OPTIMIZATION OF FORTIFIED PARBOILING PROCESS OF WHITE RICE WITH *Clitoria ternatea* FLOWER EXTRACT AND ITS CHARACTERIZATION OF PHYSICOCHEMICAL, COOKING PROPERTIES AND STORAGE STABILITY

MUHAMMAD EZZUDIN BIN RAMLI

UNIVERSITI SAINS MALAYSIA

2019

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by

MUHAMMAD EZZUDIN BIN RAMLI

Thesis submitted in fulfillment of the requirements for the degree of Master of Science

May 2019

ACKNOWLEDGEMENT

In the name of Allah, The Most Gracious, The Most Merciful, Shalawat and Salam for the Holy Prophet Muhammad P.B.U.H. and to all His ahlul bayt and to His companion, not to forget, to all Muslim too.

Firstly, I would love to express my utmost gratitude to my supervisor Dr. Rabeta Binti Mohd Salleh for trusting and giving me this golden opportunity to learn under her supervison. I am also grateful for her patience, guidance and commitment in seeing this research is finally submitted. In addition, the financial support of this work by the Ministry of Higher Education through Fundamental Research Grant Scheme (FRGS) with grant number 203/PTEKIND/6711526 is gratefully acknowledged. I am also greatly indebted to my co-supervisors Dr. Sapina Binti Abdullah @ Uthumporn Utra and Dr. Musfirah Binti Zulkurnain for devoting their precious time and efforts in completing this research. Their guidance and generosity in providing helps and knowledge throughout this journey has amazingly inspired me.

I particularly want to thank to my best friends Qasimie, Rohini, Raiya, Pavel, Najihah, Leila, Radzi, Rumaisa, Syuzeliana, Hui Yin, Raihan, Hafiz and Syazana who helped me tremendously during my experimental work. I am very lucky having them as my lab mates who always encourage and support me to have passion in this master project. Their advices were such a valuable thing that I got as a friend. Besides that, thanks to the entire Food Technology Division, USM especially lab assistant En. Maarof, En. Ghoni, En. Rahim, En. Firdaus, Pn. Mazura and Pn. Noraini for helping me throughout this journey.

Further, I would like to express my special thanks to my parents, Ramli Bin Bakar and Raha Binti Ramin and not to forget my family members, Nor Edayu Binti Ramli, Nor Elinatasya Binti Ramli and also Rayyan Adam Mikail Binti Mohd Hafifi for their trust, love, prayer, support and financial aid. I also place on record, my sense of gratitude to one and all who directly or indirectly, have lent their helping hand throughout this journey. Thank you very much.

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LIST OF ABBREVIATIONS

CL	Chain Length
CT	*Clitoria ternatea*
DMSO	Dimethyl Sulfoxide
DOA	Department of Agriculture
DP	Degree of polymerization
DPPH	1,1-diphenyl-2-picrylhydrazyl
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	Food and Agriculture Organization of the United Nations Statistical Databases
FC	Folin-Ciocalteu
FRAP	Ferric Reducing Antioxidant Power
GR	Glutinous Rice
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
TPTZ	2,4,6-Tri(2-pyridyl)-s-triazine
USDA	United States Department of Agriculture

LIST OF SYMBOLS

α	Alpha
-b*	Yellowness
°C	Degree Celsius
-a*	Greenness
L*	Lightness
µg/g	Microgram per gram
mg/g	Miligram per gram
%	Percentage
kg	Kilogram

PENGOPTIMUMAN PROSES FORTIFIKASI SEPARUH MASAK BERAS PUTIH DENGAN EKSTRAK BUNGA *Clitoria ternatea* DAN PENCIRIAN FIZIKOKIMIA, SIFAT MEMASAK DAN KESTABILAN PENYIMPANANNYA

ABSTRAK

Ekstrak bunga *Clitoria ternatea* biasanya digunakan untuk membuat nasi berwarna biru yang popular dalam makanan masyarakat Melayu iaitu 'Nasi Kerabu'. Proses fortifikasi separuh masak telah dihasilkan. Fortifikasi flavonoid dan pewarnaan biru pada beras putih telah dihasilkan dengan proses separuh masak menggunakan ekstrak bunga *Clitoria ternatea*. Fizikokimia, sifat memasak dan stabiliti penyimpanan beras yang difortifikasi dikaji dan dibandingkan dengan beras mentah dan beras putih yang diseparuh masak sebagai kawalan. Dalam fasa 1, kaedah fortifikasi diperkenalkan ketika perendaman beras putih dengan ekstrak bunga telang (1:100 berat: isipadu) untuk menggantikan air sebelum dikukus dan dikeringkan. Proses fortifikasi separuh masak melibatkan tiga jenis varieti beras yang berbeza iaitu MRQ 74, MRQ 76 dan beras pulut, semua beras ini dioptimumkan menggunakan reka bentuk faktorial penuh dengan dua proses parameter iaitu suhu rendaman (50, 60, and 70 °C) dan masa rendaman (10, 20, and 30 minit) untuk mendapatkan jumlah kandungan maksimum flavonoid (TFC) di dalam beras yang telah difortifikasi. Hasil kajian menunjukkan jumlah kandungan flavonoid meningkat apabila suhu dan tempoh masa rendaman bertambah untuk ke semua varieti beras. MRQ 74 menunjukkan model lurus dengan maksimum TFC, 3150.0 μg/g, manakala MRQ 76 dan beras pulut menunjukkan model kuadratik dengan maksimum TFC masing-masing ialah 4466.7 and 6111.1 μg/g pada keadaan optimum rendaman 70 °C dan 30 minit. Menariknya, maksimum TFC adalah berkolerasi songsang dengan kandungan amilosanya (Korelasi Pearson = -0.999). Dalam fasa 2, fizikokimia dan sifat memasak beras yang telah difortifikasi separuh masak dibandingkan dengan beras mentah dan beras separuh masak (dikawal). Proses separuh masak menurunkan secara signifikan (p<0.05) ketumpatan pukal dan 1000 berat bijian beras tanpa mengira proses fortifikasi untuk kesemua varieti. Tambahan pula, proses separuh masak juga secara signifikannya meningkatkan nisbah panjang-lebar dan kapasiti penyerapan air untuk kesemua beras yang berkolerasi terus dengan kandungan amilosa. Proses separuh masak dapat mengurangkan secara signifikan masa memasak tetapi meningkatkan kekerasannya. Dalam fasa 3, jumlah keseluruhan fenolik, jumlah keseluruhan flavonoid, aktiviti memerangkap DPPH dan aktiviti pengurangan ferik (APF) diukur selama 12 minggu waktu penyimpanan dalam keadaan bungkusan vakum. Walau bagaimanapun, jumlah kandungan fenolik, jumlah kandungan flavonoid dan aktiviti-aktiviti antioksida untuk kesemua varieti beras menurun secara signifikan (p<0.05) selepas 12 minggu penyimpanan. Kesimpulannya, fortifikasi flavonoid dan pewarnaan biru untuk beras putih boleh dicapai melalui proses fortifikasi separuh masak untuk meningkatkan kualiti memasak dan aktiviti antioksida tanpa berkompromi dengan kualiti fizikal bijian.

OPTIMIZATION OF FORTIFIED PARBOILING PROCESS OF WHITE RICE WITH *Clitoria ternatea* FLOWERS EXTRACT AND CHARACTERIZATION OF PHYSICOCHEMICAL, COOKING PROPERTIES AND STORAGE STABILITY

ABSTRACT

Clitoria ternatea flower extract is commonly used in making blue rice of popular Malay delicacy “Nasi Kerabu”. A fortified parboil process was developed. Flavonoid fortification and blue coloration of white rice was developed by parboiling process using the *Clitoria ternatea* (CT) flower extract. The physicochemical, cooking properties and storage stability of the fortified rice were studied and compared with raw and white parboiled rice as control. In Phase 1, the fortification method was introduced during soaking of white rice with CT extract (1:100 w/v) to replace water prior to steaming and drying. The fortified parboiling process of three different varieties of rice namely MRQ 74, MRQ 76 and glutinous rice was optimized using full factorial design with two processing parameters: soaking temperatures (50, 60, and 70 °C) and soaking time (10, 20, and 30 minutes) to obtain maximum total flavonoid content (TFC) of the fortified rice. The TFC increased with increase in soaking temperature and time for all varieties of rice. MRQ 74 showed linear model with maximum TFC of 3150.0 μg/g, while MRQ 76 and glutinous rice showed quadratic model with maximum TFC of 4466.7μg/g and 6111.1 μg/g, respectively at optimum soaking condition of 70 °C and 30 minutes. Interestingly, the maximum TFC was inversely correlated to their amylose content (Pearson correlation=-0.999). In Phase 2, the physicochemical and cooking properties of the fortified parboiled rice were compared with raw and parboiled rice (control). The parboiling process significantly decreased (p<0.05) bulk density and 1000 grain weight of the rice regardless of fortification for all rice varieties. Moreover, the parboiling treatment significantly increased the length-breadth ratio and water absorption capacity of all rice varieties which directly correlated to their amylose content. The parboiling treatment significantly reduced cooking but increased their hardness. Interestingly, the TFC increased significantly after cooking for all rice varieties. In Phase 3, the total phenolic contents, total flavonoid content, DPPH scavenging activity and ferric reducing activity (FRAP) were measured over 12 weeks of storage in vacuum packed condition. However, the total phenolic contents, total flavonoid contents and antioxidant activities of all rice varieties decreased significantly (p<0.05) after 12 weeks of storage. In conclusion, flavonoid fortification and blue coloration of white rice can be achieved via fortified parboiling process to increase antioxidant activities and cooking quality without compromising physical grain quality.

CHAPTER 1

INTRODUCTION

1.1 Research background

Rice (*Oryza sativa*) is a staple food more than half of the world population (Food and Agriculture Organization of the United Nations (FAO), 2004) where more than 90% is cultivated and consumed in Asia (Mitchell, 2009). It is highly preferred by consumers as it has significant health benefits, unique organoleptic and acceptable sensory characteristics such as taste, odor texture and color (Bhat and Riar, 2017). Moreover, it contains about 80% carbohydrate (Thomas *et al*., 2013) which contributes to 21% of global human per capita energy (Maclean *et al*., 2002). Besides that, rice also provides vitamin such as B_1, B_2, B_3, B_5, B_6, B_7 and B_{12} (Kyritsi *et al*., 2011) and a phenolic compound such as ferulic acid (Walter *et al*., 2013).

However, most of the nutrients present in rice are destroyed or lost during processing contributing to the decreasing of the nutritional value (USDA Nutrient Data Laboratory, 2005; Mohapatra and Bal, 2006). Generally, rough rice will be converted into brown rice by a dehulling process which separated the hull from brown rice (Buggenhout *et al*., 2013). During this process, up to 14%, 2% and 47% of vitamin B_1, B_3, and B_7, respectively were lost (Abbas *et al*., 2011). Moreover, milling process will turn brown rice into white rice by removing the bran layer on the rice endosperm results in further loss of vitamin B_1, B_3, and B_7 up to 33%, 79%, and 10%. Besides that, phenolic and anthocyanins compound were also reported lost during milling as the friction and abrasion forces were applied (Min *et al*., 2012; Walter *et al*., 2013).

So, to overcome this problem, this is where food fortification can be applied. Food fortification is an addition of one or more nutrients to any food during processing to increase the level of a specific nutrient, restore the nutrient loss and improve health quality of targeted groups without making significant changes in dietary habits (Allen *et al*., 2006). There are few types of rice fortification technologies have been developed to enhance nutrient content in rice such as dusting, coating, extrusion, and parboiling (Piccoli *et al*., 2012). However, in this research, only parboiling technique was employed because it is a low-cost process, no large investment needed and no alteration of dietary behavior. Furthermore, fortification via parboiling method has successfully done by previous researchers resulting in significant increase of nutrients such as folic acid (Kam *et al*., 2012), calcium (Sirisoontaralak *et al*., 2016), phytochemicals (Igoumenidis *et al*., 2016) and iodine (Tulyathan *et al*., 2007). Moreover, a survey done by Lau *et al*. (2012) revealed that Malaysian consumers nowadays are demanding for the healthy food available in the markets. For example, fortified version food product which can claim to have direct health benefits in preventing and curing chronic diseases such as cardiovascular, diabetes and cancer (Hasler, 2002).

Parboiling rice is a hydrothermal process which combines several steps including hydration, heating, and drying (Bhattacharya, 2004) on rough (Patindol *et al*., 2008), brown (Parnsakhorn and Noomhorm, 2008) or white rice (Houssou *et al*., 2016). Hydration is a process where the water molecules move into the rice grain via diffusion method when there are existing of moisture gradient in the rice system (Yu *et al*., 2017). Next, rice heated above the gelatinization temperature through steaming or boiling resulting in recrystallization of starch molecules that led to a change in physicochemical properties of rice (Ayamdoo *et al*., 2013). Then, drying is done to reduce the moisture content in the rice grains less than 14% either by sun or oven drying as it is a suitable condition for further processing and storage (Genkawa *et al*., 2011). Besides that, parboiling offers several advantages over the unparboiled rice such as increased milling yield (Danbaba *et al*., 2014), reduced cooking time (Buggenhout *et al*., 2013), increased micronutrients contents (Kam *et al*., 2012; Kyritsi *et al*., 2011) and enzyme inactivation (Heinemann *et al*., 2008).

1.2 Rationale of the study

Many researchers had been conducted on meeting the growing demand for healthy food. Healthy food staples are vital to reduce the risk of developing chronic diseases such as cancer, diabetes mellitus, and coronary heart disease in the world population. In line with this objective, researchers have presented different methods and concepts to innovate healthy food.

Recently, a lot of researches have been carried out on fortification of rice through a parboiling process, but, it is limited to micronutrients such as zinc, calcium, and vitamin only. Moreover, there is no study have been conducted on fortified rice with *Clitoria ternatea* flower extract as a fortificant and also colouring agent through the parboiling process. Furthermore, no standardized condition has been agreed on the parboiling conditions as different rice variety will have different grain structure such as total amylose content, cell structure, proteins, and lipids which play an essential role in determining the quality of the parboiled rice.

1.3 Research objectives

The main objective of this study was to develop fortified rice with *Clitoria ternatea* flower extract (Nasi Kerabu) through parboiling process in order to restore the nutrient loss during processing and to introduce natural colourant. Meanwhile, the specific objectives were as follows:

1. To optimize fortified parboiling process for maximum total flavonoid content in rice variety of MRQ 74, MRQ 76 and glutinous rice.
2. To determine the effect of parboiling on physicochemical and cooking quality of rice.
3. To evaluate the stability of phenolic, flavonoid and antioxidant activities in fortified parboiled rice during storage.

CHAPTER 2

LITERATURE REVIEW

2.1 Rice

Rice (*Oryza sativa*) is one of the leading crops in the world and is consumed worlwide. Nine percent of the total growing areas in this world is cultivated with paddy which importance socioeconomic for people who are living in the rural area (Maclean *et al*, 2002). Besides that, rice also plays an important role as a primary source of the nutrient such as carbohydrate, proteins, minerals, fibers, and vitamins (Bouis *et al*, 2003; Yokoyama, 2004) in Asia, South America and Africa (Heinemann *et al*, 2006). Nutritional composition and cooking quality of rice are mainly depending on the genetic, environmental conditions, degree of milling, polishing as well as the storage duration (Giri and Laxmi, 2000; Singh *et al*, 2005). Moreover, rice starch has unique characteristics such as hypoallergenic, spreadable, bland and creamy taste (Wani *et al*., 2012). These unique characteristics make rice starch valuable in various food applications such as confectionary products (Alvarez-Jubete *et al*., 2010), soups (Wongsagonsup *et al*., 2014) and noodles (Choy *et al*., 2012).

2.1.1 Rice production

Food and Agriculture Organization of the United Nations Statistical Databases FAOSTAT (2015) reported that the world rice production in the year of 2014 was 741 million tons. China was the largest producer in the world with 206 million tons followed by India and Indonesia with 154 and 70 million tons, respectively. Meanwhile, in Malaysia, production of rice was at 2.64 million tons (Food and

Agriculture Database, 2014) where Kedah state was the largest producer with 1.03 million tons (Department of Agriculture, 2014). The rice production would be expected to increase by 50% in 2050 in order to meet the future demands of 9.3 billion of the world population (Sheehy and Mitchell, 2011).

2.1.2 General rice processing

In general rice processing, raw rice is converted into brown rice by a dehulling process. The dehulling process is the separation of the outer layer from the raw rice by using a rubber roller at the control speed (Buggenhout *et al,* 2013). Moreover, the distance between the roller and raw rice determines the dehulling efficiency of raw rice when stress such as tension, compression and friction forces was applied (Juma Omar and Yamashita, 1987). So, as the distance decreases, the rice experiences more stress resulting in higher broken rice (Buggenhout *et al*, 2014) and loss of vitamin B1, B3 and B7 up to to 14%, 2%, and 47%, respectively (Walter *et al*., 2013).

Meanwhile, from the brown rice, it will turn into white rice by a milling process. The milling process is the last stage of post-harvest where the bran layer firmly bonded to the endosperm detached by mechanical stress (Afzalinia, *et al,* 2004). Mechanical stress such as friction milling will force brown rice against each other and metal screen plate. Then frictional force created in between brown rice and also between metal screen plate resulting in further loss of vitamin B_1, B_3, and B_7 up to 33%, 79%, and 10%, respectively (Bond, 2004; Walter *et al*., 2013). Besides that, nutritional components such as phenolic and anthocyanins compound lost during this process as the bran layer contains abundance of protein, fibers, lipids, minerals, vitamins and phytochemicals (Abdel-Aal *et al*., 2006; Liu, 2007).

2.1.3 Rice grain structure

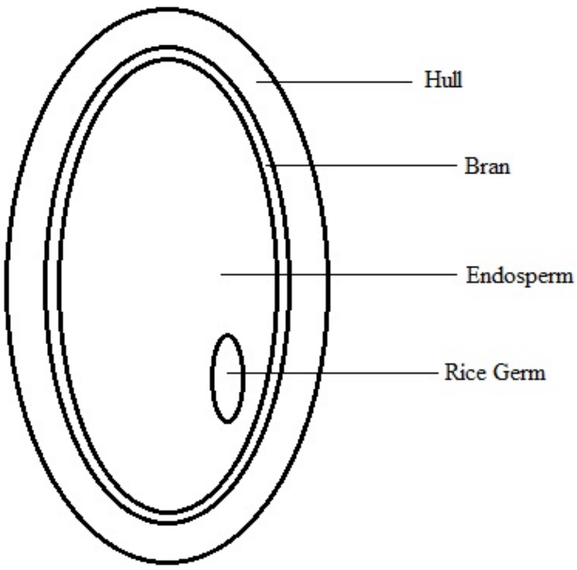

Figure 2.1: Rice structure

Rice harvested as paddy grain consists of hull, bran and endosperm (Saikia and Deka, 2011). Endosperm is the most significant part of the rice grain consists 80% of starch and have been identified as a major factor influencing the physicochemical and cooking properties of rice (Bocevska *et al*., 2009). Moreover, Vandeputte and Delcour (2004) mentioned that rice starch is existing in discrete particles as starch granules. It is the smallest granules exist in cereal grains with the size range from 2-7 µm with smooth surface structure and polyhedral in shape. Besides that, the rice starch occurs in the form of semi-crystalline granules consist of two polysaccharides namely amylose and amylopectin. Both amylose and amylopectin represent approximately 98-99% (dry weight) of the starch granules and <1% of minor components such as lipids, minerals and protein (Tester *et al*., 2004).

2.1.3(a) Amylose

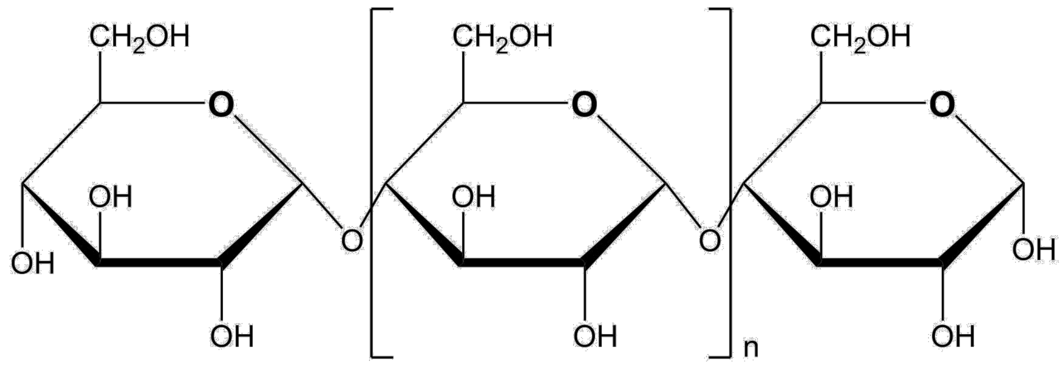

Figure 2.2: Amylose structure

Amylose is a linear polymer of α−1,4−linked D-glucopyranosyl units with few branches (< 0.1%) of α−1,6−linked D-glucopyranosyl units by glycosidic bonds (Gunning *et al*., 2003; Hizukuri *et al*., 1981). Park *et al*. (2007) reported that amylose has a molecular weight in rice grain ranging from 3.73-3.90 X 10^5 g/mol for short/medium and long rice, respectively. In addition, the degree of polymerization (DP) in amylose around 920-1100 glucose units with a low degree of branching (<0.1%), 2-5 chains which contain approximately 250-370 glucose unit. Moreover, the locations of amylose can be divided into three regions which are amorphous lamellae, amorphous growth ring or it interspersed with amylopectin molecules in starch granules (Hoover *et al*., 2010).

Amylose is a helical structure where the hydrogen atom located in the interior part of the helix act as hydrophilic compounds allowing the amylose to form clathrate (an inclusion complex) with fatty acid, alcohol and iodine (Fennema, 1996). Besides that, the amylose forms a complex with iodine (polyiodide) to determine the 'apparent amylose' in a sample by changing the colour (calorimetry method) into blue-black

(Bhattacharya, 2009; Juliano *et al*., 1981). The differences of amylose content in rice varieties might be related to the cultivar type, growing zones, climatic and soil conditions during rice grain development (Bao *et al*., 2004; Tester and Karkalas, 2001). Then, the rice cultivar was further categorized according to their amylose content as shown in Table 2.1. Based on the amylose content, the rice varieties can be categorized into low (waxy), intermediate and high amylose (non-waxy) content of rice. The total amylose content of different rice varieties may vary depending on different botanical source as it affected by the climatic conditions and soil type and (Asaoka *et al*., 1985; Morrison and Azudin, 1987).

Table 2.1: Type of rice variety and amylose content. (Adapted from Lawal *et al*., 2011; Yu *et al*., 2012)

Level of amylose	Amylose Content (%)
Waxy	0-2
Very Low	3-12
Low	13-20
Intermediate	21-25
High	>25

2.1.3(b) Amylopectin

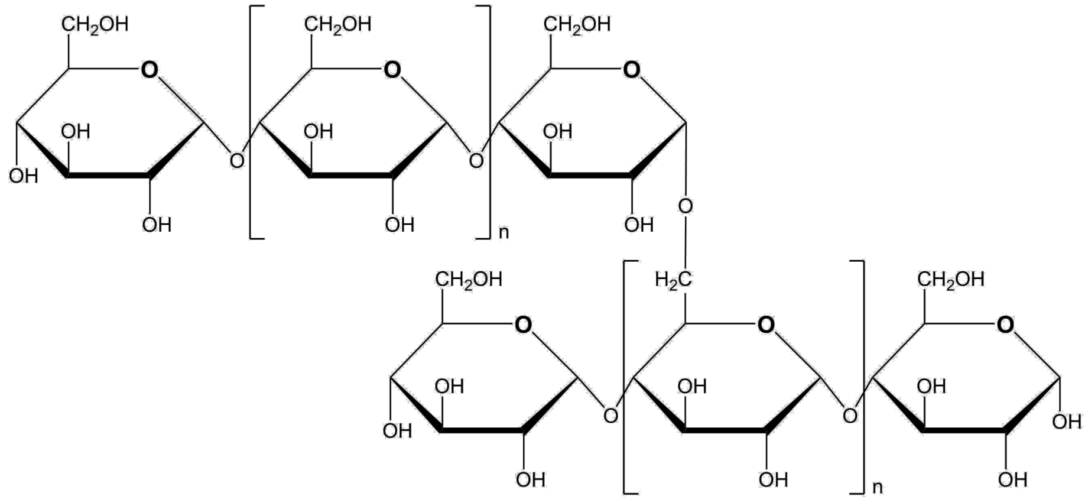

Figure 2.3: Amylopectin structure

Amylopectin is a massive polymer structures consisting of α−1,4−linked D-glucopyranosyl units branched with 5-6% of α−1,6−l linked D-glucopyranosyl units giving it a highly branched (Gilbert *et al.*, 2013; Hoover, 2001). Amylopectin has a molecular weight at 1.10 X 10^8 g/mol, 1.81 X 10^8 g/mol and 2.47 X 10^8 g/mol for long grain, short/medium grain and waxy grain, respectively (Park *et al.*, 2007). Besides, it has a degree of polymerization (DP) around 8200-12800, 19-23 chain length with 10-100 chains (Manners, 1979).

The basic structure of amylopectin might be classified into three types named A, B and C (Wang *et al.*, 1998). A chain (outer chain) is the shortest (CL 6-15) and unsubstituted (Copeland *et al.*, 2009) linked by α−1,6−linked D-glucopyranosyl to B chain. Besides that, B chain is substituted by other chains which further classified into B1, B2, B3 and B4 with the chain length ranging from 15 to 50. Meanwhile, C chain exists as single per amylopectin molecule containing reducing terminal and carries other chains (Donald, 2004). Table 2.2 shows summary characteristics of amylose and amylopectin components in rice starch.

Table 2.2: Summary characteristics of amylose and amylopectin components in rice starch

Characteristics	Amylose	Amylopectin
General structure	Linear	Branched
Linkage	$\alpha-1,4$ and few $\alpha-1,6$	$\alpha-1,4$ and $\alpha-1,6$
Molecular Weight (g/mol)	$3–4 \times 10^5$	$1–2 \times 10^8$
Degree of polymerization	920-1100	8200-12800
Average Chain Length (CL)	250-370	19-23
Number of Chain	2-5	10-100

2.1.3(c) Minor component

Several minor (non-starchy) components are associated with starch granule including lipids, proteins, minerals and moisture. Lipid made up of free fatty acids (FFA) and lysophospholipids (LPL) that are correlated to the amylose content in rice (Buléon *et al*., 1998). In addition, a recent study by Baldwin *et al*. (1997) observed that a significant number of lipids on the surface of starch granules and had been identified as a palmitic and linoleic acid. High amylose rice (non-waxy) contains more lipid that embedded and free in starch granules compared to low amylose rice (waxy) (Choudhury and Juliano, 1980). Besides that, the hydrophobic nature of lipids is negatively correlated with the swelling capacity of granules as less water can be absorbed into the starch granules during soaking (Vasanthan and Bhatty, 1996).

Next, the proteins in rice are mostly concentrated on the outer surface of starch granule and in the bran layer of brown rice (Champagne *et al*., 2004). Starch proteins can be categorized into two namely surface and integral protein. Surface protein is a protein which can readily be extracted in aqueous solution. Meanwhile, integral protein is a protein that only extractable when a certain amount of heat is applied near or above the gelatinization temperature (Morrison and Karkalas, 1990). In addition, integral protein is covalently bound in amylose and amylopectin structure as it embedded in the granule, while the surface protein has loosely existed on the surface of the granule (Thomas and Atwell, 1999). Besides that, proteins have been identified as significant component in water absorption as it plays an important role in regulating the water diffusion into starch granule and also control the granule swelling (Matveev *et al*., 2000).

Ash is relatively related to the amount of minerals content such as phosphorous, magnesium, calcium, potassium and sodium is less than 0.4% dry basis present in rice (Tester *et al*., 2004). It is varies depending on the rice variety, agronomic procedures and also milling practices done on the rice (Thomas and Atwell, 1999). Phosphorous plays an essential role in starch functional properties such as viscosity, paste clarity, consistency and paste stability (Bao *et al*., 2004; Blennow *et al*., 2000). There are two primary forms of phosphorous found in rice starch are phosphate monoesters and phospholipids. In high amylose rice (non-waxy), the phospholipid is the dominant form of phosphorous with 0.048% hereas in low amylose rice (waxy) phosphorous is identified in the form of phosphate-monoesters with 0.003% (Jane, 1996; Lim *et al*., 1995). Lastly, the rice starch usually contains around 10-12% of moisture (Tester *et al*., 2004).

2.2 Starch gelatinization

Gelatinization is an endothermic process occurs when starch is heated in the presence of sufficient amount of water resulting in disruption of molecular ordered in starch granule (Wani *et al*., 2012). Moreover, gelatinization process involving two stages before occurring. The first stage is the absorption of water from the surrounding and rapid expansion in the starch granules (Atwell *et al*., 1988). Next, the swelling of the amorphous regions exerts a stress on the crystalline structure causes destabilize of the starch granules and break the hydrogen bond (Jenkins and Donald, 1998). Once the crystalline structure loses their order, the starch granules can swell much bigger from its original size. Thus, resulting in polymer molecule, amylose to solubilize and leach out from the swollen starch granules and increased the viscosity (BeMiller, 2007; Parker and Ring, 2001).

Moreover, several irreversible changes in starch granules during gelatinization such as swelling, crystalline structure melting, loss of birefringence and starch solubilization can be observed when the high temperature was applied (Atwell *et al*., 1988). So, several analytical methods have emerged to determine the starch gelatinization such as differential scanning calorimetry (DSC) (Acquistucci *et al*., 2009; Wickramasinghe and Noda, 2008), microscopy (Li *et al*., 2013), X-ray diffraction (Chung *et al*., 2011) and enzymatic analysis (Uthumporn *et al*., 2010). Moreover, DSC is a widely used method in determining the gelatinization temperature of starch because it is less time consuming, used minimal amount of sample (5-10 mg) and applicable over a wide range of water content (Nakazawa *et al*., 1985). From DSC, thermal properties such as gelatinization enthalpy (ΔH), gelatinization onset (T_o), peak (T_p) and conclusion temperature (T_c) can be identified.

2.3 Retrogradation

Starch retrogradation is defined as a reassociation of amylose and amylopectin into an ordered molecule structure during cooling (Atwell *et al*., 1988). This is because during cooling less energy is available to keep the solubilized starch molecules (amylose and amylopectin) to be apart (Thomas and Atwell, 1999). Besides that, retrogradation process occurs when the hydrogen atom has a stronger tendency to reassociate with the hydroxyl group from the adjacent starch molecules which further determines the elasticity, firmness and textural staling in a starch system (Atwell *et al*., 1988). The rate of retrogradation in the sample depends on the amylose content, amount of lipid and molecular weight (Billiaderis and Zawistowski, 1990) that can be measured by using several analytical methods such as DSC (Chung *et al*., 2011; Lawal *et al*., 2011), rheology (Vandeputte *et al*., 2003) and also nuclear magnetic resonance (NMR) (Qi *et al*., 2003; Yao *et al*., 2003). Furthermore, the rate of amylose retrogradation in non-waxy rice is faster than the waxy rice because the gelation of amylose is prominent when compared to the amylopectin (Baik *et al*., 1997; Gidley, 1989)

2.4 Rice fortification

Food fortification is defined as the addition of one or more micronutrient to food during processing to increase the level of a specific nutrient, restore the nutrient loss and improve the health quality without changing the dietary habit (Allen *et al*., 2006; FAO, 2004).The successful of food fortification is strongly related to the right choice of food to be fortified as it is regularly eat in recommended amount, palatable and culturally accepted (Piccoli *et al*., 2012). Rice represents a perfect food vehicle for food fortification because it is consumed by all population groups including elderly and children, affordable and cultivated in many regions (Gallagher *et al*., 2004). Moreover, white milled rice is considered as an excellent choice of food to be fortified food for developing flavours, aromas and colours due to the inert taste of the rice (Igoumenidis *et al*., 2016).

Rice fortification can be categorized into two types which are whole grain and powder (Dexter, 1998; Misaki and Yasumatsu, 1985). Whole grain fortification is done by applying the nutrient to rice either externally or mixed, while, for powder is an addition of the nutrient to rice flour followed by the extrusion process. Furthermore, according to Piccoli *et al*. (2012), the basic terminology on rice fortification are fortificant and fortified rice. Fortificant is the selected micronutrient in a particular chemical form that would be use in fortifying selected food product meanwhile fortified rice is rice that has been added with specific micronutrient through a technology. Table 2.3 shows the four main technologies for rice fortification and some of their characteristics.

Table 2.3: Summary method of rice fortification (Adapted from Piccoli *et al*., 2012)

	Technology			
	Parboiling	Dusting	Coating	Extrusion
Fortification method	Soaking rice grain with micronutrient, followed by steaming and drying	Dusting rice grains with micronutrient premix powder	Spray fortificant on the surface of rice grain added with coating agent such as waxes and gums	Dough made from rice flour mixed with fortificant is passed through a simple pasta press or single/twin screw extruder
Characteristics of fortified grain	The fortificant penetrate into the endosperm during soaking	Micronutrient added lost during rinsing and washing	Colour of fortified rice depends on fortificant but have distinctive colour, smell and taste	Grains are opaque and slightly off-colour
Examples of micronutrient added	Iodine, Vitamin, Phytochemicals	Iron, Vitamin B_1, Vitamin B_3, Folic Acid	Turmeric, Vitamin B_1, Vitamin B_3, Vitamin B_{12}, Zinc	Vitamin B_1, Vitamin B_3, Vitamin B_{12}, selenium, folic acid
Country commonly used rice fortification	India, Bangladesh, Thailand	USA	USA	Brazil, China, Philippine, India

2.4.1 Fortification using parboiling

Recent studies by Piccoli *et al*. (2012) showed that fortification of rice through parboiling process is a recommended method to increase or restore the nutrient loss during rice processing. Besides that, parboiling process is very cost- effective vehicle in addressing the nutrient deficiencies in the general population, especially in the remote area (Prom-u-thai *et al*, 2010). The consumers preference for parboiled rice in South Asia is based on the 'traditional taste' of parboiled rice that is less sticky and fluffier compared to non-parboiled rice (Oli *et al*., 2014). Moreover, for the sensitive health consumers, parboiled rice is the right choice to consume as it contains higher nutritional properties such as vitamin (Kyritsi *et al*., 2011), mineral (Prom-u-thai *et al*., 2010; Sirisoontaralak *et al*., 2016; Tulyathan *et al*., 2007) and antioxidant properties (Igoumenidis *et al*., 2016). Furthermore, the other reasons why rice is subjected to parboil are to increase the head rice yield during milling, to enhance or to restore the nutrient loss during processing and to improve the shelf-life by inactivation of enzymes (Oli *et al*., 2014).

2.5 Telang (*Clitoria ternatea*)

2.5.1 Botanical descriptions and nutritional value

Plants have long been used to treat diseases because they contain many natural ingredients, such as phenolic compounds. *Clitoria ternatea* (CT), commonly known as telang (Malaysia), butterfly pea, kordofan pea (Sudan), cunha (Brazil) or pokindang (Philippines) is from the Fabaceae family. The plant is 90.00 to 162.00 cm tall. It is a long-lived perennial herb with an erect habit (Kalamani and Gomez, 2001). Besides that, according to Gomez and Kalamani (2003), CT has solitary flowers with vivid, deep-blue and white colouration. The flowers are also 6.00 to 12.00 cm long. Furthermore, it also contains six to eight brown or black-coloured seeds per pod which are slightly pubescent or glabrous. Moreover, the butterfly pea is self-pollinated by nature but it also exists because of cross-pollination due to the identification of genotype segregation (Cook *et al*., 2005).

Figure 2.4: Telang flower

Many reports have been published on the medicinal effects of CT such as antipyretic, analgesic and anti-inflammatory (Mukherjee *et al*., 2008). These properties are good for one's health and well-being because they help to reduce health disorders. In addition, this flower is also used as a source of food for livestock. It is highly preferred by livestock because of its mild and acceptable taste over other types of legumes (Gomez and Kalamani, 2003). Furthermore, proximate and mineral value of CT flowers has been reported by Neda *et al*. (2013) as summarized in Table 2.4 and Table 2.5.

Table 2.4: Proximate analysis of telang flower (Adapted from (Neda *et al*., 2013)).

Proximate	Value (%)
Moisture	92.40±0.10
Ash	0.45±0.15
Fat	2.50±0.10
Protein	0.32±0.03
Crude Fibre	2.10±0.20
Carbohydrate	2.23±0.30

Table 2.5: Mineral analysis of telang flower (Adapted from (Neda *et al*., 2013)).

Mineral	Value (mg/g)
Calcium	3.10
Magnesium	2.23
Potassium	1.25

2.5.2 Active compounds

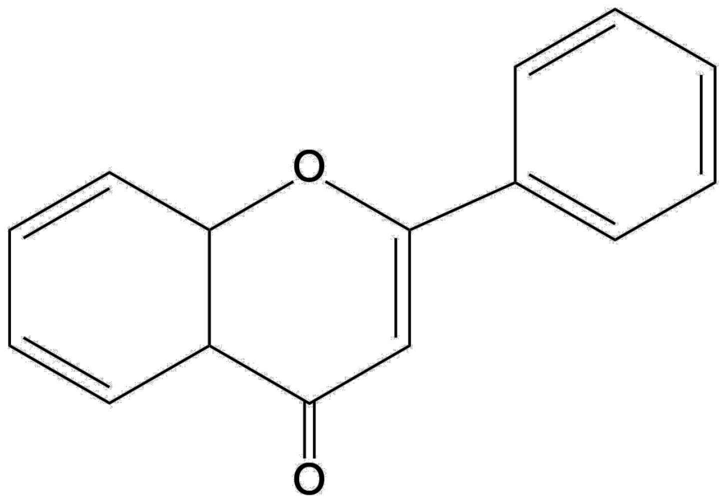

Figure 2.5: Basic structure of flavonoid

The major active compounds identified by LC/MS/MS in CT flower petals are flavonoids and anthocyanins with the total amount at 20.07 nmol/ mg petal and 5.40 nmol/ mg petal, respectively. For the flavonoid, the flavonol glycosides founds are quercetin 3-(2^G-rhamnosylrutinoside), quercetin 3-neohesperidoside, quercetin 3- (2″-rhamnosyl-6″-malonyl) glucoside, quercetin 3-rutinoside, quercetin 3-glucoside, kaempferol 3-(2^G-rhamnosylrutinoside), kaempferol 3-neohesperidoside, kaempferol 3-rutinoside, kaempferol 3-glucoside, kaempferol 3-(2″- rhamnosyl-6″-malonyl) glucoside, and myricetin 3-(2^G-rhamnosylrutinoside). Moreover, for the anthocyanins compounds found are ternatin A1, ternatin A2, ternatin A3, ternatin B1, ternatin B2, ternatin B3, ternatin B4, ternatin C1, ternatin C2, ternatin C3, ternatin C4, ternatin C5, ternatin D1, ternatin D2 and ternatin D3 (Kazuma *et al*., 2003).

2.5.3 Biological effects on human health

The telang tree is found to possess a significant number of advantages and natural properties against several diseases and ailments in the human body based on the research conducted. Some scientific researchers have claimed that this plant is beneficial in the prevention of diabetes (Gunjan *et al*., 2010) and asthma *(Chauhan et al*., 2012; Taur and Patil, 2011). In addition, it also contains high antioxidant activity (Rabeta and An Nabil, 2013), possess the ability to heal many infectious diseases, possess high anti-helmintic activity in roots (Khadatkar *et al*., 2008) and leaf extracts (Salhan *et al*., 2011; Sarojini, 2012) as well as serving as a strong hepatoprotective agent against paracetamol (Nithianantham *et al*., 2011) and carbon tetrachloride, CCl_4 (Shanmugasundram *et al*., 2010). The potential effects of *Clitoria ternatea* in human health are summarized in Table 2.6.

Table 2.6: The potential effects of *Clitoria ternatea* in human health

Properties	CT Part	Findings	References
Antioxidant	Flower	Aqueous extracts showed higher scavenging activity compared to methanol	Rabeta and An Nabil, 2013
Antidiabetic	Flower	The glucose level tested in diabetic rats significantly decreased after 14 days of administering with CT flower extract with 150mg/kg body weight	Gunjan *et al*., 2010
Antibacterial	Leaves	The methanolic and petroleum ether extracts are the greatest protection against *Bacillus cereus*, *Salmonella typhi, Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*	Anand *et al*., 2011; Shekhawat and Vijayvergia, 2010
Antihelmintic	Leaves and Root	Increasing the concentration of the extracts from 10.00-100.00 mg/ml, the time taken for the earthworms to be paralyzed and dead were decreasing	Khadatkar *et al*., 2008; Salhan *et al*., 2011; Sarojini, 2012
Hepatoprotective	Leaves	The treated group with CT extracts was observed to possess a reduced level of enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and bilirubin compared to paracetamol-treated group.	Nithianantham *et al*., 2011
Cognitive	Root	Administered with 100mg/kg of aqueous root extract significantly increased acetylcholine (Ach) content in hippocampus of neonatal	Rai *et al*., 2001

2.5.4 Application in foods

Telang tree is widely used as a natural colourant in food nowadays (Mukherjee *et al*., 2008; Nanasombat *et al*., 2015). In India (Kerala) and Philippines, the aerial part of CT plants such as young shoots, leaves, flowers and pods are eaten as vegetables (Mukherjee *et al*., 2008). Besides that, due to the attractive and stable colour of flower, it also makes extensive use as a natural colouring agent in food industries. For example, the flowers are employed to impart a bright blue colour to the rice which known as blue rice or nasi kerabu (Muhammad Ezzudin and Rabeta, 2018). Nasi kerabu is a famous dish in Kelantan eaten with grilled chicken or fried fish coated with flour, fish crackers, salted egg and other local herbs. Moreover, due to the original colour of telang flower, it is also used in Baba and Nyonya culture in imparting their cuisines such as in kuih chang, pulut inti (glutinous rice cakes with sweet coconut) and pulut tatai (steamed blue glutinous rice cakes) (Ng and Karim, 2016).

2.6 Parboiling process

Parboiling is a hydrothermal process comprises of soaking, steaming and drying (Bhattacharya, 2004). It is usually performed either on paddy (rough rice) (Prom-u-thai *et al*., 2010), dehulled (brown rice) (Parnsakhorn and Noomhorm, 2008) or milled (white rice) (Miah *et al*., 2002; Mridula *et al*., 2015) for improving rice quality and milling yield.

Soaking is a hydration process to control the movement of water into the rice grain by diffusion process. According to Oli *et al*. (2014), water in the system will diffused from higher to lower water concentration until it is achieving the equilibrium state. Besides that, rate of diffusion is strongly related on the processing method, variety, time and temperature. During soaking, water will diffuse rapidly inside the grain preventing from the fermentation and unfavourable food products (flavour, colour and odour) when heat is applied (Amato *et al*., 2002). Furthermore, soaking is very critical step in rice parboiling as it is major contributor in altering the physical, chemical and cooking properties of parboiled rice (Naivikul, 2007).

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Next step is steaming. Steaming is a thermal treatment to further gelatinized the rice starch after done the soaking step. For the laboratory scale, steaming at the atmospheric pressure is the most widely used compared to mild (80-100°C) and severe heating (above 100°C) conditions. The crystalline structure in the rice granules changed into an amorphous form resulting in the irreversible and swelling of the starch granules (gelatinization) (Taghinezhad *et al*., 2016). Moreover, drying is the final process in rice parboiling process. During drying, the moisture content is reduced to 12-14 % for safe storage and prevent from microbial and fungal attack (Buggenhout *et al*., 2013). Drying process can be performed either by traditional practice such as sun drying or for conventional practice is hot air drying. In Southeast Asia, hot air drying is the most popular method used because it reduces the drying time, improves the grain quality and reduces the drying cost (Le and Jittanit, 2015).