TEXTURAL PROPERTIES OF GLUTINOUS RICE FLOUR GELS AND DODOL AS INFLUENCED BY HIGH DIASTASE HONEY

by

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Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy

July 2019

ACKNOWLEDGEMENT

First of all, I would like to express my sincere gratitude to my main supervisor, Prof. Dato' Dr. Azhar Mat Easa for his invaluable advice, encouragement, guidance, patience, and understanding throughout the course of this research and writing of this dissertation. He has been supportive and giving me much independence in doing my research.

My utmost appreciation goes also to my co-supervisors, Dr. Tan Thuan Chew, and Dr. Lee Lai Kuan for their time, guidance and the freedom they offered in pursuing my study. Their insightful comments have certainly helped a lot in the writing of my dissertation.

I would also like to dedicate my gratefulness to my friends and laboratory mates especially Assoc. Prof. Dr Gan Chee Yuen, Kah Aik, Chin Hong, Sze Chi, Yee Yaw, Yan Kitt, Dayang, Shima, Maisa, Syuze, Hui Yin and Lee Fen, who have been helping with my problems and for the conducive work atmosphere they provided throughout these years. Thank you for supporting me in times of stress and difficulty. I appreciate too the help from the laboratory assistants especially Mr. Firdaus, Mr. Ghoni, Mr. Maarof, Mr. Shamsul, Madam Aida and Madam Mazura. Their valuable assistance and cooperation has certainly contributed to the success of this study.

I wish to give my acknowledgement of the financial aid and research fund granted by MyBrain MyPhD and Universiti Sains Malaysia Research University Grant (Grant No: 1001.PTEKIND.8011023), respectively. Their kind offerings have enabled me to focus on my study without financial worries.

Most importantly, none of this would have been possible without the love and patience of my family. I am thankful to them for being ever supportive in all my endeavours. I know I always have a family that I can count on during times of adversity.

Lastly, my deepest gratitude goes to all people whom I have met along the way and who have contributed to the completion of my research.

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LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
DSC	Differential scanning calorimetry
DMSO	Dimethyl sulfoxide
DN	Diastase number
DRI	Differential refractive index
FT-IR	Fourier-transform infrared spectroscopy
GRF	Glutinous rice flour
HBH	Honey bee honey
HMF	Hydroxymethylfurfural
HPLC	High performance liquid chromatography
KBH	Kelulut bee honey
KBr	Potassium bromide
LVR	Linear viscoelastic region
MEC	Multiple extrusion cell
ND	Not detected
NM	Not measured
pH	Potential of hydrogen ion
SME	Small medium enterprises
SNI	Indonesian National Standard
TPA	Texture profile analysis
TSS	Total soluble solids

SIFAT TEKSTUR JEL TEPUNG BERAS PULUT DAN DODOL YANG DIPENGARUHI OLEH MADU BERDIASTASE TINGGI

ABSTRAK

Dodol adalah konfeksi yang kenyal, berkanji dan manis yang biasanya dihidangkan semasa perayaan di Malaysia. Kemerosotan tekstur dodol sering dikaitkan dengan proses pengerasan yang disebabkan oleh retrogradasi kanji. Kajian ini bertujuan untuk mengkaji kemungkinan untuk menggunakan madu lebah berdiastase tinggi untuk mengurangkan pengerasan dodol semasa penyimpanan dan membandingkan penerimaan dodol yang ditambahkan dengan madu. Pelbagai jenis jel tepung beras pulut atau dodol yang ditambahkan dengan madu telah disediakan. Parameter kelikatan, reologi dan kekerasan untuk jel tepung beras pulut konvensional (congel), jel tepung beras pulut madu lebah (honeygel), jel tepung beras pulut madu kelulut (kelugel), jel tepung beras pulut madu lebah yang dipanaskan (honeygel terpanas), jel tepung beras pulut madu kelulut yang dipanaskan (kelugel terpanas) dan jel tepung beras pulut gula (sugargel) telah dinilai. Berbanding jel lain, hanya honeygel menunjukkan penurunan kelikatan yang ketara ($p<0.05$) apabila jel dikekalkan pada 55 ° C selama 30 min dan nilai kekerasan yang terendah (0.64 N) semasa penyimpanan selama 7 hari. Honeygel adalah ($p<0.05$) paling kurang elastik (bersifat seperti cecair) dengan nilai G' (modulus elastik) yang paling rendah, berbanding dengan congel dan kelugel. Penurunan nilai G' adalah sebanyak 5.37% dari 6.33 hingga 5.99 Pa selepas ujian selama 30 minit. Sifat ini disebabkan oleh kandungan diastase yang agak tinggi dalam madu lebah mentah. Eksperimen yang sama diulangi pada dodol. Dodol konvensional (condol), dodol madu lebah (honeydol) dan dodol madu kelulut (keludol) telah dinilai untuk profil tekstur (kekerasan, kelekatan, keanjalan, kesatuan, kekenyalan, ketahanan), sel penyemperitan berbilang (W_{inf}, w_1, n_1), relaksasi tekanan (F_{max}, k_1, k_2) dan analisis terma (T_{onset}, T_{peak}, ΔH). Berbanding dengan condol dan keludol, struktur honeydol dengan retrogradasi yang kurang adalah lebih mudah untuk dipecahkan. Hal ini terbukti dengan kekerasan, keanjalan, kesatuan, kekenyalan, work 1st, W_{inf}, w_1, F_{max}, ΔH yang paling rendah ($p<0.05$) dan nilai n_1, T_{onset} dan T_{peak} tertinggi. Penilaian hedonic terhadap sifat-sifat sensori (penampilan, warna, aroma, rasa, tekstur, kekenyalan dan penerimaan keseluruhan) dari condol, honeydol dan keludol dijalankan dalam kalangan kumpulan pra-tua (n = 30) yang berumur 40-59 tahun. Tahap penerimaan honeydol adalah sama seperti keludol dan condol, kecuali nilai tekstur, kekenyalan dan penerimaan keseluruhan yang lebih rendah ($p<0.05$). Kesimpulannya, madu lebah berdiastase tinggi berpotensi untuk digunakan sebagai bahan pelembut dalam pengilangan dodol.

TEXTURAL PROPERTIES OF GLUTINOUS RICE FLOUR GELS AND DODOL AS INFLUENCED BY HIGH DIASTASE HONEY

ABSTRACT

Dodol is a chewy, starchy and sweet confection commonly served during festivals in Malaysia. Texture deterioration of dodol is often associated to the hardening process caused by retrogradation of starch. Present study aimed to investigate the feasibility of using high diastase raw bee honey to reduce hardening of dodol during storage and compare the acceptance of honey-added dodol. A range of glutinous rice flour (GRF) gels or dodol incorporated with honey were prepared. Conventional GRF (congel), honey bee honey-GRF (honeygel), kelulut bee honey-GRF (kelugel), heated honey bee honey-GRF (heated honeygel), heated kelulut bee honey-GRF (heated kelugel), and sugar-GRF gels were assessed for viscosity, rheological and hardness parameters. Compared to other gels, only honeygel showed appreciable drop in viscosity when the gels were maintained at 55 °C for 30 min and significantly ($p<0.05$) lowest hardness value (0.64 N) during storage for 7 days. Honeygel was significantly ($p<0.05$) less elastic (more liquid-like) with the lowest G' (elastic modulus) value, as compared to congel and kelugel. There was a 5.37% decrease in G' value from 6.33 to 5.99 Pa after 30 min of testing. This behaviour was thought to be due mainly to the relatively high diastase content of the raw bee honey. Similar experiments were repeated in dodol. Conventional dodol (condol), honey bee honey-dodol (honeydol) and kelulut bee honey-dodol (keludol) were assessed for texture profile (hardness, adhesiveness, springiness, cohesiveness, gumminess, resilience), multiple extrusion (W_{inf}, w_l, n_l), stress relaxation (F_{max}, k_1, k_2) and thermal analysis (T_{onset}, T_{peak}, ΔH) parameters. As compared to condol and keludol, structure of honeydol with the least retrogradation was

easier to be broken down. This was evidenced by its lowest hardness, springiness, cohesiveness, gumminess, work 1st, W_{inf}, w_1, F_{max}, ΔH ($p<0.05$) and the highest n_1, T_{onset} and T_{peak} values. Hedonic evaluation of sensory attributes (appearance, colour, aroma, taste, texture, chewiness and overall acceptability) of condol, honeydol and keludol was conducted among the pre-elderly group (n=30) aged 40-59 years. Honeydol was as acceptable as keludol and condol, except for its texture, chewiness and overall acceptability with significantly ($p<0.05$) lower values. To conclude, high diastase honey bee honey has potential to be used as a softening additive in dodol manufacturing.

CHAPTER 1

INTRODUCTION

1.1 Background

Dodol, as a sticky sweet rice-based dessert is made up of a few main ingredients such as glutinous rice flour (GRF), coconut milk, white sugar, palm sugar, and sometimes with the addition of permitted food additives and flavours (Karim & Bhat, 2013; Chuah et al., 2007). Traditional cooking of dodol requires 7-8 hours of constant stirring until the liquid mixture turns into a sticky and viscoelastic mass. It is the sticky-like texture that makes the product to be recognised as dodol, but it is preferred in a firm texture and does not stick to fingers. A good quality dodol has a desirable quality characteristics of low firmness and high cohesiveness. Traditional dodol was found to be stickier and tastier as compared to commercial dodol which requires less labour and shorter cooking time (Nasaruddin et al., 2012).

Often regarded as traditional food being served during festivals, dodol is categorised as intermediate moisture food (IMF) with water content between 10-50% and water activity (a_w) in the range of 0.65-0.90. Dodol is a more stable traditional Malaysian starch-based IMF as compared to wajik and lempok (Seow & Thevamalar, 1988). Although rapid microbial spoilage is unlikely to occur in dodol with a_w at 0.69 (Nasaruddin et al., 2012), this product is susceptible to yeasts and moulds growth that may occur at a_w 0.60 and above. Other than surface moulds growth, issues of hardening and objectionable flavour arise from rancidity upon storage contribute to the short shelf life of dodol with only approximately 2-3 weeks (Zahid et al., 2012). In addition, a lot of dodol was thrown as waste in industry as well as supermarket due to the hardening issue.

Dodol is not regularly eaten and it is not known as a nutritious food. It is a snack that is normally being consumed during festivals. Apart from the dodol taste, texture is an essential quality characteristic that contributes to consumers' satisfaction and acceptance of food. Knowing that dodol is attributed by its unique mouth-feel properties, previous researches have studied the effects of processing such as cooking time, sugar content and temperature on textural properties of traditional dodol. It was found that increase of cooking time and sugar level has caused an increase in firmness, consistency, cohesiveness and index of viscosity but the reverse trend was observed when temperature was increased (Nasaruddin et al., 2012; Chuah et al., 2007). The data are certainly useful to improve the dodol quality to achieve the desirable characteristics of low firmness and high cohesiveness.

An enzymatic dodol processing technology was developed by Malaysia Agricultural Research and Development Institute (MARDI) in an attempt to overcome quality issues in dodol by incorporation of bacterial amylase enzyme and heat treatment (Zahid et al., 2012; Hamzah et al., 2007). Amylase enzyme has been known to be present in honey and it originates from bee as salivary secretions (Babacan et al., 2002; Vit & Pulcini, 1996). Characterisation of honey amylase found its optimum temperature and pH to be 55°C and within the range of 4.6-5.3, respectively (Babacan & Rand, 2007). Incorporation of honey amylase will be effective as the pH values of dodol are in the range of 5-6 (Chuah et al., 2007).

Addition of honey to starch matrix resulted in starch thinning and a significant viscosity loss, and there was no effect on viscosity observed if the honey has been preheated prior to addition. This clearly explained that starch degradation has taken place due to the presence of amylase activity (Babacan et al., 2002). Amylase hydrolyses starch and reduce the viscosity of starch solutions that starch fragments produced in the finished gel also reduce the rate of starch retrogradation (Torley et al., 2004). Incorporation of high diastase honey into dodol may offer a natural yet effective way to enhance its textural quality by inhibiting starch retrogradation (hardening) to prolong and maintain the desirable low firmness quality during storage. Raw honey containing active diastase was used in present study as there is no other form of diastase (eg. purified diastase) available in the market.

1.2 Problem statement

Deterioration of dodol's textural quality (hardening issue) occurs over time due to starch retrogradation that causes problem in its acceptability to the consumers. Hardened dodol affects the eating pleasure of consumers due to the difficulty in chewing.

1.3 Objectives

The general objective of present study is to prepare a texture-modified dodol with low firmness. Specific objectives of the study are listed as follows:

1. To assess the effect of different honey types on GRF gel.
2. To estimate the effective softening concentration of honey using GRF gel model.
3. To compare the rheological properties of GRF gel as influenced by heating treatment and honey types.
4. To study the textural, thermal, microstructural and chemical properties of GRF gel as influenced by honey types.

5. To compare the composition, physical and sensory properties of conventional dodol (as a control) and honey-added dodol.

6. To compare the textural, structural breakdown, thermal and chemical properties of conventional dodol and honey-added dodol.

1.4 Hypothesis

Raw honey, containing naturally occurring diastase (amylase) that hydrolyses the starch molecules into lower molecular weight short chains. Starch retrogardation can be inhibited due to the weakened starch network that yields low viscosity and weaker gel starchy products that eventually affect the textural qualities during storage. The proposed appproach to solve hardening issue using raw honey aids the commercial development of dodol, that the hardened dodol thrown as waste can be minimised.

1.5 Thesis organisation

Chapter 1 is a general introduction on the background of dodol and the challenges faced by the dodol industry due to the hardening issue. The proposed solution is presented that supports the application of raw honey containing active diastase for enzymatic hydrolysis of starch in dodol to delay the retrogradation process. Besides, the rationale, problem statement, objectives and hypothesis of this study are briefly discussed.

The emphasis of present project is on the active diastase presents in raw honey to hydrolyse the starch chains into shorter chains in dodol, which delays starch retrogradation. The general literature review of the ingredients for dodol cooking and composition of dodol and honey that may influence the starch retrogradation are presented in Chapter 2.

Chapter 3 shows the prelimininary study on commercial dodol and honey to establish the fundamental of present study. Commercial dodol and honey were characterised to get a rough picture of their quality characteristics. GRF gel model was established to estimate the effective softening concentration of the honey selected. The concentration decided was applied in the next chapters.

Chapter 4 and 5 describe and compare the effect of raw honey containing active diastase and the pre-heated honey that its diastase had been inactivated, on the rheological, textural, thermal, microstructural and chemical properties of GRF gel model. These two chapters provide evidences of the softening and anti-retrograding effect of honey diastase in GRF gel model to suggest the application of raw honey in dodol cooking.

In Chapter 6 and 7, raw honey was added in dodol cooking to assess the honey diastase effect in real food product. Chemical composition, physical parameters and sensory analysis of conventional dodol and honey-added dodol were compared. In addition, textural, structural breakdown, stress relaxation, thermal, microstructural and chemical properties of conventional dodol and honey-added dodol were assessed to verify and support the previous findings obtained using GRF gel model.

Last but not least, a general discussion of the findings, overall conclusions on the whole study and recommendations for the future study are given in Chapter 8.

CHAPTER 2

LITERATURE REVIEW

2.1 Dodol

2.1.1 Background

Dodol is one of the famous local traditional foods in Malaysia, Indonesia, Thailand and Philippines (Karim & Bhat, 2013). Production of dodol is considered as a small scale food processing (Budi et al., 2016; Damardjati, 1995). Traditional foods used to be prepared by households only for their own use. However, transformation of home-based industry to small medium enterprises (SME) occurs owing to the demand for convenience foods. Traditional foods that are complex to prepare are now being produced industrially, and to be sold as ready-to-serve products (Damardjati, 1995).

Dodol produced by SME is currently purchased as a souvenir as well as a Malay wedding door gift (Raji et al., 2017; Budi et al., 2016; Djatna & Luthfiyanti, 2015; Muhammad et al., 2013). It may symbolise fertility, appreciation, blessing and sweet life to bridegroom (Muhammad et al., 2013) and to promote unity and strengthen relationships (Karim & Bhat, 2013). Dodol is also well-liked by many during festive seasons especially during religious Muslim festivals (Karim & Bhat, 2013; Rosniyana et al., 2010).

There was a growing interest in improving the quality and nutritional values of dodol due to its great potential commercial value (Chuah et al., 2007). To add nutritional values to dodol, seaweed (*Eucheuma cottonii*) (Yuliasih & Wendrawan, 2013; Astawan et al., 2004), roselle (*hibiscus sabdariffa*) (Wulandari, 2009), nutmeg

(Breemer et al., 2010), stabilised rice bran flour (Rosniyana et al., 2010), squash and carrot (Nugraheni et al., 2011), Cassava (*Dioscorea Alata*) (Malawat & Hidayah, 2013), jack fruit (*Artocarpus heterophyllus*) seed flour (Wulandari et al., 2014) and cow milk (Ahda et al., 2018) were either substituted in part in dodol formulation or used to replace the existing ingredient. The processing approaches are promising and have yielded a final product with enhanced nutritional quality such as increased fiber, minerals and vitamins content.

According to the values reported by National Sub-committee in Protein Food Habits Research and Development Malaysia, and Nutriweb Malaysia of Ministry of Health Malaysia, a traditional dodol have a moisture content of 24.4%, protein 2.9%, fat 4.7%, carbohydrate 66.9%, ash 1.0%, fibre 0.1%, and energy value of 322 kcal per 100 g of dodol (Nasaruddin et al., 2012; Chuah et al., 2007). Owing to the high carbohydrate content in dodol, there was an attempt to develop this high energy food as an "Emergency Food Product" with mung bean flour, palm oil, margarine, soybean protein isolate, and glycerol other than the main ingredients. The final product must provide 700 kcal per serving of 171.87 g of dodol. It provides a wider range of food choices to meet the required daily energy level at 2100 kcal during emergency conditions, with the assumption to consume it thrice daily (Syamsir & Sitanggang, 2010).

Recently, versatile dodol premix was also introduced that it facilitates the dodol preparation process as well as cutting down the cost and production time. Preparation of fresh quality dodol can be easily done using a kitchen microwave oven (Zainun & Rokiah, 2010).

2.1.2 Cooking process

Dodol is one of the most common and well-accepted traditional Malaysian starch-based IMF. However, current production technologies used are not well developed to ensure their long-term survival to compete with the large-scale production of Western-style convenience foods. There is a need for such small-scale indigenous food technologies to be rapidly upgraded by the application of modern scientific principles. The dearth of detailed scientific studies and documentation where dodol is concerned is a contributory factor limiting the scope for improvement of this food (Seow & Thevamalar, 1988).

Preparation of traditional IMF involves the mixing or blending of ingredients, cooking with continuos stirring, moulding or extruding, cutting and packaging and most of these processing steps involve manual labour (Seow & Thevamalar, 1988). Dodol, as a sticky sweet rice-based dessert is made up of a few main ingredients such as glutinous rice flour (GRF), coconut milk, white sugar, palm sugar, and sometimes with the addition of permitted food additives and flavours (Karim & Bhat, 2013; Chuah et al., 2007). Traditional dodol is prepared by mixing and cooking GRF, coconut milk, white sugar and brown sugar (Seow & Thevamalar, 1988).

Traditional cooking of dodol is simple but it requires 7-8 hours of constant stirring under slow fire using firewood until the liquid mixture turns into a sticky and viscoelastic mass (Figure 2.1). It is the sticky-like texture that makes the product to be recognised as dodol, but it is preferred in a firm texture and does not stick to fingers. Traditional dodol was found to be stickier and tastier as compared to commercial dodol which requires less labour and shorter cooking time (Nasaruddin et al., 2012). When the desired consistency of dodol is achieved, the hot mass is allowed to age before it is moulded and cut into various shapes and sizes (Seow & Thevamalar, 1988). Dodol is commonly packed into tetrahedral shaped using plastic material or filled into container (Figure 2.2).

Figure 2.1 Long hours of traditional dodol cooking that involves manual labour (Google image)

Figure 2.2 Commercial dodol packed into tetrahedral shaped (Google image)

2.1.3 Dodol ingredients

Four main basic ingredients of dodol are glutinous rice flour, palm sugar, white sugar and coconut milk. Each ingredient plays a different role in dodol cooking that contributes to the physical changes along its long cooking process as well as the dark brown colour of the final product.

2.1.3(a) Glutinous rice flour

Glutinous rice, or better known as sweet rice or sticky rice, is a staple crop commonly cultivated in mainland of Southeast Asia (Golomb, 1976). Owing to its sticky and desirable natural sweet taste, it is normally used in making traditional snacks or desserts in Asia. For instance, waxy rice has been used in making mochi in Taiwan and Japan (Chuang & Yeh, 2006), hangwa and injeolmi in Korea (Choi et al., 2001), dodol in Malaysia and Indonesia (Chuah et al., 2007), and Bhoja chaul in India (Dutta et al., 2016).

Starch is the main constituent in glutinous rice flour (GRF) other than trace amount of protein and lipids. Starch is made up of two types of long chains polymer, i.e. amylose which is a linear α(1-4) glucan chain, and amylopectin, the highly branched molecules which consists of an α(1-4) glucan chain with α(1-6) branch points (Burey et al., 2009). Mass of amylopectin is larger than that of amylose, that these structural differences caused the differences in their functionality and properties significantly. It was deduced that different types of starches had been tried in the old days that eventually led to the choice of GRF to produce a dodol with desired smooth and elastic textural attribtues (Karim & Bhat, 2013).

Glutinous rice is a type of waxy rice, that waxy starch generally contains more than 95% of amylopectin (Thomas & Atwell, 1997). The presence of waxy starch in

GRF results in a long and stringy paste during dodol cooking. This is the major role of GRF in preparation of dodol as it imparts the elastic and chewy texture, which is the desirable characteristic of a dodol (Karim & Bhat, 2013). Dodol has the characteristic that it is slightly sticky to teeth and to certain extents of stickiness are desirable. Hence, the desirable characteristic of a good dodol is that it does not dissolve easily but remains in the mouth without rapid breakdown, and it can be easily detached from teeth during chewing. A good example of food that shares the similarities is cooked rice cake (mochi). Mochi is quite easy to deform but it is difficult to cut off completely (Kohyama et al., 2007). A desired mochi is the one with moderate extensibility and firmness, which corresponds to viscosity and elasticity, respectively (Isono et al., 1990).

Nonwaxy starch in regular rice flour, on the other hand, shows a less stringy paste during cooking. Therefore, it is not uncommon to include regular rice flour in dodol formulation. Incorporation of regular rice flour aims to modulate the overall texture of the final product to assure that it is not too chewy or too firm. The ratio of GRF to regular rice flour commonly used in traditional dodol is at 6:1 (Karim & Bhat, 2013).

Starch in GRF plays a major role in physical changes as indicated by the increase of thickness during the initial cooking process of dodol. This is attributed to the series of changes that take place during starch gelatinisation. Gelatinisation begins with the swelling of starch granules during heating, and breaking of intra- and inter-molecular hydrogen bonds within, follows by leaching of amylose (Singh et al., 2007; Chuah et al., 2007). Further increase of temperature causes disintegration of starch granular structure and dissolution of amylopectin with a higher degree of orientation which is evident with the increase of viscosity (Chung et al., 2006; Thomas & Atwell, 1997), a stage where the starch paste becomes thicker. Glutinous rice starch is a waxy type of cereal starch that usually swell at lower temperatures than other similar starches (Karim & Bhat, 2013).

2.1.3(b) Palm sugar and white sugar

Palm sugar is a processed sugar obtained from the flower sap of coconut or sago palm sold in the form of hard cakes (Raji et al., 2017; Karim & Bhat, 2013). Palm sugar is more commonly known as gula Melaka in Malaysia. Both gula Melaka and white sugar are incorporated into dodol cooking to impart sweetness as well as its characteristics flavour and the dark brown appearance (Karim & Bhat, 2013). Sugar which is hygroscopic in nature plays a major role in controlling water activity (a_w) in food. Hygroscopic refers to the water absorbing ability from the surrounding environment, which aids in preservation and shelf life extension purposes. Water is controlled that the environment is not suitable for chemical or biochemical reactions, that food spoilage can be prevented through inhibition of microbial proliferation (Goldfein & Slavin, 2015). Moreover, the function of sugar of a humectant extends shelf life of baked foods by preventing staleness of drying out of products (Spillane, 2006).

Gula Melaka contains reducing sugars (fructose and glucose) and sucrose, which the reducing sugars together with amino acids, participate in a chemical reaction called Maillard reaction (Karim & Bhat, 2013). Maillard reaction produces by-products that contribute to the development of flavour and colour during the dodol cooking process. Both Maillard reaction and caramelisation of sugars have taken place simultaneously that resulted in the formation of yellow-brown colour (Chen et al., 2009; Benjakul et al., 2005). Sugar caramelisation is often associated with toasting or dry heating in the presence of sugars at high concentration (Karim & Bhat, 2013).

Although sugar caramelisation enhances brown colour, degree of brownness is often associated to the extent of Maillard reaction (Fayle & Gerrard, 2002; Morales & van Boekel, 1998). It is worth noting that gula Melaka itself contains the products produced by both Maillard reaction and sugar caramelisation as the sugar mass is processed at high temperatures during the production process (Karim & Bhat, 2013).

2.1.3(c) Coconut milk

Coconut milk is a common and basic ingredient in Southeast Asian dishes and Malay cooking, as well as a key ingredient in dodol (Raji et al., 2017; Karim & Bhat, 2013). It is more commonly known as santan in Malaysia. Santan can be extracted by grating a mature coconut flesh by adding a little amount of warm water for maximum output (Raji et al., 2017). A freshly extracted santan contains 47-56% water, 27-40% fat and 3-4% protein. Hence, the fat content in santan plays a few major and functional roles in appearance and physical changes of dodol. Fat content imparts the shiny surface and overall flavour and mouthfeel of dodol. In addition, fatty acid-amylose complex formed during cooking delays starch retrogradation, which modulates the hardness and elasticity of dodol (Karim & Bhat, 2013).

Amylose is an unique linear polymers of starch that has the ability to interact with linear polar or non polar compounds to form a molecular inclusion complex, under favourable conditions (Marinopoulou et al., 2016). Complexation of amylose and fatty acid chains may occur during starch gelatinisation, which delays starch swelling by restricting water penetration and postponing water transport into starch granules, and prevents further amylose solubilisation. Subsequently, this fatty acid-amylose complex impedes the formation of amylose-amylose double helix and crystallisation of a heated starch sample upon cooling, which retards the starch retrogradation process (Putseys et al., 2010). Amylose-lipid complexes can also inhibits amylopectin recrystallisation by interacting with outer amylopectin branches (Fu et al., 2015).

2.1.3(d) Other ingredients

Other ingredients can be incorporated during dodol cooking, such as the addition of pandan (*Pandanus amaryllifolius*) leave or permitted food additives and flavours (Karim & Bhat, 2013; Chuah et al., 2007). Pandan leave is an optional ingredient that imparts characteristically fragrant aroma and flavour to dodol, rather than the colour. Green colour of pandan extract is masked by the dark brown colour of the final product. Pandan extract contains essential oils, tocopherols and tocotrienols, carotenoids, quercetin and alkaloids. The characteristic flavour of dodol was reflected by a complex interactions between these compounds with the Maillard reaction products (Karim & Bhat, 2013). Potassium sorbate is sometimes added at permitted level to extend the shelf life of a dodol.

2.1.4 Deterioration of textural quality (Hardening issue)

The attributes of dodol such as texture, colour and flavour are resulted by a complex series of reactions from a few basic ingredients. GRF is the main ingredient which plays the central role in controlling the textural attribute, whilst gula Melaka, white sugar and santan contribute to the characteristic flavours and colour. Dodol presents in a viscous and sticky mass immediately after cooking. Upon cooling, the mass will gradually achieve a firmer gel-like texture (hardening) that can be cut into freestanding shapes (Karim & Bhat, 2013).

This hardening phenomenon is called starch retrogradation, which attributed to the reassociation and rearrangement of starch molecules to a crystalline structure.

Hardening of the products during storage is one of the major problems encountered with traditional starch-based IMF, even in the absence of water (Seow & Thevamalar, 1988). It was suggested to adjust the ratio of GRF to regular rice flour in such a way that the final outcome of starch retrogradation can yield a desirably soft and chewy texture. High amount of regular rice flour causes a firmer and less chewy texture, whilst high amount of GRF results in a stringy, too soft and sticky dodol (Karim & Bhat, 2013).

Starch consists of two types of long chains polymer, i.e. amylose which is a linear α(1-4) glucan chain, and amylopectin, the highly branched molecules which consists of an α(1-4) glucan chain with α(1-6) branch points (Burey et al., 2009) (Figure 2.3). Mass of amylopectin is larger than that of amylose, that these structural differences caused the differences in their functionality and properties significantly. Native amylose contains 500-6000 glucose units depending on the botanical origin. The average molecular weight of chains with about 500 degree of polymerisation is between 10^5 to 10^6 g/mol. The amylose content varies according to the botanical origin of starch. It varies between 0% (waxy maize or waxy rice starch) and 70-80% (high amylose maize and wrinkled pea starch). Amylopectin is the main constituent of most starches and it has a very high molecular weight from 10^7 to 10^8 g/mol. As starch is a major component of dodol, it is expected that any changes in the gelatinised starch fraction that occur during storage will affect their textural properties and hence their acceptability and shelf life (Perez-Rea & Antezana-Gomez, 2018; Seow & Thevamalar, 1988). Retrogradation of starch takes place after thermal processing, when the disorganised starch macromolecules are kept at either low temperature (eg. 4 °C) or room temperature without previous dehydration or drying treatment (del Carmen Robles-Ramírez et al., 2012).

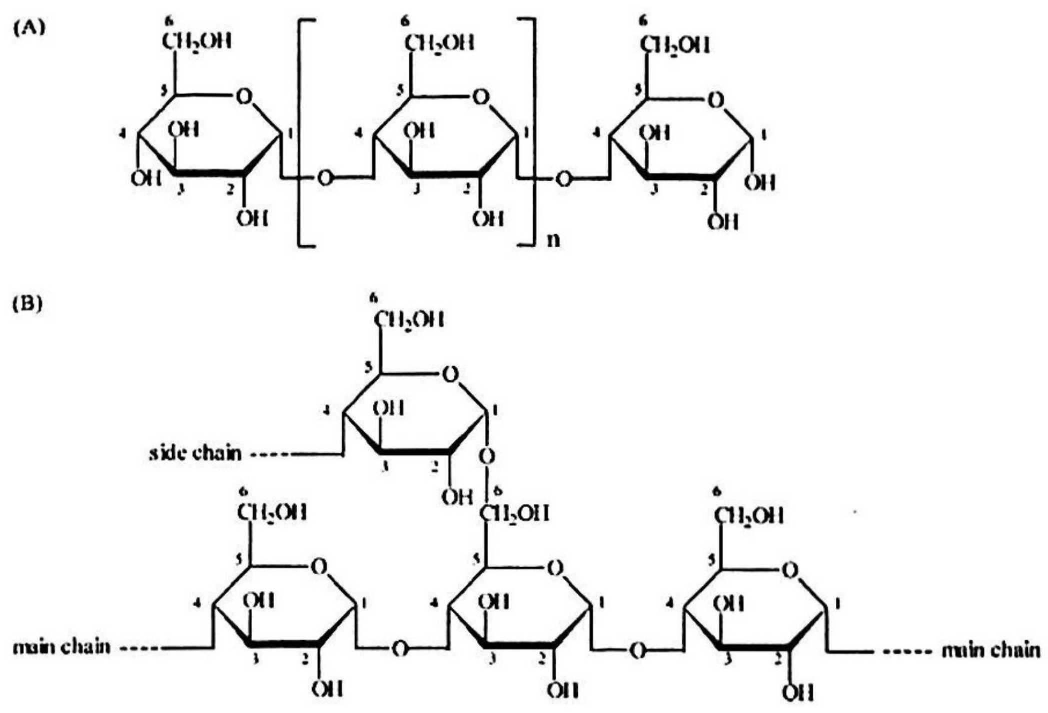

Figure 2.3 Structures of amylose (A) and amylopectin (B) (Herrero-Martínez et al., 2004)

Retrogradation of starch is generally a spontaneous crystallisation process with the system moving towards a thermodynamically more stable condition (Seow & Thevamalar, 1988). Starch retrogradation consists of short-term retrogradation and long-term retrogradation (Fu et al., 2015). Initial stage of starch retrogradation starts with the gel forming, which is due to the reassociation of linear chain amylose. Amylose retrogradation happens rapidly in starch-rich products that it takes only a few hours which is a short-term retrogradation (del Carmen Robles-Ramírez et al., 2012). The second stage of retrogradation (long-term retrogradation) occurs slowly during storage is due to the reassociation and recrystallisation of amylopectin to form a more order structure (Wang et al., 2015). Restoration of crystallinity reinforces the rigidity of starch network and the continuous starch network induced by the formation of double helices of outer branches and recrystallised amylopectin, respectively (Perez-Rea & Antezana-Gomez, 2018; Hug-Iten et al., 2003). It is worth noting that starch retrogradation is only favoured by certain length of starch chains, with the optimum size ranges from 14-24 degree of polymerisation (Shi & Seib, 1992). Hardening of dodol is mostly due to retrogradation of amylopectin during aging as the main ingredient, i.e. GRF contains around 95% of amylopectin (Thomas & Atwell, 1997).

Starch retrogradation occurs with the molecules in the dodol rearranged into a more ordered manner (from amorphous to crystalline state), as well as the reinforcement of the entanglement of molecules (Chung et al., 2006; Eerlingen et al., 1994). The reassociation of starch molecules to form ordered structures in either pastes, gels or baked foods during storage, affects their texture and shelf life (Zhang et al., 2019; del Carmen Robles-Ramírez et al., 2012). There is evidence that amylopectin retrogradation is reversible by the application of heat whilst that of amylose is not. Retrogradation occurs more readily with amylose than with amylopectin since amylose is a smaller unbranched molecule. Hence, the use of waxy starches (low amylose content) in foods can decrease the level of retrogradation. If indeed the changes in textural properties during storage were due primarily to starch retrogradation, then it would be expected that, on heating the stored samples, the textural parameters would regain, at least partially to their original values in the fresh state (Kong & Singh, 2011; Seow & Thevamalar, 1988).

In industry, starch is subjected to many physical or chemical modifications to avoid starch retrogradation, but safety issues arise due to the use of questionable chemicals. Therefore, many natural additives including carbohydrates, amino acids, and polyphenols are used to prevent the retrogradation of starch (Pan et al., 2019; Liu et al., 2019; Zhang et al., 2019; Berski et al., 2018). Interestingly, addition of increasing concentrations of water soluble sugars increased the gelatinisation temperature and enthalpy, which were attributed to the stabilisation of the crystalline region in starch and immobilisation of water by sugar molecules. Low concentration of water-soluble sugars made the retrogradation of starch slower that the softness may be durable. Addition of fructose is not as effective as glucose and sucrose, and there could be interaction effects between the sugars in mixture of sugars that modify their sole effects. The mechanism of retrogradation inhibition is considered as follows: Sugar molecules interact with starch molecular chains to stabilise the amorphous and entangled matrix of gelatinised starch (Torley & Van der Molen, 2005; Sopade et al., 2004a; Sopade et al., 2004b; Kohyama & Nishinari, 1991).

Apart from the dodol taste, texture is an essential quality characteristic that contributes to consumers' satisfaction and acceptance of food. Knowing that dodol is attributed by its unique mouth-feel properties, recent researches have studied the effects of processing such as cooking time, sugar content, and temperature on textural properties of traditional dodol. Pseudoplastic behaviour was exhibited with the evidence of decreasing apparent viscosity with increasing shear rate, indicating strong shear-thinning behaviour of dodol. Besides, increase of cooking time and sugar level has caused an increase in firmness, consistency, cohesiveness, and index of viscosity but the reverse trend was observed when temperature was increased (Chuah et al., 2007; Nasaruddin et al., 2012). The data are certainly useful to improve the dodol quality to achieve the desirable characteristics of low firmness and high cohesiveness. Nevertheless, scientific research on the starch retrogradation of dodol (hardening issue) that affects the textural quality of product is still in paucity and this underexplored area needs further investigations.

2.1.5 Other quality issues

Dodol is categorised as intermediate moisture food (IMF) with moisture content between 10-50% and a_w in the range of 0.65-0.90. IMF is defined as a food preserved by restricting water mobility at ambient temperatures which has sufficient moisture to be consumed directly without rehydration (Prabhakar, 2014). Although rapid microbial spoilage is unlikely to occur in dodol with a_w at 0.69 (Nasaruddin et al., 2012), this product is susceptible to yeasts and moulds growth that may occur at a_w 0.60 and above (Prabhakar, 2014). Other than issues of hardening, surface moulds growth and objectionable flavour arise from rancidity upon storage contribute to the short shelf life of dodol with only approximately 2-3 weeks (Zahid et al., 2012).

To overcome quality issues in dodol, Malaysia Agricultural Research and Development Institute (MARDI) has developed an enzymatic dodol processing technology with the use of enzyme, heat treatment and effective packaging material to improve the quality of commercial dodol. The use of amylase enzyme synthesised by bacterial culture that cuts the starch chains into smaller units in a controlled manner has shown promising effect. Enzymatic dodol retains its desirable organoleptic qualities such as taste, softness, and chewiness upon storage and there is no mildew or moulds growth observed. Most importantly, its shelf life has been greatly extended that it could be stored up to 6 months (Zahid et al., 2012; Hamzah et al., 2007). Nonetheless, despite the enhanced quality, the addition of amylase into dodol has raised the cost in production that the dodol industry has to hold back on this exciting idea. There are cheaper amylase available in the market (i.e. from porcine sources) but the Halal issue arises regarding the source of the enzyme.

Owing to the use of santan with high fat content as one of the main ingredients in dodol, randicity issue due to the fat oxidation that occurs during storage is unavoidable. Interestingly, recent study found that rancidity as indicated by the thiobarbituric acid value was reduced after an edible coating breadfruit starch (*Arthocaypus altilis*) with glyerol was applied on dodol surface (Triwarsita et al., 2013). These processing approaches have extended the shelf life of dodol by solving the quality issues arise.

Currently, there is no standard or specifications established as guidelines for local dodol industry in Malaysia. Indonesian National Standard (SNI) for dodol (SNI 01-2986-1992) established by the Indonesian Standardisation Body (BSN) may serve as a reference but its emphasis is mainly on the appearance, proximate composition, heavy metals and microbiological analysis content (Badan Standardisasi Nasional, 1992). The huge variation in quality of commercial dodol is due to the use of different dodol recipes, processing parameters and practices.

2.2 Honey

2.2.1 Background

Honey is extracted by bees from the flower nectar and saccharine exudation of plants (Singh & Bath, 1997). It is a combination of by-product of nectar and the upper aero-digestive tract of a bee, as a salivary secretion. It is gathered, concentrated and modified through a dehydration process and stored inside a bee comb to ripen and mature into honey (Biluca et al., 2017; Eteraf-Oskouei & Najafi, 2013; Singh & Bath, 1997).

Honey has been widely consumed in its unprocessed state as a natural tonic foods as well as one of the oldest traditional medicines that play a vital role in treatment of human ailments since ancient times (Chew et al., 2018; Eteraf-Oskouei & Najafi, 2013; Chua et al., 2012; Mandal & Mandal, 2011). Recently, there are many research studies reported the bioactivities of honey such as antioxidant, anti-microbial, anti-mutagenic, anti-inflammatory, anticancer and wound healing properties (Chew et al., 2018; da Cunha et al., 2018; Rao et al., 2016; Alvarez-Suarez et al., 2014; Ferreira et al., 2009). However, honey is more commonly used as a sweetening additive in food applications (Kadam et al., 2010; Varga, 2006; Chick et al., 2001). Honey offers beneficial effects to human health due to the presence of various natural chemical compounds. Furthermore, honey serves as an excellent energy food, with an average content of approximately 3.5 kcal/kg (Kadam et al., 2010).

Two types of honey that are commonly consumed in Malaysia are: honey bee honey (HBH) and stingless bee honey, or it is locally known as kelulut bee honey (KBH). The most common bee species of *Apis* genera that produces bee honey is *Apis mellifera* and it is mainly found in Europe and Asia (El Sohaimy et al., 2015; Moniruzzaman et al., 2013; Guerrini et al., 2009). Other subspecies such as *Apis dorsata*, *Apis florea* and *Apis cerana* are distributed in Southern and Southeastern Asia (Kek et al., 2014). On the other hand, of over 500 species of kelulut bee found throughout the world, *Heterotrigona itama* and *Geniotrigona thoracica* are among the two *Trigona* species. which are commonly reared and found in Malaysian forests (Shamsudin et al., 2019). There are more than one type of genera for the tribe of *Meliponini*, i.e. Trigona Melipona and Scaptotrigona. These kelulut bees are native to tropical and subtropical parts of the world like Asia, Central and South America, Africa and northern Australia (Boorn et al., 2010).

HBH from *Apis* species are stored in hexagonal-shaped honey combs, whilst KBH from *Trigona* species are deposited in small resin pots clusters close to the extremities of their nests (Kek et al., 2014). It is comparatively easier to harvest KBH than HBH because kelulut bee does not sting (Nordin et al., 2018). On contrary to honey bees which are normally lost and more vulnerable to diseases, artificial hive is always constructed to increase the honey production by manipulating the colony as kelulut bee are not obnoxious in selecting place for hive building (Abd Jalil et al., 2017; Kek et al., 2014).

2.2.2 Chemical composition

Honey is a nutritious food that contains about 200 substances (Eteraf-Oskouei & Najafi, 2013). The variation in composition is mainly owing to the different floral source, geographical origins, producing species, climate and harvesting time (Biluca et al., 2016; de Rodríguez et al., 2004; Singh & Bath, 1997). Despite the various constituents present in honey, four main components that contribute to the honey characteritics and nutritional composition are sugars, protein, organic acids, vitamins and minerals.

2.2.2(a) Sugars

About 75% of sugars present in honey are monosaccharides, together with approximately 10–15% of disaccharides and trace amounts of other sugars (da Silva et al., 2016). Three patterns of sugar composition in nectar were obtained through analysis of 889 floral species, i.e. high sucrose nectar, nectar with equal amount of major sugars (i.e. fructose, glucose and sucrose), and high glucose and fructose nectar. The relationship between sugars ratio and floral species causes the variation of sugar content in honey due to different botanical sources (Doner, 1977; Percival, 1961).

Other than major sugars, determination of sugar profiles of honey found that honey contains other sugars in varying amount such as turanose, nigerose, rhamnose, maltose, kojibiose, trehalose, nigerobiose, maltotetraose, maltotriose, maltulose, melibiose, palatinose, raffinose, isomaltose, erlose and melezitose (Anjos et al., 2015; de la Fuente et al., 2011; Ruoff et al., 2006; Doner, 1977). Sugars present in honey contribute to the honey properties such as viscosity, hygroscopicity, energy value and granulation (Kamal & Klein, 2011). Addition of honey into duck jerky samples was found to retain more moisture than other humectants such as rice syrup and sorbitol, and incorporation of increased concentrations reduced the a_w of products with better stability achieved (Triyannanto & Lee, 2016).

2.2.2(b) Protein

Amino acids make up to around 1% (w/w) of the honey's constituents and their relative proportions are based on the honey's origin (Hermosín et al., 2003). Honey contains numerous amino acids such as proline, glutamic acid, aspartic acid, glutamine, histidine, glycine, threonine, alanine, a-alanine, b-alanine, phenylalanine, leucine, isoleucine, arginine, caminobutyric acid, tyrosine, valine, ornithine, lysine, serine, asparagine, methionine, cysteine and tryptophan (Kečkeš et al., 2013; Di Girolamo et al., 2012; Rebane & Herodes, 2010; Iglesias et al., 2006; Hermosín et al., 2003). A few common amino acids are proline, glutamic acid, tyrosine, alanine, phenylalanine, leucine and isoleucine, with proline being the predominant one that represents around 50-85% of amino acids (Truzzi et al., 2014; Di Girolamo et al., 2012; Iglesias et al., 2006). Proline can be used as an indicator for maturation or adulteration of honey, whereas a pure honey should have a minimum proline value at 180 mg kg^{-1} (Hermosín et al., 2003). Free amino groups of amino acids may participate in Maillard reaction with the presence of reducing sugars during heat processing or unfavourable storage conditions may produce undesirable products that affect the quality of honey (da Silva et al., 2016).

Honey also contains enzymes such as diastase, glucose oxidase, catalase, invertase, both α- and β-glucosidase, and acid phosphatase (Sak-Bosnar & Sakač, 2012; Won et al., 2008). Diastase is one of the most vital enzyme that determines honey quality. It consists of both α- and β-amylase, which cleaves the branched chains into shorter chains through α-1,4 linkages, at an internal site to produce dextrin, and in a stepwise trend initiating from the non-reducing end to produce maltose, respectively (da Silva et al., 2016; Sak-Bosnar & Sakač, 2012; Thomas & Atwell, 1997). β-amylase (EC 3.2.1.2) is an exoenzyme that hydrolyses the penultimate α-(1,4) linkage from the nonreducing end of the polymeric chains and releases the disaccharide maltose. β-amylase, acting alone, can degrade amylose completely to maltose. This enzyme will not, however, attack the α-(1,6) linkages or α-(1,4) linkages close to the α-(1,6) links; hence, it degrades only the outer chains of amylopectin and leaves a large portion (called β-limit dextrins), in which the outer chains have been degraded to stubs of two or three glucose residues adjacent to the α-(1,6) linkages (Meshram et al., 2019; Izydorczyk & Edney, 2003).

Amylase enzyme has been known to be present in honey for over 100 years and it is finally acknowledged to originate from bee as salivary secretions (Babacan et al., 2002; Vit & Pulcini, 1996). Characterisation of honey amylase found its optimum temperature and pH to be 55°C and within the range of 4.6-5.3, respectively (Babacan & Rand, 2007). Addition of honey to starch matrix resulted in starch thinning and a significant viscosity loss, and there was no effect on viscosity observed if the honey has been preheated prior to addition. This clearly explained that starch degradation has taken place due to the presence of amylase activity. Amylase hydrolyses starch and