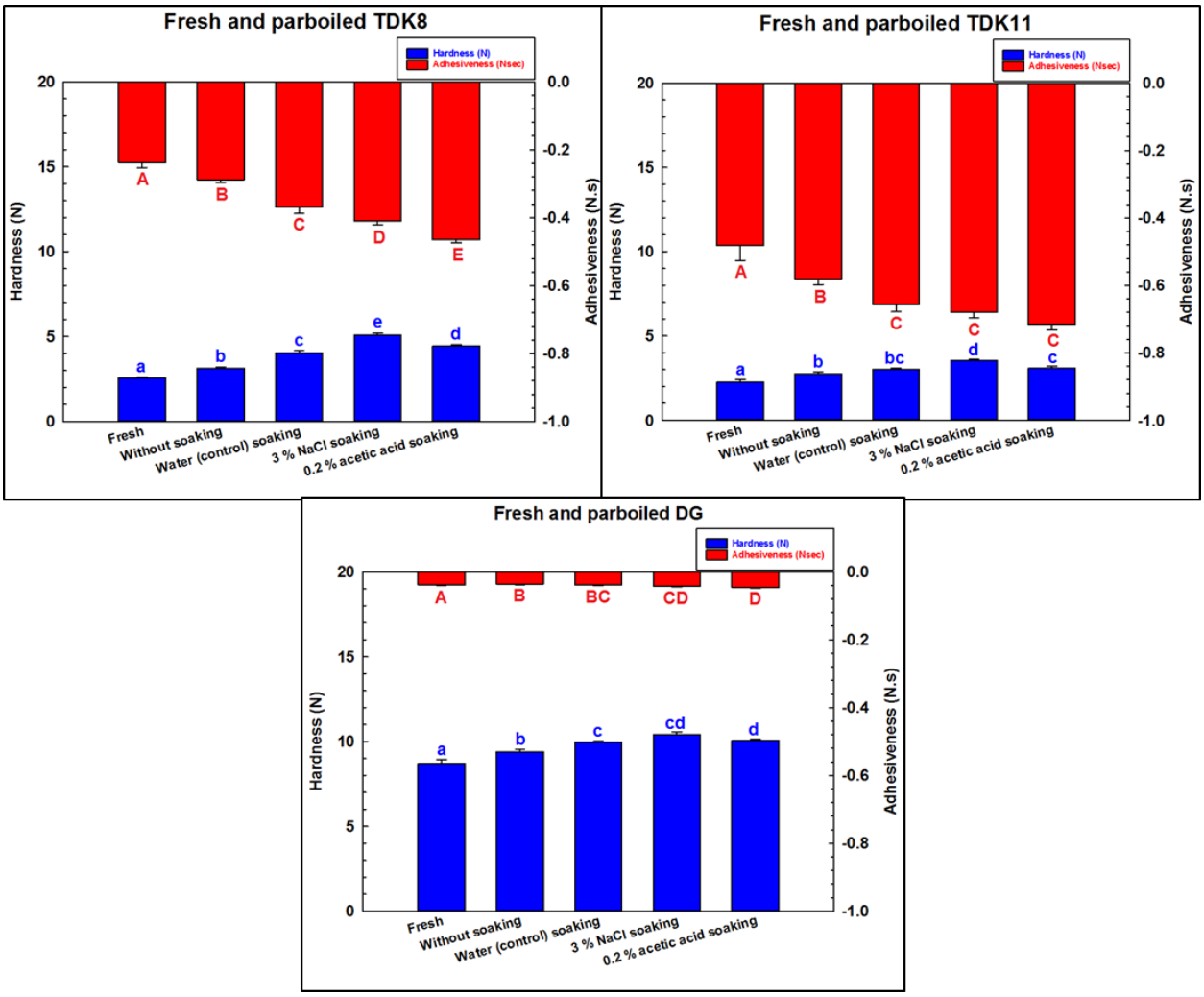

Figure 8.4 Textural profile analysis of fresh and parboiled rice grains of Thadokkham-8 (TDK8) and Doongara (DG)*

*Means ± SD (n = 3). Within each variety, significant differences are denoted by lowercase letters for hardness and uppercase letters for adhesiveness at 5% probability level.

Table 8.2 Experimental results of estimated GI, and gelatinization and retrogradation properties of fresh and parboiled rice grains of Thadokkham-8 (TDK8), Thadokkham-11 (TDK11), and Doongara (DG)*

Rice variety	Treatment	Estimated GI	Gelatinization				Retrogradation				
			T_o (ºC)	T_p (ºC)	T_c (ºC)	ΔH (Jg^{-1})	$T_{o(r)}$ (ºC)	$T_{p(r)}$ (ºC)	$T_{c(r)}$ (ºC)	$\Delta H_{(r)}$ (Jg^{-1})	R (%)
TDK8 flour	Fresh	116.2±0.2^{b}	66.4±0.0^{a}	73.7±0.4^{a}	89.9±1.6^{a}	10.4±0.5^{a}	51.7±0.0^{a}	58.5±0.5^{a}	65.2±1.3^{a}	0.2±0.0^{a}	2.1±0.1^{a}
	Without soaking	111.8±0.3^{c}	50.6±0.4^{b}	58.1±0.2^{b}	62.6±0.2^{b}	5.1±0.1^{b}	40.6±0.4^{b}	52.4±0.4^{b}	60.3±0.2^{b}	0.1±0.0^{b}	2.0±0.1^{a}
	Water (control) soaking	108.0±1.0^{d}	48.2±0.1^{c}	56.1±0.4^{c}	61.1±0.2^{b}	3.5±0.2^{c}	ND^	ND^	ND^	ND^	ND^
	3 % NaCl soaking	119.2±1.0^{a}	47.3±0.2^{d}	54.4±0.4^{d}	58.0±0.0^{c}	3.5±0.0^{c}	ND^	ND^	ND^	ND^	ND^
	0.2 % acetic acid soaking	100.4±0.2^{e}	45.8±0.2^{e}	54.0±0.0^{d}	57.4±0.3^{c}	3.0±0.1^{c}	ND^	ND^	ND^	ND^	ND^
TDK11 flour	Fresh	105.9±0.2^{b}	64.8±0.1^{a}	71.0±0.2^{a}	81.1±0.2^{a}	9.1±0.2^{a}	50.6±0.4^{a}	57.5±0.4^{a}	63.82±0.17^{a}	0.20±0.01^{a}	2.14±0.02^{a}
	Without soaking	101.5±0.3^{c}	49.5±0.5^{b}	57.1±0.1^{b}	61.1±0.2^{b}	4.4±0.2^{b}	40.1±0.1^{b}	50.6±0.4^{b}	59.06±0.17^{b}	0.10±0.00^{b}	2.15±0.00^{b}
	Water (control) soaking	97.7±1.0^{d}	47.3±0.2^{c}	54.4±0.4^{c}	58.2±0.2^{c}	3.6±0.0^{c}	ND^	ND^	ND^	ND^	ND^
	3 % NaCl soaking	108.9±1.0^{a}	45.8±0.2^{d}	53.7±0.3^{c}	57.3±0.3^{d}	3.1±0.1^{d}	ND^	ND^	ND^	ND^	ND^
	0.2 % acetic acid soaking	90.1±0.2^{e}	43.4±0.4^{e}	52.6±0.4^{d}	56.4±0.3^{e}	2.0±0.1^{e}	ND^	ND^	ND^	ND^	ND^
DG flour	Fresh	94.8±1.4^{a}	72.3±0.7^{a}	77.1±0.5^{ab}	83.0±1.5^{a}	3.4±0.0^{a}	43.7±0.2^{a}	56.4±0.0^{a}	62.9±0.2^{a}	2.0±0.0^{a}	59.2±0.0^{a}
	Without soaking	84.3±0.5^{b}	70.9±0.2^{b}	77.6±0.3^{a}	82.6±0.2^{a}	3.1±0.1^{b}	43.1±0.1^{b}	55.9±0.1^{b}	61.8±0.1^{b}	1.6±0.1^{b}	51.8±1.3^{b}
	Water (control) soaking	78.4±0.8^{c}	70.1±0.1^{bc}	76.8±0.1^{bc}	81.5±0.1^{a}	3.0±0.0^{c}	42.9±0.1^{b}	55.5±0.1^{c}	60.8±0.0^{c}	1.4±0.0^{c}	48.9±1.5^{c}
	3 % NaCl soaking	85.6±1.4^{b}	70.1±0.1^{c}	76.1±0.1^{cd}	78.7±0.2^{b}	2.4±0.1^{d}	41.0±0.1^{c}	54.9±0.1^{d}	59.9±0.0^{d}	1.1±0.0^{d}	45.7±0.3^{d}
	0.2 % acetic acid soaking	72.2±0.8^{d}	69.9±0.1^{c}	75.5±0.1^{d}	77.6±0.2^{b}	1.9±0.1^{e}	39.9±0.1^{d}	54.0±0.1^{e}	59.4±0.2^{e}	0.9±0.0^{e}	44.9±1.2^{d}

ˆNot detected.

*Means ± SD (n = 3). For a particular rice variety, means with different letters in the same column denote significant difference at 5 % probability level within each rice variety.

8.5. Conclusions

This study showed that the soaking medium affected the physicochemical properties of glutinous (TDK8 and TDK11) rice varieties. Milling efficiency of glutinous rice was improved by adding NaCl and acetic acid to the soaking water of paddy before parboiling. Induced coloration from the husk during steaming can be minimized by the bleaching effect of acetic acid used during soaking. Saline soaking can also improve water absorption of parboiled rice. NaCl and acetic acid affected the residual starch, resulting in reduced crystalline regions which may be responsible for more adhesiveness during textural profile analysis. The parboiling of rice significantly reduced the glycemic index which may be due to more resistant starch contents. This should be further investigated. Current findings showed the potential of parboiling with modified soaking techniques to improve grain quality of glutinous rice.

Chapter 9 Effects of three types of modified atmospheric packaging on the physicochemical properties of selected glutinous rice

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9.1. Abstract

The effect of modified atmospheric packaging (MAP) on the physicochemical properties of selected glutinous (Thadokkham-8 and Thadokkham-11) rice was studied and compared with non-glutinous rice (Doongara). The freshly harvested/milled grains were packed in four different MAP conditions viz. control, vacuum, CO_2, and N_2 for 12 months at room temperature (23±1ºC). Gas (N_2 or CO_2) was flushed in aluminum bags at the pressure of 300 kPa for 3 sec and subsequently hermetically sealed. Vacuum packaging was done at -100 kPa. Results showed that aging-induced changes in the starch granules were less prominent in a vacuum and/or MAP samples using CO_2 or N_2. Surface analysis showed that control storage significantly reduced the percentage of lipids and increased the percentage of proteins on the surface in all selected varieties. N_2 and CO_2 storage of TDK8 and DG slowed down the shift of properties of macromolecules and maintained the surface starch/proteins/lipids ratios during 6 months of storage. Moreover, the grains stored in a vacuum maintained the lipids with a lower proportion of proteins exposed to the surface after cooking. N_2 and CO_2 induced increase in pasting temperature but significant reduction in final viscosity when compared to control. The findings correlated well with thermal analysis. The *in situ* Thermal Mechanical Compression Test (TMCT) device cooking and texture analysis revealed that modified storage slightly slowed the aging-induced changes in the cooking quality and stickiness of glutinous rice. Among all storage conditions used vacuum was relatively the best to maintain the quality of the grain.

9.2. Introduction

The significance of food storage especially for cereals such as rice and wheat has become important because of frequent natural disasters such as floods, drought, and earthquakes to ensure food security (Tulyathan & Leeharatanaluk 2007). Rice being the primary staple food of more than half of the world's population requires adequate storage with preservation of its eating quality to ensure uninterrupted supply to end users all year round (Thongrattana 2012). Storage of rough and milled rice undergoes a wide range of physicochemical changes on the characteristics of rice such as pasting properties (Ziegler et al. 2017), thermal properties (Faruq et al. 2015; Ziegler et al. 2017), and textural changes in the cooked rice over a time period (Nawaz et al. 2017; Zhou et al. 2007b; Keawpeng & Venkatachalam 2015; Jungtheerapanich et al. 2017).

Age-related effect on the cooking quality of rice has been obvious to the consumers. Aging is very complex process and attributed to several changes (Zhou et al. 2015) in cell walls and proteins (Zhou et al. 2002a; Sodhi et al. 2003), starch/protein interaction (Tananuwong & Malila 2011), and oxidation of lipids, resulting in breakdown of products (Park et al. 2012). Loss of stickiness is one of the most noticeable changes occurring in glutinous rice (Chen et al. 2015). Several factors are responsible for this physicochemical deterioration. Previous studies have demonstrated the strong association of aging and endogenous enzymatic reactions on rice starch (Zhou et al. 2003a; Huang & Lai 2014), proteins (Zhou et al. 2003a) and lipids (Zhou et al. 2003b). Huang and Lai (2014) reported the effects of endogenous amylase on the isolated starch fine structures of aged waxy rice varieties (TNW1 and TCSW1) and reported that the structural changes in the starch fine structures with a decreasing percentage of longer chains and an increasing percentage of shorter chains of amylopectin when rice was stored for a longer period (15 months) of time. Although, the studies on rice protein suggested that there was no significant difference in the gross protein contents of fresh and aged rice (Zhou et al. 2002a), storage in ambient conditions could induce oxidation reaction of proteins, resulting in the formation of intermolecular disulphide bonds (S-S) (Teo et al. 2000). The polymerization of rice proteins due to intermolecular disulfide bond formation can increase the average molecular weight of rice storage proteins especially oryzenin (Zhou et al. 2007a). The formation of bigger protein bodies in stored rice can lead to increased final viscosity during rapid visco analysis and reduced stickiness of cooked waxy rice (Ohno & Ohisa 2005; Zhou et al. 2007b). In addition to this, previous studies (Shin et al. 1986; Park et al. 2012) have reported that there was no significant change found in the fatty acids (both unsaturated and saturated) in the propanol-water extractable lipid (PWE-L) fraction between fresh and stored rice grains. However, unsaturated fatty acids fractions in the petroleum ether extractable lipid (PEE-L) of aged rice grains was significantly ($P<0.05$) lower than that of fresh rice grains (Zhou et al. 2015). This suggests that the unsaturated fatty acids in the PEE-L fraction are more unstable and could be more responsible for the oxidation than those in PWE-L fraction during storage (Tsuzuki et al. 2014). It is now quite well defined that the lipid peroxidation during storage can cause quality deterioration of rice grains, especially, lipoxygenase-3 (LOX-3), which is one of the key enzymes catalyzing the reaction (Shin et al. 1985; Hildebrand 1989; Suzuki & Matsukura 1997). The above studies suggest that an efficient direction for improving the storage property of the rice grains can be achieved through proper storage environment.

To slow down aging-induced physicochemical changes in stored rice, storage at chilling temperatures (at or below 8^{o}C, targeting 5^{o}C) is usually reported as the best method (Pearce et al. 2001). However, cold storage is not cost effective due to initial capital investment for cooling system installation, high energy consumption during its operation, and also expensive equipment maintenance (Evans et al. 2014). Also, the suggested method cannot be effectively applied in the developing nations facing an energy shortage. Thus, modified atmospheric packaging of rice can be the best option to reduce aging-induced physicochemical changes in ambient temperature conditions and maintain the cooking quality.

In modified atmospheric packaging (MAP), extra nitrogen (N_2) or carbon dioxide (CO_2) is added to alter the ratio of oxygen (O_2), N_2, and CO_2 (Caleb et al. 2012). Altered ratio of O_2, N_2, and CO_2 in the micro-environment of the food product can maintain the physical and cooking quality of aged food product by slowing down the physicochemical changes such as the speed of oxidation reactions (Caleb et al. 2013) and other physicochemical changes. The MAP of fresh fruits and vegetables is quite well-established technique mainly focusing on the prolonging shelf life by reducing the respiration rate and microbial growth (Oliveira et al. 2015; Shaarawi & Nagy 2017). However, to the best of our knowledge MAP of rice has not been widely reported by researchers due to its least susceptibility towards microbial spoilage, having a relatively long shelf life and less importance given to the cooking quality of rice.

Good quality glutinous rice grains usually lose their shape during cooking and become very sticky. However, storage at ambient temperature induces physicochemical changes in glutinous rice, resulting in significant reduction in the stickiness during cooking. As mentioned earlier, although aging-induced changes can be minimized by storing the rice in the refrigerated condition, this method is not feasible due to the cost factor and large volume of the grains. Therefore, the objective of the present study is to investigate the effect of altered atmospheric (vacuum, N_2, and CO_2) storage on the functional properties of selected glutinous rice varieties. For this, the milled rice grains of two glutinous rice varieties viz. Thadokkham-8 (TDK8) and Thadokkham-11 (TDK11) were selected and stored in four different modified atmospheric conditions for one year. For comparison purpose, one non-glutinous rice variety (Doongara (DG)) was also milled and stored in the same condition. This modified storage is expected to maintain the functional quality of aged

glutinous rice grains stored at ambient temperature by reducing the rate of physicochemical changes in rice, in particular, the loss of stickiness.

9.3. Material and methods

Two *Oryza sativa* indica cultivars of glutinous rice from Lao PDR viz. Thadokkham-8 (TDK8) and Thadokkham-11 (TDK11) having 3.77 % and 3.72 % apparent amylose contents (AAC), respectively and one *O. sativa* japonica non-glutinous rice from Australia (Doongara (DG), 19.71 % (AAC)) were used in this study. The milled TDK8 with 9 % degree of milling (DOM) (harvested March/April 2015) was provided by the National Agriculture and Forestry Research Institute (NAFRI), Lao PDR, while TDK11 and DG (harvested March/April 2015) were provided by Rice Research Australia Pty Ltd (RRAPL), Mackay, QLD, Australia.

9.3.1. Paddy milling

TDK11 and DG paddies were milled to brown rice by using a rice husker (Satake, Japan). The brown rice of both cultivars was milled to white rice using an abrasive polisher (Satake, Japan). The DOM of both TDK11 and DG was maintained at 9 % to be consistent with milled TDK8 provided by NAFRI, Lao PDR.

9.3.2. Modified atmospheric packaging

The milled rice kernels (250 g per bag) of TDK8, TDK11, and DG were packed in sealed aluminum bags (West's Packaging Services, Melbourne, VIC, Australia) using various modified atmospheric conditions viz. vacuum, N_2, CO_2 by using a tabletop vacuum chamber machine equipped with gas flushing system; Vacumatic 282 (Vacumatic Australia Pty Ltd, Pakenham, VIC, Australia). N_2 and CO_2 were flushed in aluminum bags at a pressure of 300 kPa for 3 sec and subsequently hermetically sealed. Similarly, vacuum packaging was done at -100 kPa. Moreover, one control (MAP_{ctrl}) without any treatment was also kept in sealed aluminum bags for comparison. The control and MAP packets of rice grains of selected rice varieties were stored at room temperature (23±1ºC). The experimental design of the present study is presented in Appendix 7.

9.3.3. Scanning electron microscopy of a cross-section of rice kernels

The cross sections of milled rice kernels of fresh, 6 and 12 months aged TDK8, TDK11, and DG stored in the various MAP were mounted onto SEM stubs by placing them on a double-sided carbon adhesive tape. Biological materials suffer from extensive charge build-up under the electron beam; hence they need to be coated with conductive material. Thus, samples were iridium-coated for 3 min (~ 15 nm thick). The samples were examined using a Philips XL30 Field Emission Scanning Electron Microscope operating at 8 kV accelerated voltage.

9.3.4. Surface chemical analysis

The surface chemical analysis of pure rice components (starch, proteins, and lipids), milled uncooked rice kernels, and freeze-dried cooked rice kernels of fresh, 6 and 12 months aged TDK8, TDK11, and DG stored in various MAP were analyzed by using a Kratos AXIS Ultra Kratos Analytical (Manchester, UK) spectrometer with a monochromatic Al X-ray source at 150 W. This method follows the procedure previously used by our research group (Nawaz et al. 2016b). Before analysis, the samples were outgassed under vacuum for 24 hrs. The analysis started with a survey scan from 0 to 1200 eV with a dwell time of 100 ms, pass energy of 160 eV at steps of 1 eV, with a single sweep. For the high-resolution analysis, the number of sweeps was increased, the pass energy was lowered to 20 eV, at steps of 50 meV, and the dwell time was increased to 250 ms. Data was acquired using a Kratos Axis ULTRA X-ray photoelectron spectrometer, incorporating a 165 m hemispherical electron energy analyzer. The incident radiation was Monochromatic Al X-rays (1486.6 eV) at 225 W (15 kV, 15 ma). Survey (wide) scans were taken at analyzer pass energy of 160 eV, and multiplex (narrow) higher resolution scans at 80 eV. Base pressure in the analysis chamber was 1.33×10^{-7} Pa and, during sample analysis, 1.33×10^{-6} Pa. XPS was applied to measure the relative atomic concentrations of carbon, nitrogen, and oxygen in the layer of the samples to a maximum thickness of 10 nm.

The surface composition of samples was calculated by using the relative elemental compositions of samples and pure rice components in a set of linear relations in a matrix formula according to the method developed by Fäldt et al. (1993). The matrix formula was adjusted by components/macromolecules to be analyzed. In the current study, the calculations were made by using a matrix, as presented in equations 9.1, 9.2, and 9.3 for the three rice components.

$$I^{C}{}_{sample} = I^{C}{}_{starch}\gamma_{starch} + I^{C}{}_{proteins}\gamma_{proteins} + I^{C}{}_{lipids}\gamma_{lipids} \quad \text{Eq. 9.1}$$

$$I^{N}{}_{sample} = I^{N}{}_{starch}\gamma_{starch} + I^{N}{}_{proteins}\gamma_{proteins} + I^{N}{}_{lipids}\gamma_{lipids} \quad \text{Eq. 9.2}$$

$$I^{O}{}_{sample} = I^{O}{}_{starch}\gamma_{starch} + I^{O}{}_{proteins}\gamma_{proteins} + I^{O}{}_{lipids}\gamma_{lipids} \quad \text{Eq. 9.3}$$

Where $I^{C}{}_{strach,}$ $I^{N}{}_{strach}$, $I^{O}{}_{starch}$, $I^{C}{}_{proteins}$, $I^{N}{}_{proteins}$, $I^{O}{}_{proteins}$, $I^{C}{}_{lipids}$, $I^{N}{}_{lipids}$, and $I^{O}{}_{lipids}$ are the relative contents of atomic elements (C, N, and O) measured on the surface of the pure rice components, presented in Table 5.2 in Chapter 5. $I^{C}{}_{sample}$, $I^{N}{}_{sample}$, and $I^{O}{}_{sample}$ are the relative contents of elements found by XPS for the sample. The parameters γ_{starch}, $\gamma_{proteins}$, and γ_{lipids} are unknown values corresponding to approximately 100 % component/macromolecules surface contents (starch, proteins, and lipids).

9.3.5. X-ray diffraction

The milled kernels of fresh, 6 and 12 months aged TDK8, TDK11, and DG stored in the various MAP were ground to flour using a disc mill (Good Friends of the Guangzhou Machinery Co. Ltd., Guangzhou, China) according to the method previously reported by Nawaz et al. (2016b). The flour particles were sieved through 500 µm sieve to attain particle size ≤ 500 µm. X-ray diffraction pattern measurement of rice flour was analyzed by using Bruker Advance D8 X-Ray diffractometer equipped with a LynxEye detector and Cu-kα (1.54 Å) radiation. The accelerating voltage and current of 30 kV and 30 mA, respectively, in combination with scan rate 2°/min, were used. The diffractograms were recorded in a 2θ ranged from 10° to 35° with sampling width of 0.02°. Traces were analyzed using the Diffrac^{plus} Evaluation Package Release V3.1, PDF-2 Release 2014.

The percentage of crystallinity was calculated with normalized values of the intensities at each diffraction angle, using the method of Htoon et al. (2009). The ratio of the upper diffraction peak area taken as the crystalline portion, to total diffraction area (two-phase model), represented the percentage of crystallinity. The diffractograms were smoothed by 13 points using Traces version 3.01 software (Diffraction Technology Pty LTD, Mitchell, ACT, Australia) before calculating the percentage of crystallinity.

9.3.6. Pasting properties

Pasting properties of fresh and aged rice (6 and 12 months under various MAP) flour (particle size ≤ 500 µm) of all three rice varieties were determined according to the AACC International Method

61-02.01 using a Rapid Visco Analyzer (RVA-4 model Thermocline Windows Control and analysis software, Version 1.2 (New Port Scientific, Sydney, Australia)) (AACC 1999). To calculate the sample size for RVA, the moisture contents of all the flour samples were measured according to the AACC International Method 44-40.01(AACC 1999) by using a vacuum oven. Rice flour (3.01 g, 12.4 % moisture basis) was mixed with 25.0 g MilliQ water in the RVA canister. A programmed heating and cooling cycle were used, the samples were held at 50°C for 1 min, heated to 95°C in 3.45 min, held at 95°C for 2.7 min before cooling to 50°C in 3.91 min and holding at 50°C for 1.24 min. Pasting temperature (P_{temp}), Peak viscosity (V_p), Trough viscosity (V_t), Breakdown (BD), Final viscosity (V_f) and Setback (SB) were recorded.

9.3.7. Gelatinization and retrogradation properties

Differential Scanning Calorimeter (DSC) (Mettler Toledo, Schwerzenbach, Switzerland) with internal coolant and nitrogen/air purge gas was used to determine the gelatinization characteristics of rice flours (particle size ≤ 500 µm) of fresh and aged rice (6 and 12 months under the various MAP). The DSC was calibrated for the heat flow using indium as standard. Rice flour (approx. 4 mg, dry weight basis) was weighed into an aluminum pan and 6 µL MilliQ water was added. The pan was hermetically sealed and equilibrated at room temperature for 30 min, then scanned at the heating rate of 15°C/min from 0 to 100°C with the empty sealed pan as a reference. The onset (T_o), peak (T_p) and conclusion (T_c) temperatures, and enthalpy (ΔH) of gelatinization were determined by Star^e Software Version 9.1 (Mettler Toledo).

After cooling, the scanned samples pans were placed in a refrigerator at 4±1°C for 7 days. Retrogradation properties were measured by rescanning these samples at the rate of 15°C/min from 0 to 100°C. The onset ($T_{o(r)}$), peak ($T_{p(r)}$) and conclusion ($T_{c(r)}$) temperatures, and enthalpy of retrograded starch ($\Delta H_{(r)}$) were determined. The percentage of retrogradation (R %) was calculated according to equation 9.4.

$$R\% = \left[{}^{\Delta H_{(r)}}/_{\Delta H} \right] \times 100 \qquad \text{Eq. 9.4}$$

9.3.8. Textural profile analysis

Fresh and aged (6 and 12 months under various MAP conditions) milled rice grains (5 g) were added in 15 mL of MilliQ water (rice to water ratio =1:3) in 50 mL glass beaker. The beaker with

the rice and water was placed in a water bath at 95±1ºC. Cooking was continued until there was no ungelatinized white belly observed in rice kernel cross section (data not shown). Analysis of textural attributes was performed on a TA-XT*plus* Texture Analyzer (Stable Microsystems, UK) using 35-mm circular probe as previously described (Nawaz et al. 2016c). Three cooked grains were placed on the flat stage, and the textural property was determined. The texture analyzer settings were as follows: pre-test speed, 2.00 mm/sec; post-test speed, 2.00 mm/sec; distance, 2.00 mm; time, 10.00 sec; (auto) trigger force, 0.05 N. From the force-time curve obtained, textural attributes of hardness (height of the force peak on cycle 1, N) and adhesiveness (negative force area of the first cycle, N.s) were computed using the EXPONENT Stable Micro Systems software supplied with instrument. The textural values reported are the averages of three different determinations.

9.3.9. *In situ* TMCT cooking analysis

The *in situ* rate of water absorption, grain softening and gelatinization time during cooking of fresh and aged milled rice kernels was analyzed by using a temperature controlled aluminum block (50x50x25 mm) attached to a texture analyzer; TA-XT*plus* Texture Analyzer (Stable Microsystems, UK) according to the optimized method reported by Nawaz et al. (2017). The experiment assembly is shown in Fig. 4.1 in Chapter 4. The sample block was heated to a rice cooking temperature of 95±1ºC and held it at this temperature. A single layer of 0.5 g of rice kernels and 1.5 mL of deionized water (rice to water ratio = 1:3) was put on the sample block. The soaked samples were compressed by a 35 mm Teflon probe at a steady force of 0.15 N attached to the texture analyzer. The texture analyzer recorded the change in distance of probe until rice sample has taken up all available water and fully cooked (indicated by the vibration in probe due to the evaporation of water from the cooked grain). The cooking rate of the grains was estimated by measuring the slope of the initial linear part of the *in situ* Thermal Mechanical Compression Test (TMCT) cooking curves.

9.3.10. Statistical analysis

All treatments were replicated three times and the data presented are their mean values. The reported data for the surface chemical composition of raw and cooked rice kernels, X-ray diffraction, pasting properties, gelatinization and retrogradation, textural profile analysis, and *in situ* TMCT cooking analysis for each variety was analyzed separately by analysis of variance using

Minitab R17 (Minitab® for Windows Release 17, Minitab Inc, Chicago, USA) in order to determine significant differences. The data was then analyzed using Tukey's pair-wise comparison, at 5 % level of significance, to compare the different treatments.

9.4. Results and discussion

9.4.1. Results

9.4.1.1. Microstructures of fresh and aged rice kernels

The microstructures of fresh and aged (6 and 12 months under various MAP conditions) rice kernels of selected glutinous (TDK8 and TDK11) and non-glutinous (DG) rice varieties are presented in Fig. 9.1. Scanning electron micrographs of fresh rice kernels have shown that the endosperm was mainly packed with polyhedral starch granules with an average size of 6-7 µm in TDK8 and TDK11 and 8-10 µm in DG.

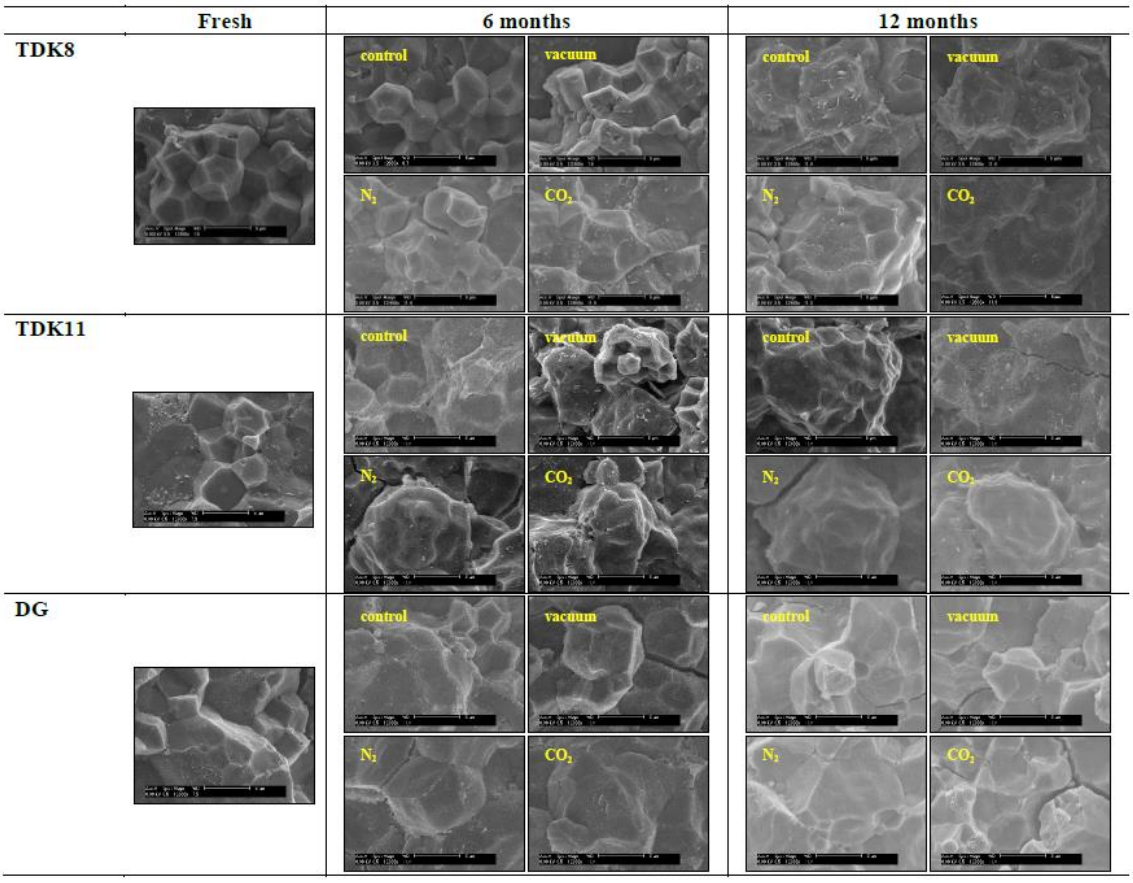

Figure 9.1 Scanning electron micrographs (SEM) of cross sections of fresh and aged (6 and 12 months under various MAP conditions) milled rice grains of Thadokkham-8 (TDK8), Thadokkham-11 (TDK11) and Doongara (DG). Scale bar on each image = 5 µm

9.4.1.2. Surface composition of uncooked and cooked grains of fresh and aged rice

The relative elemental and calculated macromolecules (starch, proteins, and lipids) of the upper 10 nm surface layer of uncooked and cooked fresh and aged (6 and 12 months under various MAP conditions) rice grains of selected glutinous (TDK8 and TDK11) and non-glutinous (DG) rice varieties are presented in Table 9.1 and Table 9.2. XPS spectra showed that the surface composition of rice kernels of selected rice varieties mainly consisted of C, N, and O. The surface of uncooked grains of fresh glutinous rice varieties (TDK8 and TDK11) had more lipids (52.5 % and 57.7 %, respectively) followed by proteins (34.2 % and 41.1 %, respectively) and less concentration of starch (13.3 % and 1.2 %, respectively). The surface of non-glutinous uncooked fresh DG rice grains showed more lipids (51.7 %) followed by starch (25.2 %) and proteins (23.1 %) as shown in Table 9.1. The control storage conditions induced significantly ($P<0.05$) higher concentration of surface proteins and reduced surface lipids in all selected rice varieties (Table 9.1).

The XPS spectra of cooked rice grains showed diversity among all rice varieties. Three peaks corresponding to C, N, and O were detected in TDK8. However, four peaks corresponding to C, N, O, and Mn were detected in TDK11 as shown in Table 9.2. In addition to this, five peaks corresponding to C, N, O, B, and Si were detected in DG. The relative elemental composition for C, N, and O of cooked rice samples did not fit in the matrix formula developed for dry samples due to the complex nature of gelatinized starch, denatured proteins, and starch/proteins/lipids interaction. Therefore, high-resolution XPS scans for C_{1s}, N_{1s}, and O_{1s} peaks of cooked fresh and aged rice grains of selected rice varieties were conducted. The deconvolution of C_{1s} showed six distinct sub-peaks (C-C, C-COOH, C-N, C-O, C=O, and O-C=O) in all samples, corresponding to various macromolecules such as polysaccharides, proteins or lipids side chains. The XPS survey and high-resolution scans of fresh cooked TDK8, TDK11, and DG are presented in Appendix 8, 9 and 10, respectively. Moreover, the calculated values for C/O and C/N stoichiometry for cooked rice samples were also calculated and compared with C/O and C/N stoichiometric values of pure rice starch, rice protein and rice lipid (presented in Table 5.2 in Chapter 5).

In the present study, the calculated C/O and C/N stoichiometry of fresh cooked glutinous rice were 2.7 and 14.9, respectively for TDK8 and 3.6 and 12.4, respectively for TDK11 (Table 9.2). However, the calculated C/O and C/N stoichiometry of fresh cooked non-glutinous rice (DG) were

3.6 and 26.6, respectively (Table 9.2) indicating that lipids mainly covered the grain surface with some proteins. The deconvolution of C_{1s} of fresh and aged cooked TDK8 stored under various MAP conditions for 6 and 12 months showed that the modifications in the storage environment might lead to significant ($P<0.05$) variations in the surface of the cooked rice.

Table 9.1 Relative elemental and calculated surface composition (%) of uncooked rice kernels of fresh and aged (6 and 12 months under various MAP conditions) milled rice grains of Thadokkham-8 (TDK8), Thadokkham-11 (TDK11) and Doongara (DG)*

Rice variety	Aging	MAP	Elemental atomic percentage (%)			Surface composition (%) after using matrix formula		
			C	N	O	Starch	Protein	Lipids
TDK8	Fresh	-	82.9±0.3^{a}	2.3±0.2^{a}	14.8±0.1^{a}	13.3±0.4^{a}	34.2±3.2^{a}	52.5±2.8^{a}
	6 months	control	80.5±0.0^{c}	2.8±0.1^{ab}	16.8±0.2^{c}	17.1±1.2^{bc}	41.0±2.3^{ab}	43.2±3.4^{b}
		vacuum	80.5±0.5^{c}	2.9±0.1^{ab}	16.7±0.4^{c}	16.7±0.8^{b}	42.9±1.7^{ab}	40.4±2.5^{b}
		N_2	81.6±0.3^{b}	2.6±0.4^{a}	15.9±0.1^{b}	15.5±2.0^{ab}	38.4±6.5^{a}	46.2±4.5^{ab}
		CO_2	80.5±0.5^{c}	2.3±0.1^{a}	17.2±0.3^{cd}	20.5±0.5^{d}	34.0±2.1^{a}	45.5±2.6^{ab}
	12 months	control	78.3±0.5^{d}	4.0±0.4^{d}	17.7±0.1^{ce}	15.3±1.2^{ab}	60.5±5.8^{d}	24.2±4.7^{c}
		vacuum	78.0±0.1^{de}	3.4±0.0^{bc}	18.7±0.0^{f}	21.0±0.3^{d}	50.1±1.2^{bc}	28.9±0.8^{c}
		N_2	78.3±0.2^{de}	3.9±0.2^{cd}	17.8±0.1^{e}	15.8±0.3^{ab}	59.5±2.3^{cd}	24.7±2.0^{c}
		CO_2	77.4±0.1^{e}	3.8±0.1^{cd}	18.2±0.2^{f}	19.7±0.7^{cd}	57.1±1.0^{cd}	23.3±0.2^{c}
	F value		95.0	30.9	118.1	21.8	28.8	43.6
TDK11	Fresh	-	85.9±1.5^{a}	2.6±0.6^{a}	11.5±0.9^{a}	1.2±0.3^{a}	41.1±9.4^{a}	57.7±9.7^{a}
	6 months	control	81.4±0.1^{abc}	4.7±0.0^{bc}	14.0±0.1^{ab}	0.4±0.4^{a}	73.8±0.1^{bc}	25.9±0.3^{bcd}
		vacuum	81.6±0.5^{abc}	5.0±0.2^{bc}	13.4±0.7^{ab}	0.1±0.1^{a}	78.6±3.1^{c}	24.0±0.3^{bcd}
		N_2	81.7±0.2^{ab}	4.9±0.4^{bc}	13.5±0.1^{ab}	0.0±0.0^{a}	76.4±5.7^{bc}	25.5±3.9^{bcd}
		CO_2	82.0±1.3^{ab}	4.5±0.4^{bc}	13.6±0.9^{ab}	0.3±0.5^{a}	70.3±6.8^{c}	29.6±7.7^{bc}
	12 months	control	76.4±3.1^{cde}	5.4±1.0^{c}	18.2±2.0^{cd}	6.7±5.9^{ab}	83.2±16.0^{c}	9.9±12.4^{cd}
		vacuum	80.4±3.5^{bcd}	3.2±0.9^{ab}	16.4±2.6^{bc}	14.7±4.2^{b}	47.8±14.2^{ab}	37.5±18.4^{ab}
		N_2	75.6±0.2^{de}	5.6±0.5^{c}	18.9±0.7^{cd}	12.6±4.1^{b}	85.4±7.8^{c}	2.6±2.9^{d}
		CO_2	74.6±2.3^{e}	5.1±0.9^{c}	20.2±1.4^{d}	15.2±5.0^{b}	78.2±13.5^{c}	7.0±9.4^{cd}
	F value		11.6	7.4	16.4	13.0	7.4	10.3
DG	Fresh	-	80.6±0.3^{c}	1.7±0.2^{c}	17.8±0.1^{c}	25.2±0.6^{c}	23.1±3.1^{b}	51.7±2.5^{ab}
	6 months	control	84.0±0.6^{a}	2.7±0.1^{ab}	13.4±0.6^{a}	7.1±1.7^{a}	40.7±0.6^{cd}	52.2±2.2^{ab}
		vacuum	84.3±0.8^{a}	2.5±0.3^{b}	13.3±0.6^{a}	7.7±0.7^{a}	37.7±3.8^{c}	54.7±4.5^{ab}
		N_2	84.5±0.7^{a}	2.7±0.3^{ab}	12.8±0.4^{a}	5.1±0.1^{a}	41.6±4.6^{cd}	53.3±4.5^{a}
		CO_2	83.5±0.1^{a}	3.0±0.1^{a}	13.5±0.1^{a}	5.9±0.6^{a}	46.8±1.7^{d}	47.3±1.0^{ab}
	12 months	control	75.7±0.1^{f}	1.5±0.2^{cd}	22.8±0.1^{e}	42.0±1.4^{e}	18.5±4.0^{ab}	39.5±2.6^{c}
		vacuum	77.2±0.5^{e}	1.0±0.0^{d}	21.8±0.5^{e}	40.9±1.5^{e}	10.9±0.3^{a}	48.2±1.7^{ab}
		N_2	78.7±0.0^{d}	1.8±0.1^{c}	19.6±0.2^{d}	30.7±1.1^{d}	23.8±2.3^{b}	45.5±1.2^{bc}
		CO_2	81.9±0.3^{b}	2.4±0.0^{b}	15.8±0.3^{b}	16.1±1.1^{b}	34.7±0.5^{c}	49.3±0.6^{ab}
	F value		155.9	42.9	348.1	578.4	58.5	9.2

*Means ± SD (n = 3, df = 8). For a particular variety, means with different letters in the same column denote significant difference at 5 % probability level within each rice variety.

Table 9.2 Relative elemental and calculated surface composition (%) of cooked rice kernels of fresh and aged (6 and 12 months under various MAP conditions) milled rice grains of Thadokkham-8 (TDK8), Thadokkham-11 (TDK11) and Doongara (DG)*

Rice variety	Aging	MAP	Elemental atomic percentage (%)									Stoichiometry	
			C							N	O	C/O	C/N
			C	C-C	C-N	C=O	O-C=O	C-COOH	C-O				
TDK8	Fresh	-	69.3±0.4^{a}	35.0±1.0^{ab}	34.9±0.7^{a}	9.3±1.0^{a}	4.3±1.2^{b}	4.3±1.2^{b}	12.1±0.4^{a}	4.7±0.2^{a}	26.1±0.3^{a}	2.7	14.9
	6 months	control	61.8±1.6^{cde}	28.1±6.6^{abc}	20.9±0.4^{c}	13.9±0.8^{bcd}	2.6±0.5^{cd}	2.6±0.5^{cd}	32.0±4.4^{bc}	3.3±1.5^{ab}	35.5±2.5^{bc}	1.7	18.9
		vacuum	60.9±0.7^{de}	18.3±3.6^{c}	17.0±2.4^{d}	12.5±1.4^{bc}	4.1±0.1^{bc}	4.1±0.1^{bc}	44.1±7.2^{c}	3.4±1.4^{ab}	35.5±1.8^{bc}	1.7	17.9
		N_2	62.4±0.7^{cde}	22.1±0.3^{bc}	29.3±0.3^{b}	11.8±1.4^{ab}	1.2±0.3^{de}	1.1±0.2^{e}	34.6±0.4^{bc}	4.5±0.2^{a}	33.2±0.4^{bc}	1.9	13.9
		CO_2	60.1±0.5^{e}	36.1±11.5^{a}	22.6±0.7^{c}	14.3±1.7^{bcd}	0.4±0.4^{e}	0.4±0.4^{e}	26.1±11.7^{b}	3.9±2.0^{ab}	36.0±1.5^{bc}	1.7	15.5
	12 months	control	63.4±1.0^{bcd}	25.9±0.3^{abc}	22.8±0.5^{c}	14.9±0.2^{cd}	1.5±0.2^{de}	1.5±0.2^{de}	34.0±0.2^{bc}	5.0±0.1^{a}	31.2±0.8^{b}	2.0	12.8
		vacuum	66.3±0.0^{b}	29.5±0.5^{abc}	26.8±0.8^{b}	12.2±0.3^{abc}	3.0±0.5^{bc}	3.0±0.4^{bc}	25.3±0.7^{ab}	2.5±2.5^{ab}	31.3±2.4^{b}	2.1	27.0
		N_2	64.2±2.1^{bc}	33.1±0.5^{ab}	10.2±0.8^{e}	14.2±1.2^{bcd}	6.6±0.6^{a}	6.7±0.5^{a}	29.1±0.9^{b}	2.4±1.1^{ab}	33.4±3.2^{bc}	1.9	26.6
		CO_2	62.6±0.8^{cde}	34.1±1.1^{ab}	5.4±1.4^{f}	16.4±0.6^{d}	7.3±0.4^{a}	7.3±0.4^{a}	29.4±0.9^{b}	0.4±0.4^{b}	37.0±1.2^{c}	1.7	148.9
	F value		22.2	5.3	216.8	11.3	59.2	65.1	9.6	3.5	10.1		
TDK11	Fresh	-	73.5±1.8^{b}	52.3±0.1^{a}	12.6±3.5^{a}	8.8±1.2^{bc}	3.3±0.4^{a}	3.3±0.4^{a}	19.8±3.3^{c}	5.9±1.1^{ab}	20.6±0.7^{b}	3.6	12.4
	6 months	control	68.1±0.3^{c}	1.4±1.4^{f}	46.8±1.3^{d}	8.5±4.0^{bc}	0.6±0.6^{c}	0.0±0.0^{d}	42.7±0.7^{b}	6.5±0.2^{a}	25.4±0.5^{c}	2.7	10.4
		vacuum	81.6±0.5^{a}	13.8±0.5^{b}	68.3±0.1^{e}	5.8±0.4^{c}	2.5±0.3^{ab}	2.5±0.3^{ab}	7.1±0.6^{e}	5.0±0.2^{abc}	13.4±0.6^{a}	6.1	16.4
		N_2	81.8±0.1^{a}	11.9±0.6^{cd}	69.3±1.1^{e}	5.2±0.6^{c}	3.2±1.0^{a}	3.3±1.1^{a}	7.0±0.0^{e}	4.8±0.3^{bc}	13.4±0.2^{a}	6.1	17.0
		CO_2	82.0±1.3^{a}	12.1±0.3^{c}	71.0±0.0^{e}	7.0±0.6^{c}	1.0±1.0^{c}	1.0±0.0^{cd}	7.9±0.5^{e}	4.5±0.4^{bc}	13.6±0.9^{a}	6.0	18.3
	12 months	control	67.7±0.2^{cd}	10.3±0.3^{d}	36.1±1.0^{c}	12.1±0.1^{b}	1.1±0.1^{c}	1.0±0.0^{cd}	39.1±0.9^{b}	3.8±0.3^{cd}	28.6±0.2^{cd}	2.4	18.1
		vacuum	65.5±0.2^{cde}	7.2±0.3^{e}	37.6±0.4^{c}	11.6±0.6^{b}	1.4±0.1^{bc}	1.4±0.1^{bc}	40.6±0.7^{b}	3.5±0.1^{cd}	30.9±0.2^{de}	2.1	19.0
		N_2	62.8±0.6^{e}	13.8±0.3^{b}	31.6±0.6^{b}	0.2±0.2^{d}	0.4±0.4^{c}	0.5±0.5^{cd}	53.4±0.9^{a}	2.6±0.1^{de}	34.6±0.7^{f}	1.8	24.6
		CO_2	64.8±2.3^{de}	0.5±0.5^{f}	30.7±0.3^{b}	52.5±1.4^{a}	0.5±0.5^{c}	0.6±0.6^{cd}	14.8±0.8^{d}	1.7±1.0^{e}	33.5±3.3^{ef}	1.9	37.3
	F value		154.9	2040.7	680.0	312.9	17.0	19.7	611.8	24.1	158.3		
DG	Fresh	-	75.9±0.4^{abc}	14.7±1.9^{a}	61.0±2.2^{a}	6.1±0.3^{a}	1.5±0.0^{b}	1.5±0.0^{a}	15.2±0.1^{ab}	2.9±0.4^{ab}	21.2±0.8^{abc}	3.6	26.6
	6 months	control	76.4±1.2^{ab}	24.1±0.9^{d}	34.6±0.5^{c}	7.5±1.3^{ab}	4.3±0.3^{c}	4.4±0.2^{b}	22.0±0.0^{d}	3.6±0.5^{a}	20.0±0.7^{a}	3.8	21.2
		vacuum	73.6±0.8^{abcd}	21.0±1.0^{bc}	19.8±0.9^{e}	17.2±0.2^{e}	6.3±0.3^{d}	6.3±0.2^{c}	29.4±0.6^{f}	2.6±0.1^{abc}	23.8±0.9^{cde}	3.1	28.3
		N_2	70.7±1.2^{d}	23.2±0.4^{cd}	19.1±0.9^{e}	18.0±0.7^{e}	6.0±0.1^{d}	6.0±0.0^{c}	27.7±0.3^{ef}	2.0±0.5^{bc}	27.3±1.7^{f}	2.6	35.2
		CO_2	74.0±0.7^{abcd}	30.6±0.6^{e}	34.6±0.5^{d}	10.1±0.4^{cd}	4.2±0.3^{c}	4.3±0.3^{b}	16.2±0.8^{b}	2.4±0.1^{bc}	23.6±0.7^{bcde}	3.1	30.6
	12 months	control	76.9±2.0^{a}	23.7±0.7^{d}	12.3±0.3^{f}	11.5±0.5^{d}	0.7±0.7^{ab}	0.8±0.8^{a}	51.0±1.0^{g}	2.9±0.6^{ab}	20.2±1.4^{ab}	3.8	26.7
		vacuum	74.8±2.1^{abc}	19.5±0.5^{b}	58.3±1.3^{a}	5.9±0.1^{a}	1.2±0.2^{ab}	1.3±0.2^{a}	13.8±0.3^{a}	3.1±0.0^{ab}	22.1±2.1^{abcd}	3.4	24.2
		N_2	73.1±1.0^{bcd}	18.9±0.1^{b}	50.9±0.9^{b}	8.4±0.7^{b}	1.3±0.2^{ab}	1.3±0.2^{a}	19.3±0.3^{c}	2.3±0.5^{bc}	24.9±0.7^{def}	2.9	31.6
		CO_2	72.7±0.4^{cd}	15.2±0.2^{a}	48.7±0.3^{b}	8.7±0.3^{bc}	0.5±0.5^{a}	0.6±0.6^{a}	26.4±1.4^{e}	1.7±0.3^{c}	25.6±0.7^{ef}	2.8	42.4
	F value		7.6	97.8	865.3	173.9	139.7	122.8	821.5	6.6	13.4		

*Means ± SD (n = 3, df = 8). For a particular variety, means with different letters in the same column denote significant difference at 5 % probability level within each rice variety.

9.4.1.3. X-ray diffraction pattern of fresh and aged rice flour

The x-ray diffraction pattern of fresh and aged (6 and 12 months under various MAP conditions) rice flour of selected glutinous (TDK8 and TDK11) and non-glutinous (DG) rice varieties are presented in Fig. 9.2. As expected, all the three selected rice varieties exhibited A-type diffraction (XRD) pattern detected with main peaks at 14.9º, 16.9º, and 22.8º. The crystallinity for TDK8 and TDK11 was recorded as 50 and 48 %, respectively. However, the crystallinity for DG was recorded as 45 %. Moreover, no significant ($P>0.05$) change in crystallinity was recorded during various MAP conditions in all three selected rice varieties.

9.4.1.4. Pasting properties of fresh and aged rice flour

The pasting properties of fresh and aged (6 and 12 months under various MAP conditions) rice flour of selected glutinous (TDK8 and TDK11) and non-glutinous (DG) rice varieties are presented in Table 9.3. The pasting temperature (P_{temp}) of flour of fresh rice grains of selected glutinous (TDK8 and TDK11) and non-glutinous (DG) rice varieties was significantly ($P<0.05$) lower than aged the flours. However, vacuum storage of TDK8 exhibited lower physicochemical changes which resulted in no significant ($P>0.05$) increase in P_{temp} even after 12 months of storage. On the other hand, N_2 and CO_2 storage resulted in significant ($P<0.05$) increase in P_{temp} in all selected rice varieties.

The flour of selected rice varieties showed a diverse behavior of viscosity (peak ~ V_p, breakdown ~ BD, trough ~ V_t, setback ~ SB, and final ~ V_f) during heating-cooling cycles of rapid visco analysis. TDK8 and DG showed significant ($P<0.05$) increase in V_p during aging in all MAP conditions. V_p indicates increased swelling of starch. However, TDK11 showed decreased swelling during the first 6 months of storage followed by increased V_p only for control storage after 12 months. Results showed that the MAP conditions especially vacuum and N_2 slowed down the physicochemical changes, which is reflected by significantly ($P<0.05$) lower V_f of vacuum and N_2 storage than the control in all selected rice varieties. Low V_f indicates softer texture of the final gel.

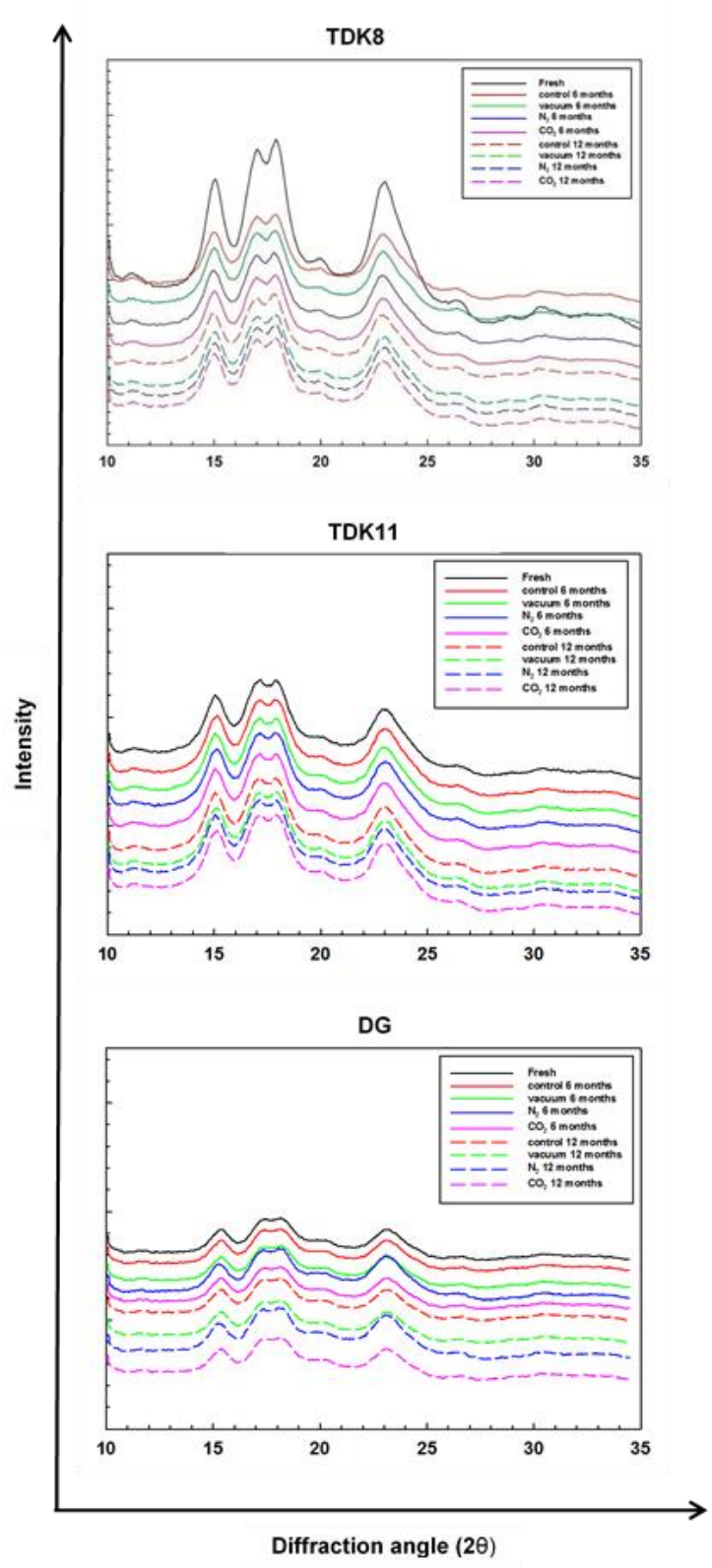

Figure 9.2 X-ray diffraction pattern of flour of fresh and aged (6 and 12 months under various MAP conditions) milled rice grains of Thadokkham-8 (TDK8), Thadokkham-11 (TDK11) and Doongara (DG)

Table 9.3 Pasting properties of rice flour of fresh and aged (6 and 12 months under various MAP conditions) milled rice grains of Thadokkham-8 (TDK8), Thadokkham-11 (TDK11) and Doongara (DG)*

Rice variety	Aging	MAP	Pasting properties					
			P_{temp} (°C)	V_p (mPa-s)	V_t (mPa-s)	BD (mPa-s)	V_f (mPa-s)	SB (mPa-s)
TDK8	Fresh	-	72.3±0.1^{a}	2371.7±47.7^{a}	1443.3±17.0^{a}	928.3±31.8^{b}	1779.7±18.0^{a}	336.3±8.4^{a}
	6 months	control	72.6±0.3^{ab}	2652.5±7.5^{cd}	1744.5±19.5^{cd}	908.0±27.0^{b}	2107.5±34.5^{cd}	363.0±15.0^{ab}
		vacuum	72.4±0.5^{ab}	2462.0±28.0^{b}	1691.5±5.5^{b}	770.5±33.5^{a}	2026.5±5.5^{b}	335.0±0.0^{a}
		N_2	72.9±0.1^{ab}	2658.0±8.0^{cd}	1771.5±2.5^{de}	886.5±10.5^{b}	2148.0±10.0^{cd}	376.5±12.5^{bc}
		CO_2	73.1±0.5^{bc}	2695.0±5.0^{cd}	1778.0±5.0^{de}	917.0±0.0^{b}	2158.3±12.6^{d}	380.3±11.6^{bc}
	12 months	control	72.6±0.2^{ab}	2939.0±40.0^{e}	1869.0±4.0^{f}	1070.0±36.0^{c}	2275.5±8.5^{f}	406.5±4.5^{cd}
		vacuum	72.5±0.1^{ab}	2623.5±26.5^{c}	1717.0±30.0^{bc}	906.5±3.5^{b}	2106.0±23.0^{c}	389.0±7.0^{bcd}
		N_2	73.7±0.2^{cd}	2654.0±17.0^{cd}	1763.0±2.5^{de}	891.0±4.0^{b}	2156.5±6.5^{cd}	393.5±14.5^{cd}
		CO_2	74.2±0.2^{d}	2719.0±31.0^{d}	1799.0±9.0^{e}	920.3±22.0^{b}	2215.5±20.5^{e}	416.5±11.5^{d}
	F value		17.6	101.5	176.1	32.8	189	21.9
TDK11	Fresh	-	68.3±0.2^{a}	2521.5±5.5^{b}	1084.0±18.0^{a}	1437.5±12.5^{a}	1377.0±7.0^{a}	293.0±11.0^{a}
	6 months	control	69.6±0.3^{b}	2077.5±44.5^{e}	1359.0±52.0^{c}	718.5±7.5^{c}	1679.0±13.0^{d}	320.0±39.0^{a}
		vacuum	69.9±0.6^{b}	1805.5±25.5^{h}	1213.5±3.5^{b}	592.0±22.0^{e}	1516.5±4.5^{b}	303.0±1.0^{a}
		N_2	70.8±0.2^{bc}	1881.5±25.5^{g}	1216.0±31.0^{b}	665.5±5.5^{d}	1586.5±36.5^{c}	370.5±5.5^{b}
		CO_2	69.9±0.2^{b}	1800.5±18.5^{h}	1784.5±6.0^{b}	657.0±12.5^{f}	1558.5±2.5^{bc}	310.5±8.5^{a}
	12 months	control	72.7±0.4^{d}	3016.0±34.0^{a}	2246.0±25.0^{f}	770.0±9.0^{b}	2732.5±12.5^{g}	486.5±12.5^{c}
		vacuum	70.8±0.7^{bc}	1984.5±8.5^{f}	1369.5±0.5^{c}	615.0±8.0^{e}	1677.0±5.0^{d}	307.5±4.5^{a}
		N_2	74.6±0.3^{e}	2441.5±6.5^{c}	1784.5±0.5^{e}	657.0±6.0^{d}	2167.5±1.5^{f}	383.0±1.0^{b}
		CO_2	72.0±0.9^{cd}	2177.0±8.5^{d}	1454.5±25.0^{d}	722.5±20.5^{c}	1838.5±37.5^{e}	384.0±22.0^{b}
	F value		48.7	708.2	719.4	1302.4	1490.7	43.5
DG	Fresh	-	75.5±0.4^{a}	1325.5±34.5^{a}	1180.0±17.0^{a}	145.5±17.5^{a}	2406.0±33.0^{a}	1226.0±16.0^{a}
	6 months	control	79.5±0.1^{b}	1618.0±21.0^{c}	1224.5±4.5^{ab}	393.5±25.5^{de}	4039.0±10.0^{d}	2814.5±14.5^{d}
		vacuum	80.2±0.5^{b}	1665.0±29.0^{cd}	1261.0±12.0^{bc}	404.0±17.0^{e}	3609.0±37.0^{b}	2348.0±25.0^{b}
		N_2	79.9±0.2^{b}	1653.0±37.0^{cd}	1229.5±18.5^{ab}	423.5±18.5^{e}	3675.5±42.5^{bc}	2446.0±24.0^{c}
		CO_2	80.4±0.2^{b}	1752.0±26.0^{d}	1273.5±24.5^{bc}	478.5±1.5^{f}	3796.5±3.5^{c}	2523.0±21.0^{c}
	12 months	control	84.0±0.4^{d}	1476.0±59.0^{b}	1254.0±39.0^{bc}	222.0±20.0^{b}	4312.5±58.5^{e}	3058.5±19.5^{e}
		vacuum	82.9±0.3^{cd}	1668.5±64.5^{cd}	1319.5±44.5^{cd}	349.0±20.0^{cd}	4515.0±92.0^{f}	3195.5±47.5^{f}
		N_2	82.6±0.2^{c}	1619.0±35.0^{c}	1246.0±13.0^{ab}	373.0±22.0^{cde}	4274.5±40.5^{e}	3028.5±27.5^{e}
		CO_2	81.7±0.9^{c}	1672.0±4.0^{cd}	1342.5±3.5^{d}	329.5±0.5^{c}	4362.0±56.0^{e}	3019.5±52.5^{e}
	F value		109.8	33.0	12.9	100.7	529.0	1203.3

*Means ± SD (n = 3, df = 8). For a particular variety, means with different letters in the same column denote significant difference at 5 % probability level within each rice variety.

9.4.1.5. Gelatinization and retrogradation properties of fresh and aged rice flour

The gelatinization and retrogradation properties of fresh and aged (6 and 12 months under various MAP conditions) rice flour of selected glutinous (TDK8 and TDK11) and non-glutinous (DG) rice varieties are presented in Table 9.4. Aging under modified atmospheric conditions resulted in slightly decreased onset (T_o), peak (T_p) and conclusion (T_c) temperatures. In general, the enthalpy

(ΔH) of gelatinization for TDK11 and DG flour was increased significantly ($P<0.05$) during aging with the more prominent increase during N_2 and CO_2 storage. Increase in ΔH can be related to the increase in P_{temp} as shown in Table 9.3. However, no significant ($P>0.05$) change in ΔH for TDK8 was observed.

Similarly, the retrogradation thermal temperatures ($T_{o(r)}$, $T_{p(r)}$, and $T_{c(r)}$) were also reduced in TDK8 and DG. The retrogradation thermal temperatures for fresh TDK8 were recorded as $T_{o(r)} \sim 51.7^{o}C$, $T_{p(r)} \sim 58.5^{o}C$, and $T_{c(r)} \sim 65.2^{o}C$. However, after 6 and 12 months of storage, the retrogradation thermal temperatures were reduced. Moreover, the retrogradation thermal temperatures for fresh DG were recorded as $T_{o(r)} \sim 43.7^{o}C$, $T_{p(r)} \sim 55.4^{o}C$, and $T_{c(r)} \sim 62.9^{o}C$ but during first 6 months of storage, no significant ($P>0.05$) reduction in thermal transition temperatures was observed. Interestingly, TDK11 did not show any retrogradation (as no retrogradation peak was detected) during the first 6 months in any MAP conditions. The results showed that vacuum condition for all rice varieties reduced the rate of physicochemical changes whether they were glutinous or non-glutinous as reflected by no significant ($P>0.05$) increase in the percentage of retrogradation (R %) (Table 9.4).

9.4.1.6. Textural profile analysis of cooked grains of fresh and aged rice

The textural profiles of cooked fresh and aged (6 and 12 months under various MAP conditions) are presented in Table 9.5. Results showed that there was a significant increase in hardness and a significant decrease in adhesiveness of cooked rice during storage under various MAP conditions in all rice varieties ($P = 0.05$). Interestingly, the rice grains stored in N_2 and CO_2 had harder texture after cooking compared to that of fresh and aged rice grains of control and vacuum storage. However, the reduction in the stickiness or adhesiveness of cooked rice grains was found relatively slower in N_2 and CO_2 than the control and vacuum storage even after 12 months of storage. The results of pasting properties especially setback (SB) and final (V_f) viscosities (Table 9.3) and thermal analysis (Table 9.4) of N_2 and CO_2 storage well correlated with TPA results.

Table 9.4 Gelatinization and retrogradation properties of rice flour of fresh and aged (6 and 12 months under various MAP conditions) milled rice grains of Thadokkham-8 (TDK8), Thadokkham-11 (TDK11) and Doongara (DG)*

Rice variety	Aging	MAP	Gelatinization				Retrogradation				
			T_o (ºC)	T_p (ºC)	T_c (ºC)	ΔH (Jg^{-1})	$T_{o(r)}$ (ºC)	$T_{p(r)}$ (ºC)	$T_{c(r)}$ (ºC)	$\Delta H_{(r)}$ (Jg^{-1})	R (%)
TDK8	Fresh	-	66.4±0.0^{ab}	73.7±0.4^{ab}	89.9±1.6^{a}	10.4±0.5^{a}	51.7±0.0^{a}	58.5±0.5^{a}	65.2±1.3^{a}	0.2±0.0^{a}	2.1±0.1^{a}
	6 months	control	65.2±0.1^{cd}	72.6±0.2^{c}	85.6±1.5^{b}	10.8±0.3^{a}	44.6±0.4^{de}	54.4±0.5^{cd}	62.7±0.8^{ab}	0.4±0.0^{ab}	3.5±0.1^{ab}
		vacuum	66.3±0.0^{abc}	73.5±0.1^{abc}	85.4±0.8^{b}	9.7±1.0^{a}	46.8±0.1^{c}	56.9±0.2^{ab}	63.6±0.2^{ab}	0.3±0.0^{ab}	2.8±0.4^{ab}
		N_2	66.8±1.0^{a}	73.9±1.0^{a}	86.9±2.4^{ab}	9.4±1.0^{a}	45.8±0.1^{cd}	56.1±1.4^{bc}	62.9±0.0^{ab}	0.3±0.1^{ab}	3.5±0.4^{abc}
		CO_2	65.1±0.1^{d}	72.7±0.2^{bc}	87.4±0.7^{ab}	10.6±0.5^{a}	41.6±0.1^{f}	53.9±0.8^{d}	64.1±1.3^{ab}	0.4±0.0^{abc}	3.8±0.1^{bc}
	12 months	control	66.3±0.1^{abc}	73.4±0.3^{abc}	84.2±0.2^{b}	9.7±0.3^{a}	47.9±0.6^{bc}	56.5±0.1^{ab}	64.0±0.3^{ab}	0.4±0.1^{abc}	4.0±0.5^{bc}
		vacuum	65.3±0.4^{bcd}	72.7±0.2^{bc}	84.2±1.4^{b}	10.8±0.5^{a}	44.0±1.1^{de}	55.3±0.8^{bcd}	62.5±1.4^{ab}	0.3±0.0^{ab}	3.1±0.0^{ab}
		N_2	65.6±0.0^{bcd}	72.5±0.0^{c}	85.1±0.7^{b}	10.3±0.7^{a}	43.1±1.8^{ef}	54.4±1.3^{cd}	61.8±1.8^{b}	0.5±0.1^{bc}	5.1±0.1^{cd}
		CO_2	66.0±0.6^{abcd}	73.3±0.1^{abc}	87.6±0.2^{ab}	9.8±1.8^{a}	49.0±1.1^{b}	56.3±0.2^{bc}	62.9±0.1^{ab}	0.7±0.3^{c}	6.5±1.4^{d}
	F value		6.8	5.1	6.5	1.0	53.1	12.5	3.1	6.1	17.2
TDK11	Fresh	-	64.3±0.1^{b}	70.6±0.1^{b}	77.4±0.1^{a}	7.7±0.1^{a}	ND^	ND^	ND^	ND^	ND^
	6 months	control	64.8±0.1^{a}	71.0±0.2^{ab}	81.1±0.2^{bc}	9.6±0.3^{bc}	ND^	ND^	ND^	ND^	ND^
		vacuum	64.2±0.1^{b}	70.9±0.1^{ab}	80.4±1.0^{bc}	8.8±0.4^{ab}	ND^	ND^	ND^	ND^	ND^
		N_2	64.3±0.2^{b}	70.8±0.1^{ab}	79.7±1.0^{ab}	9.3±0.4^{bc}	ND^	ND^	ND^	ND^	ND^
		CO_2	64.3±0.0^{b}	71.3±0.0^{a}	82.8±0.9^{c}	9.2±0.5^{bc}	ND^	ND^	ND^	ND^	ND^
	12 months	control	61.6±0.0^{e}	68.6±0.1^{d}	79.3±1.0^{ab}	10.4±0.7^{cd}	37.9±0.3^{a}	48.9±0.2^{a}	56.4±0.2^{a}	2.4±0.2^{b}	22.7±0.4^{a}
		vacuum	61.8±0.1^{d}	68.3±0.4^{de}	79.2±1.5^{ab}	10.1±0.6^{cd}	37.3±0.8^{a}	48.8±0.1^{a}	56.5±0.3^{b}	1.7±0.1^{a}	16.9±0.0^{d}
		N_2	61.1±0.0^{f}	68.0±0.2^{e}	79.4±1.2^{ab}	10.3±0.5^{cd}	37.2±0.7^{a}	48.8±0.1^{a}	57.1±0.0^{c}	3.0±0.0^{c}	28.7±1.0^{b}
		CO_2	62.2±0.0^{c}	69.1±0.1^{c}	80.2±0.5^{bc}	11.0±0.3^{d}	37.9±0.9^{a}	49.2±0.3^{a}	57.5±0.0^{d}	4.5±0.1^{d}	40.8±0.2^{c}
	F value		1232.2	165.2	8.0	14.9	5195.1	22219.3	187048.1	1097.0	5204.1
DG	Fresh	-	72.3±0.7^{a}	77.1±0.5^{a}	83.0±1.5^{ab}	3.4±0.0^{a}	43.7±0.2^{a}	56.4±0.0^{a}	62.9±0.2^{a}	2.0±0.0^{a}	59.2±0.0^{d}
	6 months	control	70.3±0.3^{b}	76.1±0.3^{b}	83.3±0.5^{a}	6.9±0.3^{bcd}	39.1±1.5^{bc}	53.4±0.0^{c}	62.4±0.1^{a}	4.7±0.1^{d}	68.4±1.3^{ab}
		vacuum	70.4±0.2^{b}	76.2±0.2^{b}	83.1±0.0^{ab}	6.6±0.1^{bc}	41.6±0.6^{ab}	54.0±0.8^{bc}	62.4±0.4^{a}	3.5±0.0^{b}	52.9±0.8^{e}
		N_2	71.0±0.1^{b}	76.6±0.1^{ab}	83.8±0.4^{a}	8.4±0.3^{e}	42.4±0.5^{ab}	54.6±0.0^{bc}	62.3±0.1^{a}	3.3±0.1^{b}	39.7±1.8^{f}
		CO_2	70.6±0.1^{b}	76.3±0.2^{b}	83.1±0.0^{a}	7.4±0.3^{cd}	39.1±1.5^{ab}	54.8±0.4^{b}	62.8±1.0^{a}	2.3±0.1^{a}	30.6±0.1^{g}
	12 months	control	68.6±0.3^{c}	74.2±0.1^{c}	81.5±0.2^{bc}	7.5±0.4^{d}	35.1±0.3^{d}	49.2±0.5^{d}	59.5±0.2^{b}	5.2±0.2^{e}	69.6±1.2^{a}
		vacuum	68.2±0.3^{c}	73.9±0.0^{c}	80.9±0.0^{c}	7.2±0.4^{cd}	36.5±0.1^{cd}	49.2±0.3^{d}	59.2±0.4^{b}	4.6±0.2^{d}	64.6±0.5^{c}
		N_2	67.9±0.0^{c}	74.0±0.1^{c}	81.0±0.4^{c}	7.7±0.4^{de}	37.6±0.2^{cd}	49.9±0.1^{d}	59.1±0.5^{b}	5.2±0.4^{e}	67.2±0.8^{abc}
		CO_2	68.3±0.0^{c}	74.0±0.1^{c}	81.0±0.0^{c}	6.2±0.3^{b}	35.1±2.9^{d}	49.2±0.7^{d}	59.2±0.4^{b}	4.1±0.1^{c}	66.4±1.1^{bc}
	F value		88.1	95.8	13.7	72.8	24.4	136.9	42.2	172.1	560.6

^Not detected.

*Means ± SD (n = 3, df = 8). For a particular variety, means with different letters in the same column denote significant difference at 5 % probability level within each rice variety.

Table 9.5 The textural profile and *in situ* TMCT cooking analysis of milled rice kernels of fresh and aged (6 and 12 months under various MAP conditions) milled rice grains of Thadokkham-8 (TDK8), Thadokkham-11 (TDK11) and Doongara (DG)*

Rice variety	Aging	MAP	Textural Profile Analysis		*In situ* TMCT cooking	
			Hardness (N)	Adhesiveness (N.s)	Rate of cooking$_{20\ min}$ (mm/sec)	Cooking time (min)
TDK8	Fresh	-	0.3±0.0^{a}	-2.79±0.14^{a}	-0.019±0.002^{a}	30.2±1.2^{a}
	6 months	control	2.3±0.2^{b}	-0.20±0.01^{cd}	-0.013±0.001^{c}	37.2±1.0^{b}
		vacuum	3.4±0.1^{c}	-0.32±0.01^{bc}	-0.017±0.004^{ab}	34.7±1.2^{b}
		N_2	4.4±0.3^{de}	-0.28±0.01^{bcd}	-0.014±0.002^{bc}	35.1±0.3^{b}
		CO_2	4.5±0.3^{ef}	-0.34±0.02^{b}	-0.014±0.000^{bc}	35.6±0.4^{b}
	12 months	control	2.8±0.2^{bc}	-0.15±0.00^{d}	-0.012±0.000^{c}	53.0±0.9^{d}
		vacuum	3.5±0.0^{cd}	-0.18±0.01^{cd}	-0.014±0.000^{abc}	47.2±1.0^{c}
		N_2	5.0±0.7^{ef}	-0.20±0.01^{cd}	-0.014±0.001^{abc}	48.8±0.6^{c}
		CO_2	5.3±0.3^{f}	-0.27±0.01^{bcd}	-0.013±0.000^{bc}	49.0±1.1^{c}
	F value		78.2	950.4	5.6	254.1
TDK11	Fresh	-	1.6±0.1^{a}	-0.33±0.02^{a}	-0.019±0.001^{a}	25.0±0.3^{a}
	6 months	control	1.6±0.0^{a}	-0.15±0.01^{cd}	-0.015±0.001^{cd}	28.1±0.6^{cd}
		vacuum	4.3±0.2^{e}	-0.33±0.03^{a}	-0.017±0.001^{a}	25.5±0.6^{a}
		N_2	3.6±0.1^{d}	-0.30±0.02^{a}	-0.017±0.001^{a}	26.8±0.3^{b}
		CO_2	3.4±0.0^{d}	-0.21±0.03^{b}	-0.016±0.001^{bc}	25.0±0.4^{a}
	12 months	control	2.7±0.1^{b}	-0.10±0.02^{e}	-0.012±0.000^{f}	32.6±0.6^{e}
		vacuum	6.1±0.1^{f}	-0.13±0.01^{de}	-0.015±0.000^{de}	28.4±0.3^{cd}
		N_2	3.6±0.0^{d}	-0.19±0.02^{bc}	-0.013±0.000^{e}	29.2±0.6^{d}
		CO_2	3.1±0.0^{c}	-0.20±0.01^{bc}	-0.013±0.000^{de}	27.4±0.3^{bc}
	F value		609.8	66.5	57.5	81.3
DG	Fresh	-	6.2±0.2^{a}	-0.14±0.02^{a}	-0.029±0.002^{a}	19.8±0.5^{a}
	6 months	control	6.3±0.0^{a}	-0.03±0.01^{efg}	-0.017±0.000^{de}	24.7±0.4^{c}
		vacuum	8.5±0.0^{cd}	-0.10±0.01^{b}	-0.022±0.001^{b}	21.8±0.6^{b}
		N_2	8.4±0.1^{c}	-0.08±0.01^{bc}	-0.019±0.001^{c}	22.6±0.6^{b}
		CO_2	8.7±0.1^{d}	-0.07±0.01^{cd}	-0.019±0.000^{cd}	22.6±0.4^{b}
	12 months	control	7.5±0.1^{b}	-0.01±0.01^{g}	-0.013±0.001^{g}	29.7±0.5^{d}
		vacuum	8.7±0.1^{d}	-0.02±0.01^{fg}	-0.015±0.001^{ef}	24.8±0.7^{c}
		N_2	9.5±0.3^{e}	-0.05±0.01^{def}	-0.015±0.000^{efg}	26.0±0.3^{c}
		CO_2	10.0±0.1^{f}	-0.05±0.01^{cde}	-0.015±0.001^{fg}	25.7±0.6^{c}
	F value		351.6	57.5	126.4	95.4

*Means ± SD (n = 3, df = 8). For a particular variety, means with different letters in the same column denote significant difference at 5 % probability level within each rice variety.

9.4.1.7. *In situ* TMCT cooking analysis of fresh and aged rice kernels

The rate of cooking (rate of softening of the grain during cooking) during first 20 min and complete cooking time of fresh and aged (6 and 12 months under various MAP conditions) rice grains using the *in situ* TMCT cooking method are shown in Table 9.5. The cooking process initiates from the starch gelatinization of the outer layers of the grain and proceeds to the inner layers of the endosperm. Moreover, this process is time-dependent (Nawaz et al. 2017). The *in situ* TMCT records this change by measuring the softness of the grain (indicated by probe movement) with the time. In general, the initial 20 min of the *in situ* TMCT cooking curves were linear in all rice samples used in the study, as shown in Appendix 11. Therefore, only the initial 20 min of cooking were used to establish the rate of cooking. Results showed that aging of rice grains significantly ($P<0.05$) reduced the rate of cooking and significantly ($P<0.05$) increased the cooking time in control storage conditions. Moreover, a similar trend was observed during the cooking of rice grains stored in MAP conditions. Findings are also well correlated with pasting temperature (P_{temp}) as shown in Table 9.3.

9.4.2. Discussion

In the current study, we have analyzed the effect of three different modified atmospheric packaging viz. vacuum, N_2 and CO_2 on the aging of selected glutinous (TDK8 and TDK11) and non-glutinous (DG) milled rice grains. Milled rice samples were packed in aluminum bags instead of plastic bags as aluminum is more impermeable to gas and water vapor than plastic (Marsh & Bugusu 2007). As aluminum bags are not cost effective, therefore, future studies on the MAP will focus on other cost-effective packaging materials.

The current findings for the microstructures of fresh grains are in agreement with previous studies that have reported larger starch granules in non-waxy rice cultivars than in waxy rice cultivars (Wani et al. 2012; Vandeputte & Delcour 2004). In the control sample, results showed aging-induced structural changes in the starch granules mainly depicted by the loss of polyhedral shape. This might be due to macromolecules (starch, proteins, and lipids) interactions, oxidation reactions and enzymatic activity (Sharp & Timme 1986). However, this structural change was seen less prominent in a vacuum and/or modified atmospheric packaged samples using an increased percentage of carbon dioxide or nitrogen. These findings revealed that the MAP conditions

(vacuum, CO_2, and N_2) might slow down the macromolecular interactions, oxidation reactions, and enzymatic activities and maintain the polyhedral shape (Wu et al. 2016).

The surface analysis of freshly milled grains showed that a nano-metric scale layer of bran oil from bran might remain on the surface of grain even after 9 % degree of milling of the grain. Similar observations were also reported in the past researches (Saad et al. 2011; Nawaz et al. 2016b). The control storage induced higher concentration of surface proteins and reduced surface lipids in all selected rice varieties. This shows some loss of lipids from the grain surface. This may be due to the oxidation of lipid or re-adsorption of lipid and oxidized lipid products to the interior of the grain as the liquid state bran oil is the main lipid of rice. Another possibility is the formation of the starch-lipid complex (Villwock et al. 1999). Ohno and Ohisa (2005) reported the outer layer of rice proteins oxidized more than inner layer during aging, resulting in a bigger size of surface protein bodies. This might have also contributed to the increased level of protein on the surface. However, N_2 and CO_2 MAP of TDK8 and DG slightly slowed down the shift of macromolecules and maintained the surface starch/proteins/lipids ratios during 6 months of storage. Detection of Mn on the surface of TDK11 might be due to accumulation in the endosperm (Sperotto et al. 2013). Moreover, the traces of B and Si in DG may be due to the bran particles intactness to the surface of endosperm (Uraguchi & Fujiwara 2011; Nawaz et al. 2016b).

From our previous research, the C/O stoichiometry values for pure rice starch, proteins and lipids were found to be 1.47, 4.76 and 11.11, respectively and the C/N stoichiometric values for the same were found to be 94.39, 12.50 and 0, respectively (Nawaz et al. 2016b). These results reflected that the surfaces of fresh cooked TDK8 and TDK11 were mainly covered by gelatinized amylopectin (branched starch) with high levels of proteins, resulting in surface adhesiveness. The surface analysis of cooked aged glutinous rice (TDK8 and TDK11) under various storage conditions showed slight decrease in C/O stoichiometric values (except TDK11 during first 6 months in vacuum, N_2 and CO_2) and slight increase in C/N stoichiometric values suggesting increase in starch and proteins and decrease in lipid exposure during cooking.

Lipids and proteins are responsible for the hardness and cohesiveness of cooked non-glutinous rice kernels (Choi et al. 2015). Interestingly, the modified storage conditions and time of storage does not affect C/O and C/N stoichiometry of DG suggesting least interaction of macro-molecules in non-glutinous rice during aging making them more resistant to changes in cooking quality during

storage as compared to glutinous rice. The rice grains in vacuum conditions slightly maintained the surface lipids with fewer proteins exposed to the surface, as more O-C=O, C-COOH, and C-O sub-peaks and less C-N sub-peak were deconvoluted. However, rice grains stored in N_2 and CO_2 showed more polysaccharides side-chains (starch) on the surface. In addition to this, rice grains in control storage showed a reduction in surface lipids possibly due to more oxidation or re-adsorption of lipids by the grains as discussed earlier.

X-ray diffraction technique is mostly used to study the change in the percentage crystallinity of starch carried out by various physical treatments such as milling and storage (Ye et al. 2016). The crystallinity depends upon the ratio of amylose and amylopectin. Higher the ratio of amylopectin higher will be the crystallinity (Park et al. 2007). Interestingly, the calculated crystallinity of the rice varieties used in this work was much higher than the reported crystallinity of similar glutinous and non-glutinous varieties in previous studies.

XRD results showed that there was no V-type crystalline peak found at 20^o in the aged glutinous and non-glutinous rice stored in various MAP conditions, showing undetectable starch-lipid complex formation. Interestingly, XRD findings are not in agreement with the XPS results where we assumed that one of the causes of reduction in surface lipids was possibly due to starch-lipid interaction. These findings may also suggest that the starch-lipid interaction may have occurred only on the exposed surface of the grain that is undetectable during XRD analysis of flour.

In rapid visco analysis, P_{temp} is the indication of water binding and initiation of gelatinization. The influence of modified atmosphere on the restricted water binding capacity of starches have been reported in the past (Cofie-Agblor et al. 1998). Higher concentrations of atmospheric CO_2 and N_2 might block the water binding sites, resulting in reduced hydrogen bonding between amylopectin branches (Noomhorm et al. 2009). This may lead to less water absorption/binding and high P_{temp}. Moreover, increased concentration of atmospheric N_2 and CO_2 may interact with water binding sites of amylopectin and restrict the gelatinization, resulting in more energy requirement to gelatinise the starch. This might be due to retarded enzymatic activity and starch/proteins/lipids interactions. The cooking process initiates from the starch gelatinization of the outer layers of the grain and proceeds to the inner layers of the endosperm. Moreover, this process is time-dependent (Nawaz et al. 2017). The *in situ* TMCT recorded this change by measuring the softness of the grain (indicated by probe movement) with the time. The atmospheric CO_2 and N_2 might retard the

recrystallisation of gelatinised starch during cooling, resulting in increased adhesiveness of cooked rice.

9.5. Conclusions

Aging-induced physicochemical changes can affect the cooking quality and textural attributes of the glutinous rice. Stickiness or adhesiveness of the cooked rice is one of the textural parameters most sensitive towards aging of rice. The findings of the present study revealed that aging-induced physicochemical changes of milled glutinous and non-glutinous rice could be slightly slowed down using the modified atmospheric packaging (MAP). Vacuum and/or higher concentration of atmospheric CO_2 and N_2 during storage can slow down the physicochemical deterioration. Overall, in relative term vacuum packaging was found more effective than any other storage conditions investigated in this work.

Chapter 10 General conclusions and recommendations for future research

10.1. General conclusions

Glutinous varieties are grown in many countries, including Lao PDR, Thailand, China, Myanmar, Vietnam, Cambodia, Japan, Bangladesh, and India. It is a staple food of Laotian people. It is usually consumed as a desert, as a breakfast cereal or as steamed rice in banana leaves in Thailand, Myanmar, Cambodia, India, China, and Vietnam. Glutinous rice contains amylopectin with a very low level (<5 %) of amylose, which contributes to a typical stickiness of cooked rice. Good quality fresh or aged glutinous rice should be reasonably sticky in texture after cooking. This research mainly focused on the underline causes of loss of stickiness of glutinous rice during storage and different processing interventions to maintain its quality. This study covered a number of aspects of glutinous rice; (i) Characterization of rehydration and gelatinization behavior of glutinous rice; (ii) Development of an *in situ* method to study the cooking kinetics; (iii) Effect of modified atmospheric storage on the stickiness attribute of glutinous rice; (iv) Characterization and modification of surface composition of raw and cooked rice grains and its correlation to stickiness property of cooked rice; (v) Pre-treatments including parboiling of glutinous rice and their effect on stickiness property. The major outcomes of this project are described below.

i. Rehydration and gelatinization behavior of rice flour is usually used as an indicator of cooking properties of rice grains. The glutinous rice flour of selected Laotian varieties viz. TDK8, TDK11, and Hom Mali Niaw were subjected to different rehydration time and temperatures and cooking times at 95^{o}C. Water uptake by the flour was directly proportional to the time/temperatures of rehydration. Starch granules showed a higher breakdown in response to extended cooking, resulting in reduced trough viscosity and reduced retrogradation. It is therefore recommended that for glutinous rice especially TDK8, extended cooking will result in a better-cooked product. This work also generated the pasting data for the most popular glutinous varieties consumed in Lao PDR.

ii. Type of rice (glutinous or non-glutinous) and aging temperature can affect the rate of water uptake and cooking properties. The physicochemical properties of rice flour are different from whole grain. Therefore, water uptake and cooking behavior of Laotian glutinous rice varieties (fresh and aged TDK8 and TDK11) was analyzed by a novel *in situ* method using Thermal Mechanical Compression Test (TMCT) attached to a texture analyzer. The *in situ* TMCT cooking method provided information on the softening of the grain due to water

uptake during soaking at various rehydration temperatures. The slope of *in situ* TMCT cooking curve can be used to estimate the rate of cooking.

iii. The textural attributes especially the stickiness of cooked rice are perceived by the surface of the grain. Therefore, it is important to study the surface of the composition. A new method was developed to quantify the surface composition of raw and cooked rice grains by using X-ray photoelectron spectroscopy (XPS). The detailed elemental composition of the upper 5-10 nm layer of rice grains and flour through XPS was used to calculate the percentage of major components on the grain surface. We found a higher percentage of proteins and lipids on the surface of grains and flour as compared to their respective percentages in bulk composition. These results for XPS analysis correlated well with CLSM microstructure analysis verifying the accuracy of the new technique.

Surface analysis showed that higher contents of protein present on the surface of milled grains might also contribute to the reduced stickiness of cooked rice. Proteins in rice are mostly glutelin which is soluble in alkali. Therefore, glutinous rice grains were washed with different concentrations of NaOH (0 to 0.2 %). We found that surface proteins of rice grains were removed with as low as 0.004 % NaOH. Protein removal by alkali washing possibly restricted the realignment of starch during retrogradation, resulting in maintaining stickiness and freshness of cooked glutinous rice. These results suggested that varying the proportion of surface proteins of the rice grain through alkali washing can be used to improve the textural attributes and sensory properties of glutinous rice.

iv. Starch modification by various pretreatments can improve the glossiness, stickiness, and softness of gelatinized starches. The intact starch of whole rice grains was esterified by using dilute acetic anhydride. Acetylation of starch in the glutinous rice grains affected the crystalline structure of starch granules which was reflected by the reduced peak and final viscosities during rapid visco analysis and reduced thermal transition temperatures and enthalpy during thermal analysis. Moreover, the texture of cooked acetylated glutinous rice grains was softer and more adhesive. *In vitro* digestion of acetylated glutinous rice showed reduced glycemic index possibly due to the increased amount of resistant starch. These results suggested that varying the acetylation of starch in the glutinous rice grain can be used to achieve the desirable textural property of intact rice grains.

v. Thermal treatment such as wet parboiling of fresh glutinous paddy may help in maintaining the stickiness and overall quality of glutinous rice. Parboiling of glutinous rice with various soaking mediums water, 3 % NaCl saline solution and 0.2 % acetic acid solution showed that saline and acetic acid soakings improved the milling efficiency. Saline and acetic acid soaking also resulted in reduced crystallinity and thermal endotherms. The amber discoloration from the husk during steaming was minimized by the bleaching effect of acetic acid used during soaking. The acetic acid soaking restricted swelling, resulting in a reduced peak (~10 % decrease) and final viscosity (~15 % decrease), whereas, the saline soaking improved water absorption, resulting in a higher peak (~10 % increase) and final viscosity (~5 % increase). Furthermore, parboiling increased hardness (2.6 to 5 N for TDK8 and 2.3 to 3.5 N for TDK11) and adhesiveness (-0.2 to -0.5 N.s for TDK8 and -0.5 to -0.7 N.s for TDK11) of glutinous rice in saline and acetic acid soaking as compared to water only soaking (-0.3 to -0.5 N.s for TDK8 and -0.6 to -0.7 N.s for TDK11). Also, the GI value of parboiled rice was reduced from 116.5 to 100.4 for TDK8 and 94.8 to 72.2 for TDK11 showing some improvements in the nutritional quality. Overall, parboiling of glutinous showed improvement in the grain quality which can have commercial potential.

vi. Cooking quality and textural attributes especially stickiness of the glutinous rice is affected by aging-induced physiochemical changes. We found that the higher concentration of atmospheric gases (CO_2 or N_2) and/or vacuum during storage can slow down aging-induced changes in milled glutinous rice and can maintain the stickiness attributes of cooked aged glutinous rice. Overall, among all the storage conditions used, the vacuum was considered the best to maintain the quality of the glutinous rice.

10.2. Future direction

i. The *in situ* TMCT cooking method was found useful to calculate the cooking kinetics of a range of samples. However, it is recommended that further work should be done to relate the *in situ* TMCT cooking data with the sensory evaluation of rice.

ii. The pre-processing treatment with alkali may induce the structural changes in amylopectin, possibly due to alkali-induced depolymerization. Therefore, the impact of alkali washing on starch molecular-structure should be studied by using advanced techniques such as Size Exclusion Chromatography.

iii. It was challenging to estimate the extent of acetylation in the whole rice grains due to technical difficulties. Soaking and subsequent drying resulted in increased internal fissure making it impossible to separate outer layers without damaging the grain core during milling. Therefore, further studies should be conducted to study the acetylation gradient across the grain.

iv. In the present study, only the fresh samples of glutinous rice were analyzed for pre-processing treatments of alkali washing, starch esterification of whole grains and parboiling. It would be interesting to see the effect of aging on the pre-processed glutinous rice.

v. In the present study, the textural attributes were assessed using a texture analyzer, and no sensory evaluation was conducted due to unavailability of the trained sensory panel. Therefore, it is recommended that all the pre-processing treatments and modified storage techniques reported in this study should be validated with the trained sensory panel.

vi. The glycemic index of pre-processed glutinous rice by using starch modification and parboiling was predicted using *in vitro* digestion method. Therefore, it is recommended that the digestibility of pre-processed glutinous rice may be tested *in vivo* using an animal model.

vii. The use of digital rice cookers with sticky rice program, pressure cooking and microwave cooking are gaining popularity around the globe. Use of these modern techniques may overcome the overnight soaking of aged glutinous rice before cooking. Therefore, it is important to study the dynamics of rice cooking under these modern cooking conditions.

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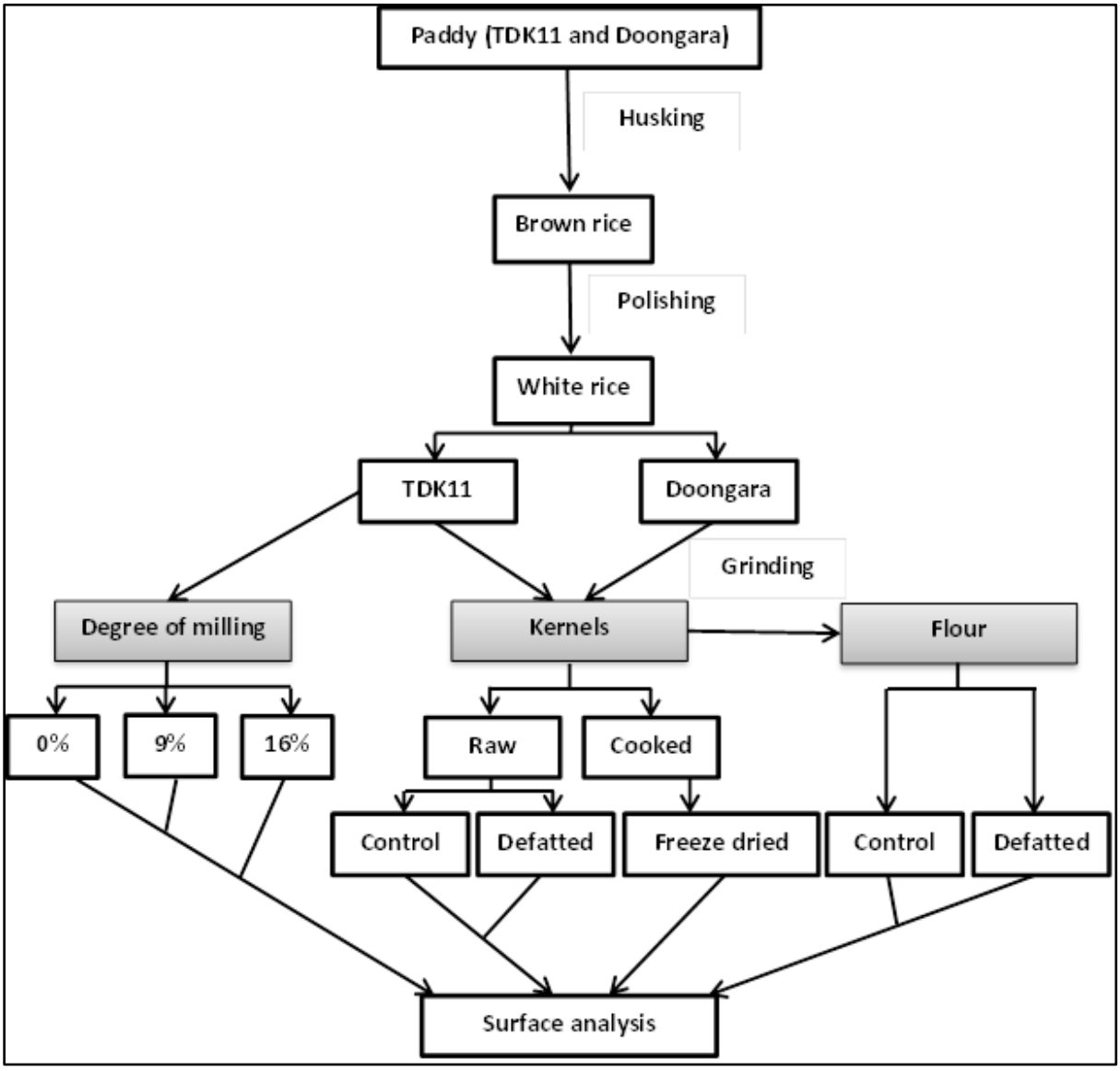
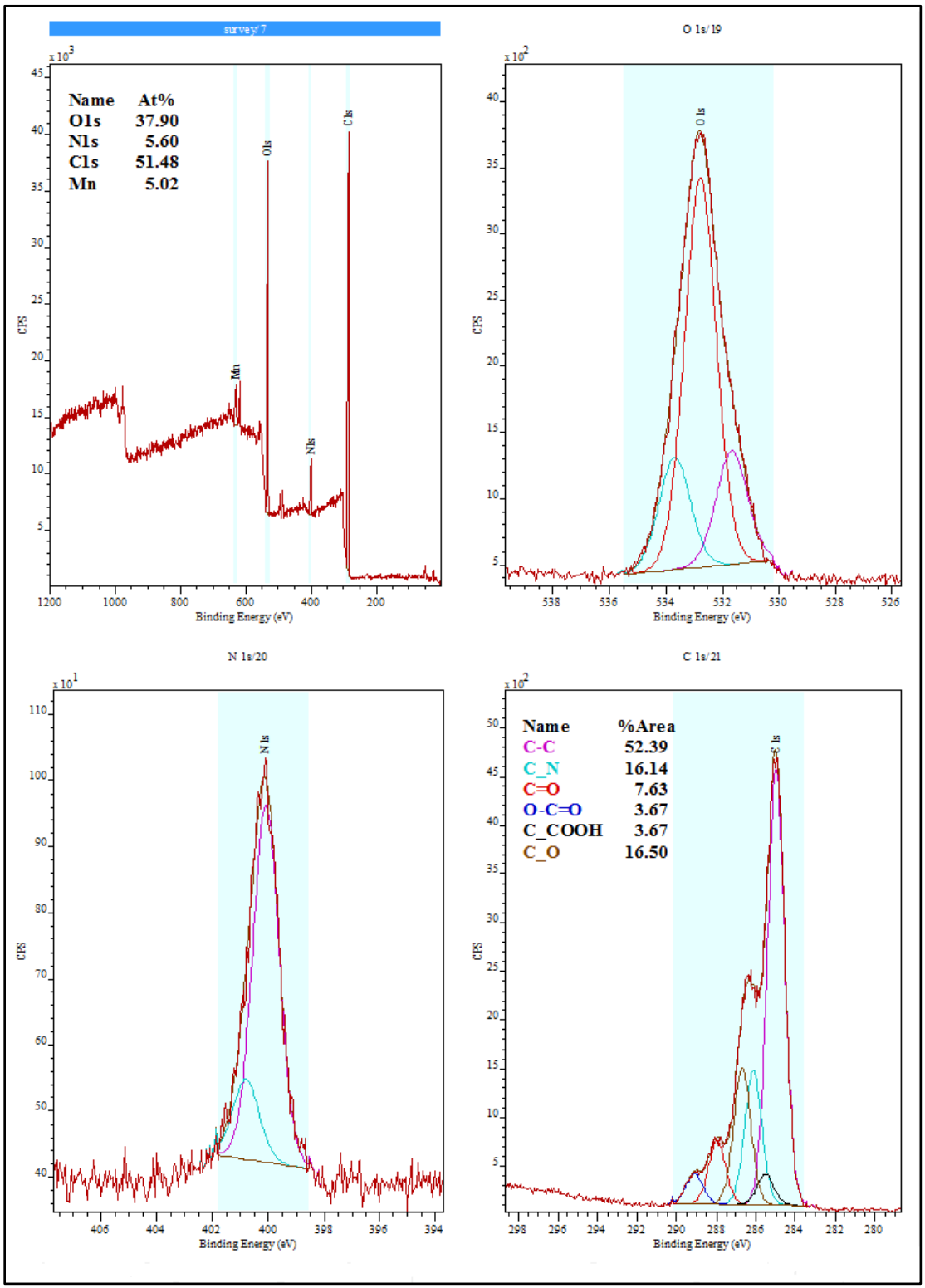
Appendices

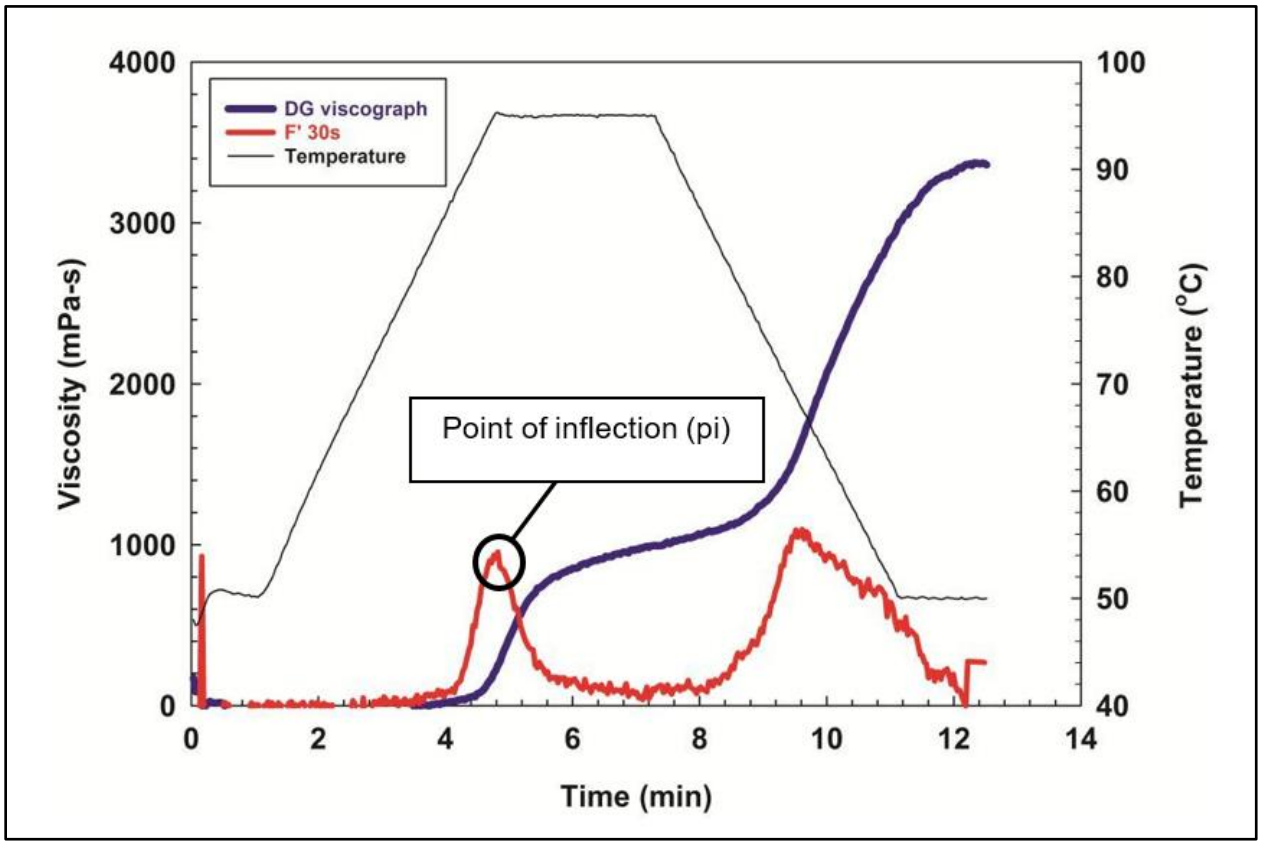
Appendix 1: The flow chart of experimental design for Chapter 5.



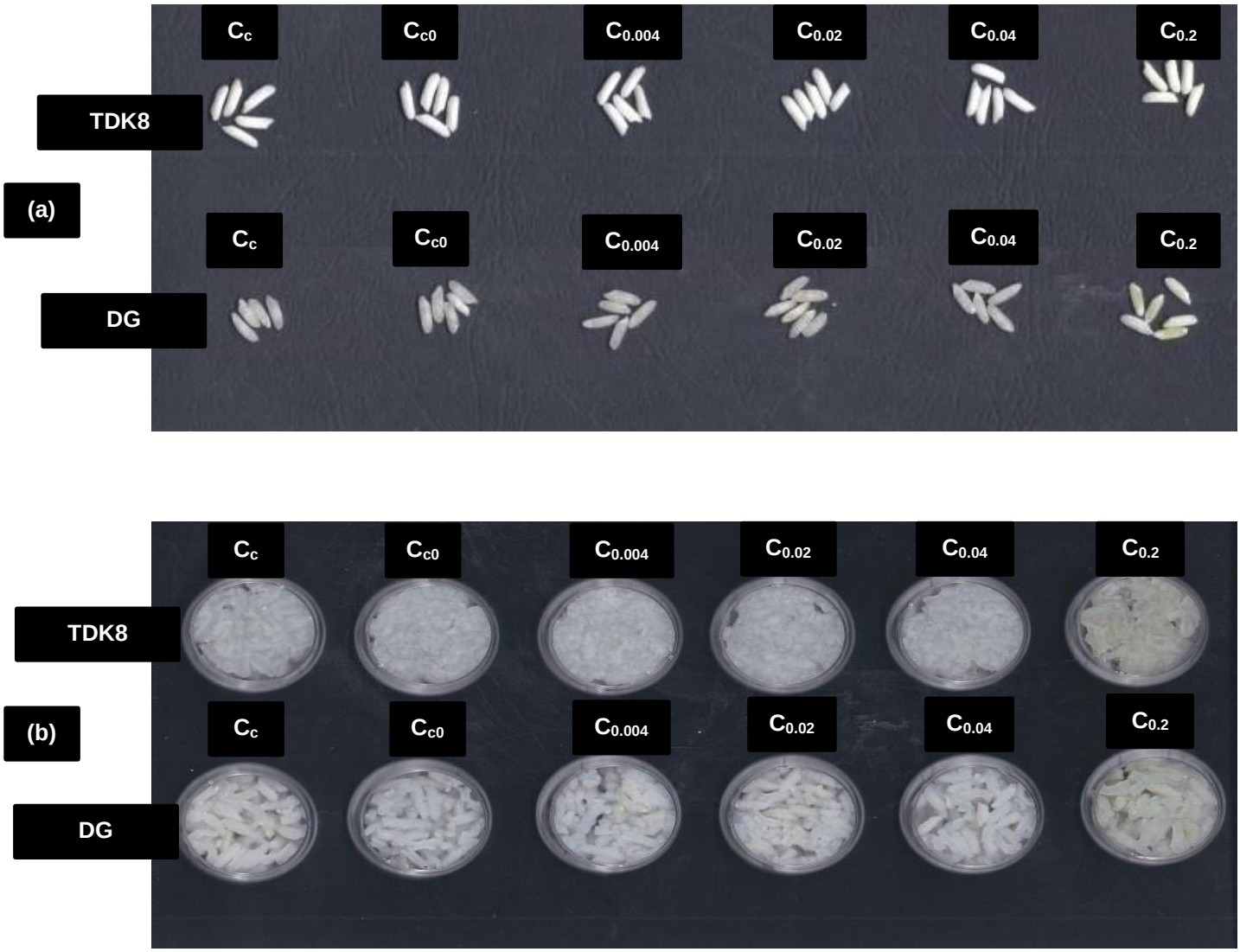
Appendix 2: Typical XPS survey and high resolution spectra of rice sample with decomposition of C_{1s} peak into distinct sub-peaks.



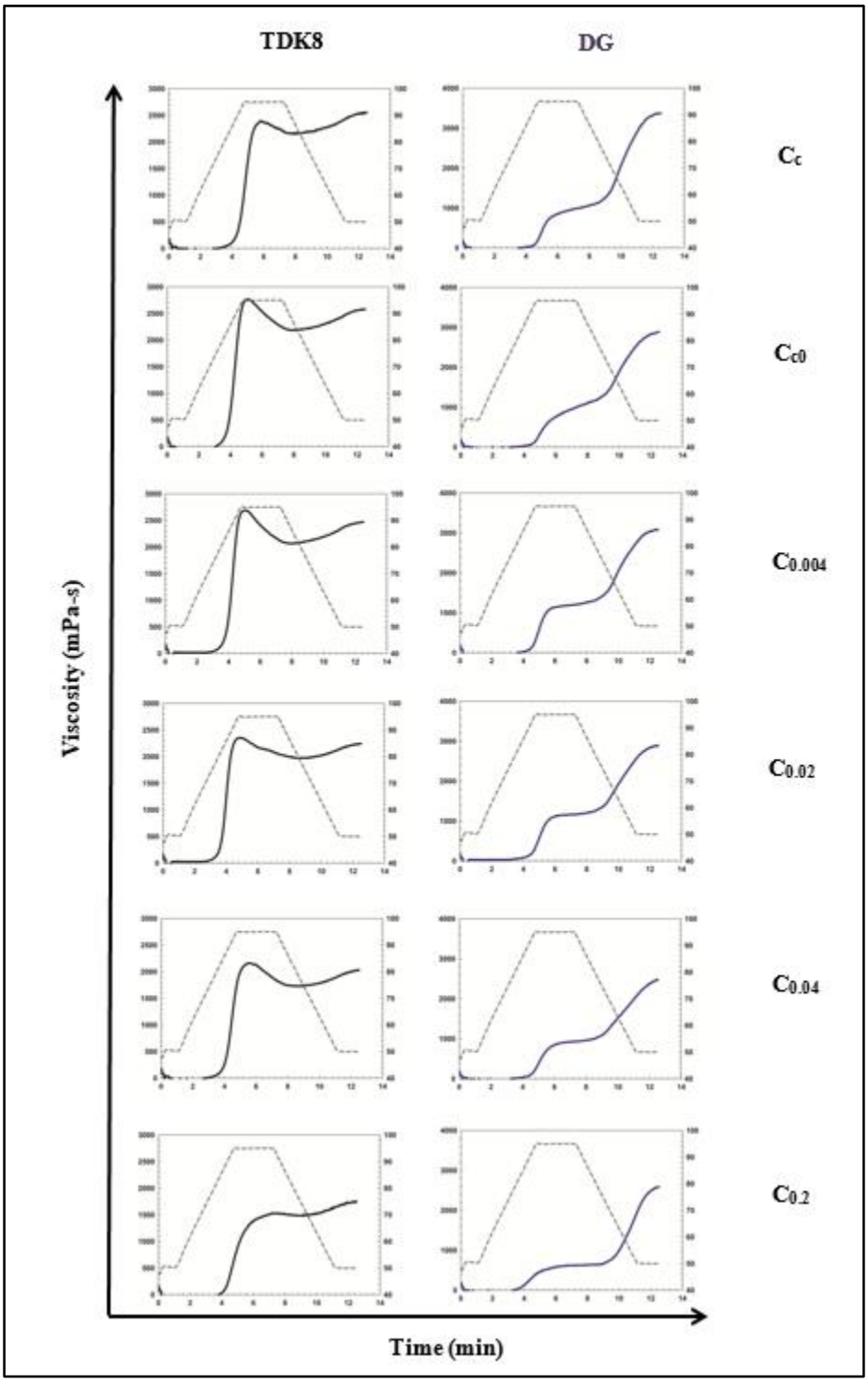
Appendix 3: Point of inflection (pi) calculation by using first derivative for every 30 sec (F´ 30 sec).



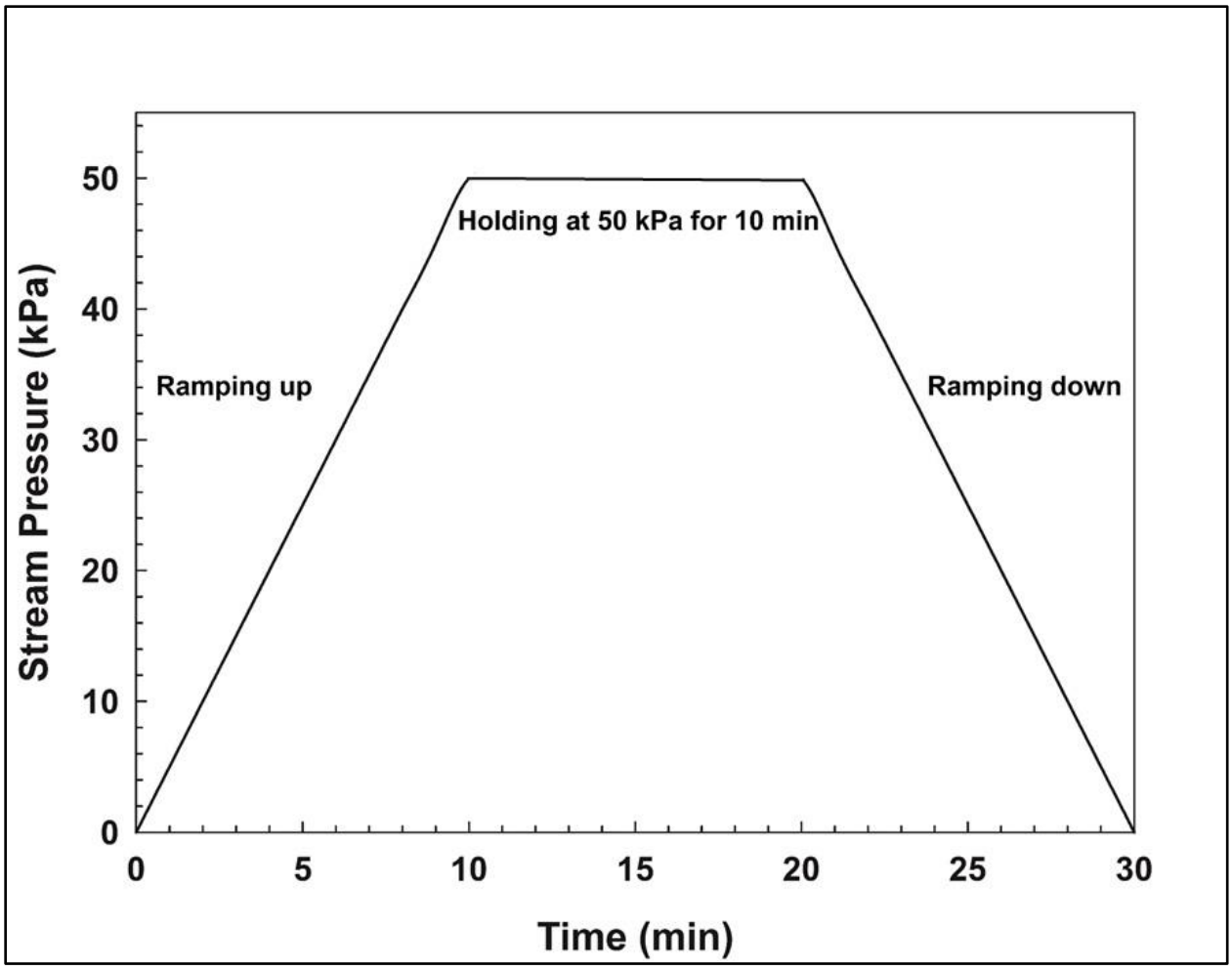
Appendix 4: Scanned images of control and alkali treated Thadokkham-8 (TDK8) and Doongara (DG); (a) uncooked rice grains, and (b) cooked rice grains.



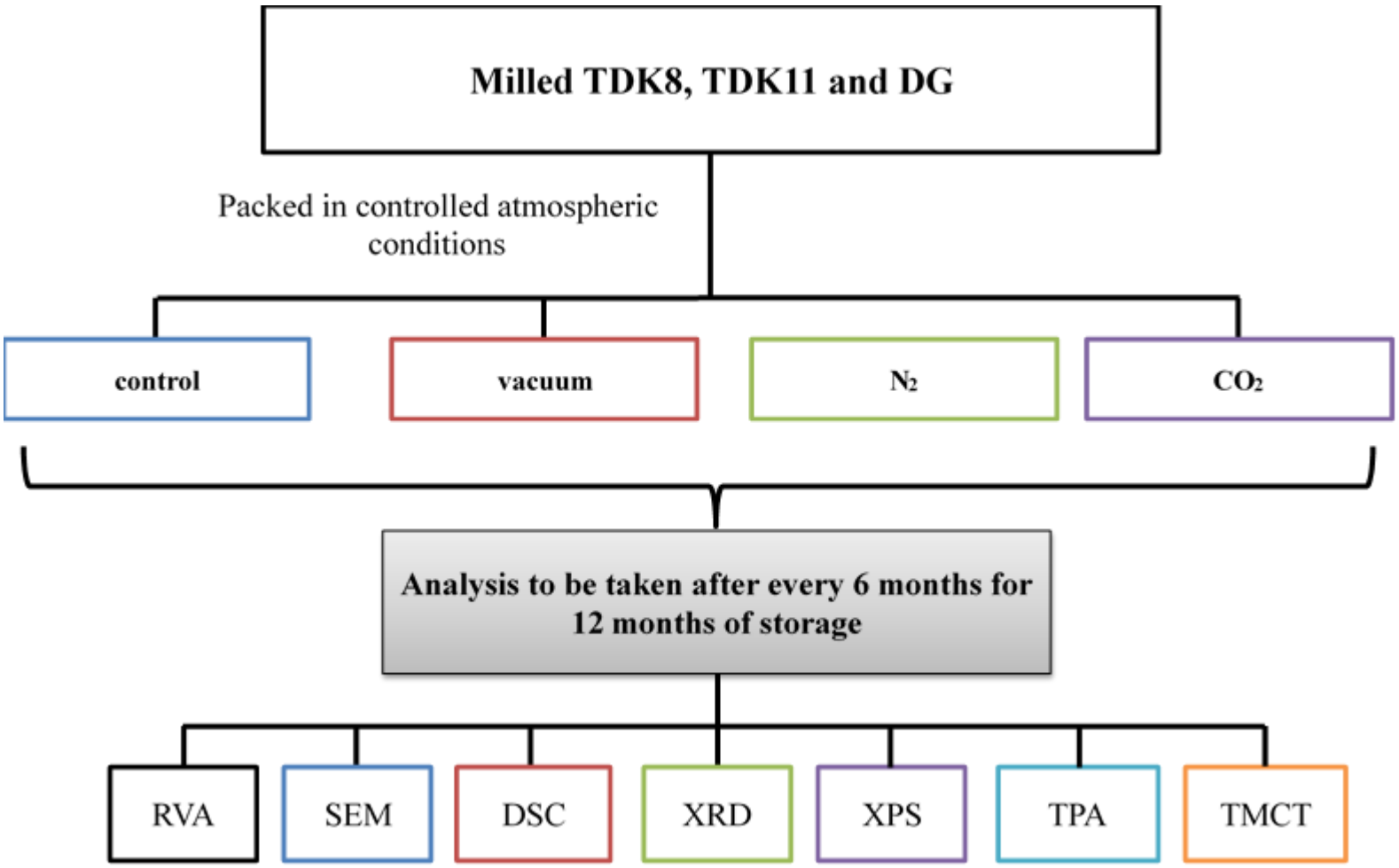
Appendix 5: RVA viscographs of control and alkali treated flour of Thadokkham-8 (TDK8) and Doongara (DG).



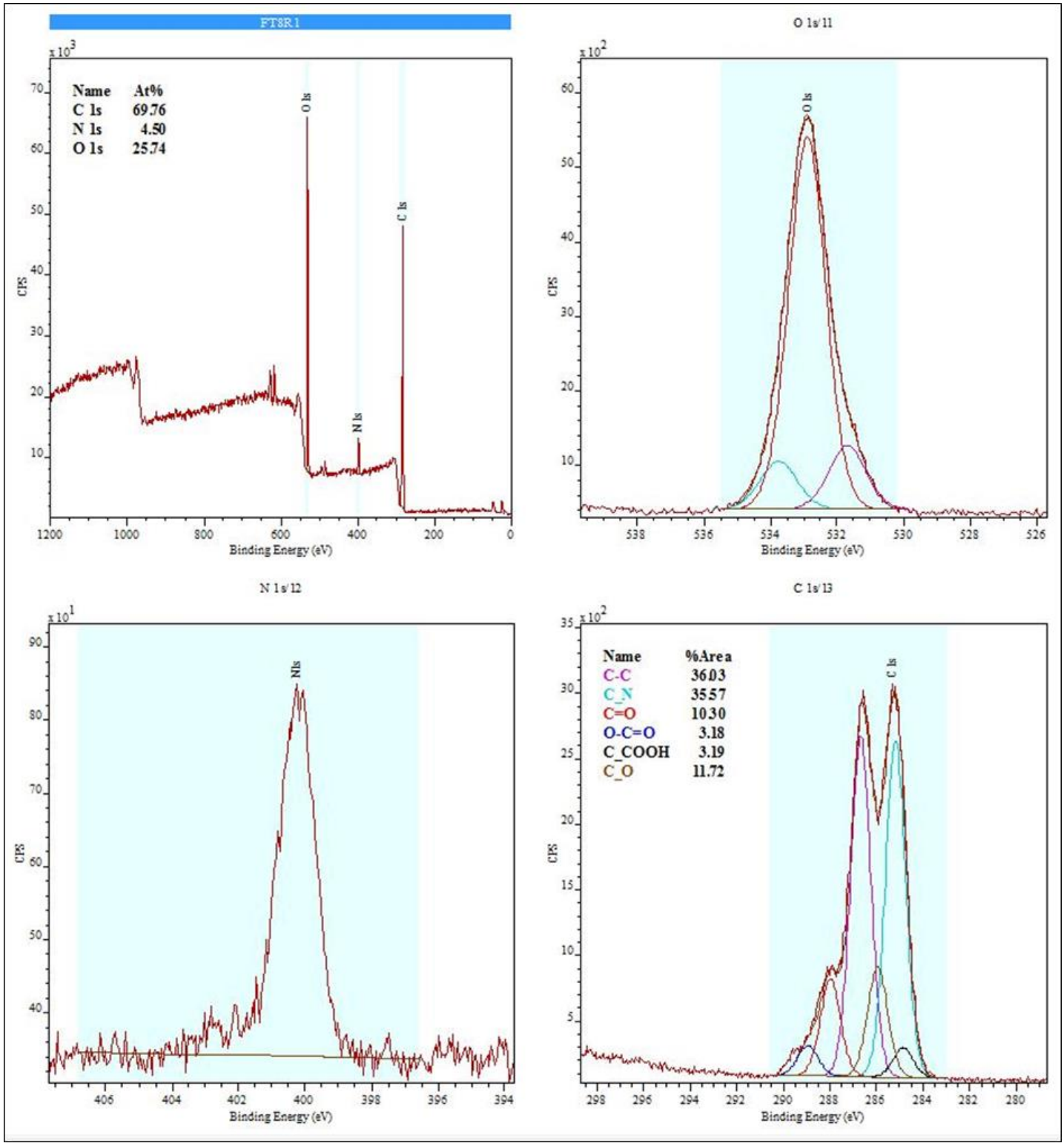
Appendix 6: Cooking curve during parboiling.



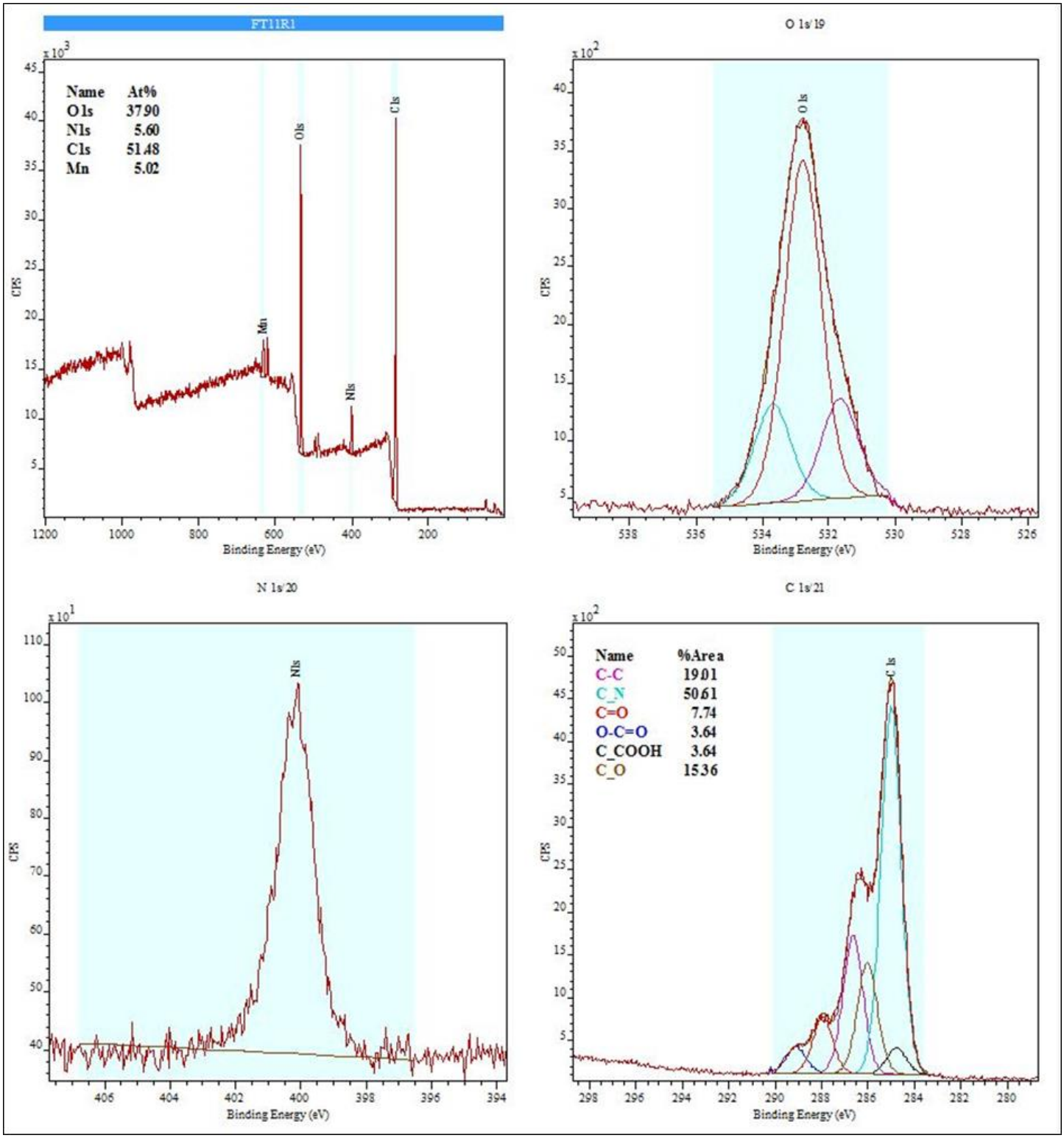
Appendix 7: Experimental design for Chapter 9.



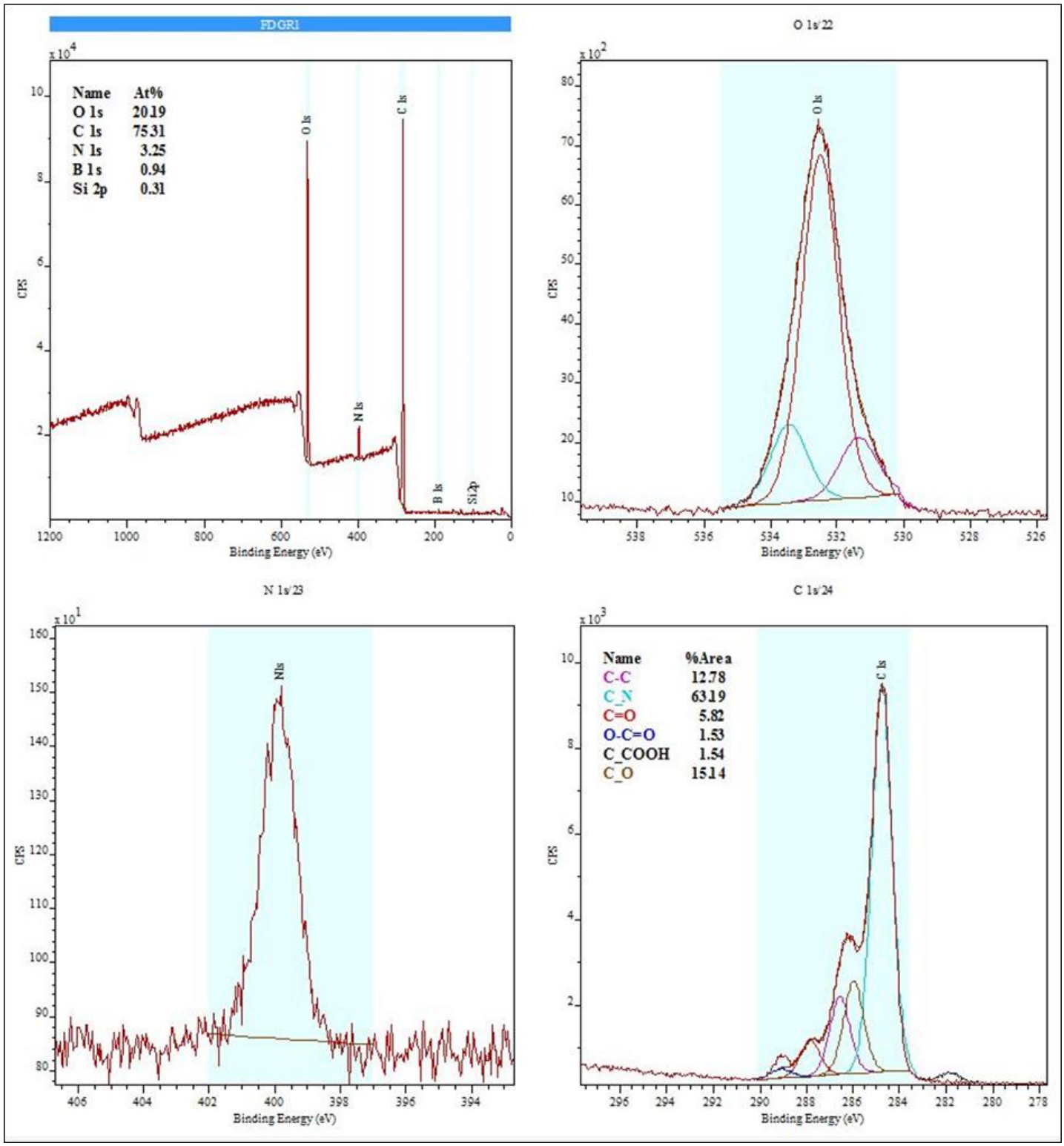
Appendix 8: XPS survey and high resolution spectra of fresh cooked Thadokkham-8 (TDK8).



Appendix 9: XPS survey and high resolution spectra of fresh cooked Thadokkham-11 (TDK11).



Appendix 10: XPS survey and high resolution spectra of fresh cooked Doongara (DG).



Appendix 11: *In situ* TMCT cooking curves of fresh and aged (six and twelve months under various MAP conditions) Thadokkham-8 (TDK8), Thadokkham-11 (TDK11), and Doongara (DG).