THE EFFECTS OF ACID DIPPING AND IN-PACK PASTEURIZATION ON THE SAFETY AND QUALITY OF FRESH RICE NOODLES

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by

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LIST OF SYMBOLS

g	Gram
mL	Millilitre
%	Percentage
±	Plus minus
^{o}C	Degree Celsius
g	Gram

LIST OF ABBREVIATIONS

AACC	American Association for Clinical Chemistry
B. cereus	*Bacillus cereus*
CFU/g	Colony forming unit per gram
cm	Centimetre
GDL	Glucono-delta lactone
GPC	Gel permeation chromatography
hr	Hour
min	Minutes
mm	Millimetre
mm/sec	Millimetre per second
MPN/g	Most probable number per gram
rpm	Rotation per minute
RVA	Rapid visco analyser

KESAN PENCELUPAN ASID DAN PEMPASTEURAN DALAM PEK TERHADAP KESELAMATAN DAN KUALITI MI BERAS SEGAR

ABSTRAK

Mi beras segar mempunyai jangka hayat yang pendek dan tidak melebihi 3 hari kerana risiko mikroorganisma dan retrogradasi kanji. Dalam kajian ini, pengawetan mi beras segar menggunakan glukono-delta lakton (GDL) sebagai celupan mandi dan pempasteuran dalam pek telah dijalankan. Hipotesis kajian ini adalah dengan menggunakan teknologi rintangan gabungan seperti mengurangkan pH permukaan mi dengan pencelupan GDL, pembungkusan vakum, dan rawatan haba pempasteuran dalam pek, mi bungkus terpasteur yang stabil dalam keadaan ambien dapat dihasilkan. Daripada analisis *Rapid visco analysis* (RVA), didapati bahawa nilai *final viscosity* (FV) dan *setback viscosity* (SB) meningkat dengan peningkatan progresif kepekatan GDL (0 %, 0.05 %, 0.1 %, 0.5 %, 1.0 % dan 1.5 % w/v). Didapati bahawa peningkatan kepekatan asid GDL dapat mendorong kadar retrogradasi kanji beras. Hasil kajian ini boleh dibuktikan dengan keputusan analisis profil tekstur (TPA), di mana kekerasan gel yang terbentuk dengan penambahan GDL adalah lebih tinggi daripada sampel gel yang tidak ditambah GDL. Dapatan ini menunjukkan bahawa retrogradasi kanji berlaku lebih pantas dengan penambahan GDL dan sangat penting dalam aplikasi praktikal. Didapati juga bahawa ketika mi beras dicelupkan di dalam larutan GDL dan dikenakan pempasteuran dalam pek, mi adalah jauh lebih mudah terlerai antara satu sama lain, tidak seperti sampel kawalan yang berubah menjadi blok selepas disimpan. Kanji molekul yang berada di permukaan mi dihipotesikan mempunyai kadar retrogradasi yang lebih tinggi daripada yang di dalam teras mi. Oleh itu, suatu lapisan kering di permukaan mi terbentuk dan dapat mengelakkan penghijrahan kelembapan

daripada teras mi. Penjelasan ini disokong oleh hasil analisis desorpsi, di mana mi GDL yang dicelupkan dan dipasteurisasi dalam pek menunjukkan pengekalan kelembapan yang lebih tinggi berbanding dengan sampel kawalan. Molekul air yang terperangkap dalam teras mi dapat membantu menangguhkan retrogradasi mi, seperti yang digambarkan oleh hasil analisis indeks retrogradasi mi yang tercelup asid. Analisis mikroorganisma mi beras yang dicelupkan asid 1.0 % dan rawatan haba ringan dalam pek (62 ^{o}C / 1 jam) didapati boleh memanjangkan jangka hayat mi beras segar. Untuk menubuhkan suatu rawatan haba protokol untuk produk mi beras segar, inokulasi patogen *Bacillus cereus* (*B. cereus*) ke dalam mi bungkus menggunakan suhu pasteurisasi yang lebih tinggi (85, 90, 95 ^{o}C) telah dijalankan. Mi yang dicelupkan dalam larutan GDL 1.0 % w/v selama 2 minit didapati mencukupi untuk mengurangkan jumlah *B. cereus* sebanyak 2 log CFU/g. Daripada analisis *D-value*, pencelupan GDL dan pempasteuran mi pada suhu 95 ^{o}C selama 27.9 minit dapat mengurangkan sel *B. cereus* sebanyak 6 log. Walau bagaimanapun, rawatan haba yang terlampau ini didapati tidak sesuai untuk aplikasi produk mi beras. Hal ini adalah kerana suhu pempasteuran yang tinggi dan masa rawatan yang panjang telah menjadikan tekstur dan penampilan mi tidak dapat diterima untuk dikomersialkan di pasaran.

THE EFFECTS OF ACID DIPPING AND IN-PACK PASTEURIZATION ON THE SAFETY AND QUALITY OF FRESH RICE NOODLES

ABSTRACT

Fresh rice noodles have short shelf life of not more than 3 days due to microbial risk and starch retrogradation. In this study, the preservation of fresh rice noodles using glucono-delta lactone (GDL) as dipping bath acidulant and in-pack pasteurization was elucidated. It was hypothesized that when GDL solubilizes in water, a low pH system with mild tart flavour would be a good dipping bath acidulant for fresh rice noodles. Whereas in-pack pasteurization that involves vacuum packaging and thermal processing can further enhance the preservation effects on the fresh rice noodles. From the rice flour pasting analysis, it was found that the final viscosity (FV) and setback (SB) values increased with progressive increase in GDL concentration (0 %, 0.05%, 0.1%, 0.5%, 1.0% and 1.5% w/v), indicating acid addition promotes retrogradation rate of rice flour. This was substantiated with the texture profile analysis (TPA) results, wherein hardness value of the gel formed with GDL were harder than the control, indicating retrogradation happened faster with GDL addition. This observation showed great practical significance. It was found that when rice noodles were dipped in GDL and subjected to in-pack pasteurization, the noodle strands were much more easily being detached from each other, unlike the control samples that turned into a block. It is speculated that the starch molecules resided on the surface retrograded faster than those counterparts resided in the core of the noodles strand. Thus, a dryer and protective layer was formed to prevent moisture migration from the core of the noodles. This explanation was supported by the desorption analysis results, where GDL-dipped and in-pack pasteurized noodles showed higher moisture retention

compared to the control samples. The retained water molecules in the core of the noodles might help to delay dehydration of the noodle strand and thus lower retrogradation index was recorded for acid dipped noodles. The microbial analysis (total plate count, yeast and mould count) of the 1.0 % w/v acid dipped rice noodles followed by in-pack mild heat treatment (62 ^{o}C/ 1 hr) for one-month storage showed a clean microbial count, indicating this combination worked in promoting shelf life of the fresh rice noodles. To establish a thermal processing protocol for this fresh rice noodles product, inoculating challenge pathogen *Bacillus cereus* (*B. cereus*) into the packed product using a higher pasteurization temperature (85, 90, 95 ^{o}C) was performed. Noodles dipped in 1.0 % w/v GDL bath for 2 mins was found adequate to reduce *B. cereus* count to around 2 log CFU/g. From the *D*-value study, acid dipping and pasteurization at 95 ^{o}C for 27.9 minutes was adequate to reduce *B. cereus* by 6-log. Nevertheless, this severe processing was found to be not suitable for rice noodles product. The high pasteurization temperature and long-time processing has rendered the noodles' texture and appearance to be unacceptable for commercialization in the market.

CHAPTER 1

INTRODUCTION

1.1 Background

Rice noodles have been a staple food since ancient times in rice-producing countries like Thailand, Vietnam, China and Malaysia. The consumption of fresh rice noodles is a common practice to the local, as the noodles are manufactured daily to cater for the local market demand. Rice noodles stored for more than 3 days are not favourable to consumers due to their significant change in texture and potential health risks. Owing to the nature of starch molecules tends to retrograde, rice noodles tend to become harder and difficult to be separated into individual strands upon storage. Moreover, fresh rice noodles are typically high in moisture content and water activity, which is favourable to microbial growth. If the rice noodles can be packed and remained texturally and microbiologically stable for more than 3 days, this would help the manufacturers to widen their distribution market.

Hurdle technology has been widely used in the food industry in securing food safety and quality during the storage process (Leistner and Gould, 2012). Combined technologies such as by modifying product pH, storage temperature, and storage atmospheric environment have been widely used in the food industry. Each technology has its own pros and cons when being adopted to resolve a particular issue pertaining to safety and quality of a particular category of food. The preservation of fresh noodles is challenging because they are susceptible to chemicals and physical treatments. A few works has been done on preserving fresh noodles especially the wheat type using irradiation (Jianming, 1998; Li *et al.*, 2011) and preservative agents (Huang *et al.*, 2007; Klinmalai *et al.*, 2017). On the other hand, Rachtanapun and Tangnonthaphat (2011) modified the packaging condition and storage temperature of fresh rice noodles.

However, the shelf life of the treated rice noodles was only 29 days with textural quality defects.

Acids have been used in preserving foods for centuries. Food is categorized into low acid food, medium acid food, acid food and high acid foods. Practically, acid can be added into the noodle dough system to lower the pH of the product to below 4.6 and act as a barrier for microbial attack. Glucono-delta-lactone (GDL) is mainly used in soy protein coagulation during tofu making, cheese curd formation, as well as enhancing heat stability of milk. Many food products has been added with GDL for preservation study such as fish fillets (Poligne and Collignan, 2000), cooked rice (Kim *et al.*, 2004), sausages (Böhnlein *et al.*, 2017; Thomas *et al.*, 2008) and vegetables (Zhou *et al.*, 2020). It was also found to be useful in prolonging the shelf life of Japanese udon, wherein GDL was added into the udon flour mix to effect a low pH dough in order to delay spoilage (Sumitra *et al.*, 2006). To the best of our knowledge, GDL has not been studied in rice noodles preservation as GDL is rarely added into the formulation of starch-based system. The acid might weaken the network of the dough or noodles system, producing a brittle noodles strand.

Heat treatment is a very effective tools in controlling the growth of microorganisms in food. The hurdles that involves hygienic food preparation, vacuum packaging, pasteurization and rapid cooling is known as in-pack pasteurization and widely applied in various food products. This includes fish products (García‑Linares *et al.*, 2004; Gonzalez-Fandos *et al.*, 2004; Stormo *et al.*, 2018; Tsironi *et al.*, 2015), meat and poultry products (Dawson, 2008; Mangalassary *et al.*, 2007; Szerman *et al.*, 2007), fruits and vegetables products (Grauwet and Shpigelman, 2018; Kathiravan *et al.*, 2014; Muñoz *et al.*, 2017), but noodles product has not yet been reported.

The heat treatments established aims to ensure the coldest spot of the product received adequate thermal processing and the rate of microbial inactivation follows a first order kinetic reaction is to be determined. *Bacillus cereus* (*B. cereus*) is a spore forming bacteria that often associated with rice noodles, pasta and pastry outbreak due to temperature abuse during handling and storage. In this project, the emetic type of *B. cereus* is used as the challenge pathogen in the ready-to eat packed rice noodles. *D* and *z*-values are the basis in thermal process calculation and is necessary in thermal processing establishment. The values obtained from the study enables a further understanding of the thermal destructive trends of *B. cereus* in ready-to eat acid dipped in pack pasteurized fresh rice noodles.

From the results obtained, the process is expected to be optimized so that an appropriate combination of temperature and time used for thermal processing is established without compromising the safety and quality issues. However, the destruction of *B. cereus* required severe processing that higher temperature of pasteurization is necessary. The inactivation of this pathogen in various food products (milk, meat and poultry, rice, peppers and others) with different types of preservation technique (thermal processing, high pressure processing, pulsed electric field, ohmic heating, radiation and others) were widely studied (Bandla *et al.*, 2012; Bermúdez-Aguirre *et al.*, 2012; Black *et al.*, 2008; Byrne *et al.*, 2006; Chai *et al.*, 2014; Choudhary *et al.*, 2011; Daryaei *et al.*, 2013; Espejo *et al.*, 2014; Evelyn and Silva, 2015; Kim *et al.*, 2017a; Kim *et al.*, 2017b; Kim *et al.*, 2013; López-Pedemonte *et al.*, 2003; Pina-Pérez *et al.*, 2009a; Pina-Pérez *et al.*, 2009b; Scurrah *et al.*, 2006; Van Opstal *et al.*, 2004). Nevertheless, the inactivation of *B. cereus* in noodles product has yet to be reported so far

1.2 Problem statement

Short shelf life (2-3 days) of fresh rice noodles due to its high susceptibility to microbial attacks and its tendency to retrograde over storage have limited the fresh rice noodles distribution market. It is a commonplace fact that the fresh rice noodles' market is confined to the geographical proximity of the noodles' producers, and many a time alluring fresh rice noodles dishes become a tourist attraction for the place at where the noodles are produced.

According to market research published by Grand View Research Inc. (Grand View Research Inc, 2016), rice noodles market size in Europe and Asia Pacific was estimated to be USD 1.69 billion in 2014, and this figure is forecasted to increase steadily through year 2022 to reach USD 3.6 billion. It is interesting to note that China and India are expected to propel the expansion of the market on a worldwide basis. The key factors influencing the expansion of this rice noodles market are attributable to consumer awareness of maintaining a healthy lifestyle. To ensure a sustainable market growth, rice noodles manufacturers are striving for technologies to support and enable expansion of distribution channels to reach a broader target market. It is challenging to distribute fresh wet rice noodles because the shelf life of the fresh wet rice noodles is relatively short (2 – 3 days) due to its high moisture content (62.51 %) and high water activity ($a_{w,}$ 0.91) (Rachtanapun and Tangnonthaphat, 2011).

Besides that, the post-cooking operations such as washing and packing using tap water are suspected to be the main points of contamination which contributes to the short shelf life of fresh rice noodles. During the production of fresh rice noodles, rice flour and water formed a cohesive dough and subjected to extrusion into boiling water. The extruded rice noodles strands get cooked in boiling water and the product is practically "sterile" since boiling water is able to eliminate any potential biological hazards. However, the hot rice noodles need to be cooled immediately using tap water to initial first stage of retrogradation and also wash off the excessive starch adhere on the surface of noodle strands. Since the noodles has been cooled off, the unhygienic conditions of the packing process might post threat to the safety of the fresh rice noodles. These explains the possible contamination points that contributes to the short shelf life of fresh rice noodles.

Besides that, over storage (> 2-3 days), rice-based noodles tend to stick into a block, become hard and brittle due to retrogradation. The realignment of amylose and amylopectin chains which squeeze out the water molecules from noodles strand resulted in the hard texture of rice noodles. The phenomenon was known as retrogradation and the recovery of individual noodle strands after storage was impossible due to the hardened noodles' poor handling property. Since texture quality is considered as one of the shelf life indicators, the changes in texture especially the retrogradation factors is relatively important besides safety issues. These two main factors lead to the short shelf life of fresh rice noodles.

1.3 Hypothesis and experiment outline

To improve the microbial quality of fresh noodles, acid dipping is more likely to be able to decrease the pH value of noodles surface to below 4.6 and reduce microbial growth. It was hypothesized that when GDL solubilizes in water, a low pH system with mild tart flavour would be good for acid dipping of fresh rice noodles. More often if a combination of hurdles such as in-pack pasteurization is used, the safety of the packed rice noodles will be improved significantly. On the other hand, the textural quality of the fresh rice noodles after acid dipping and in-pack pasteurization might change due to surface modifications of the acid and heat treatments.

The starch molecules on the surface of the noodle strand might experience partial hydrolysis effects and more short chains of amylose being freed, resulting a higher intermolecular association. The starch molecules on the surface is expected to retrograde faster than those counterparts in the core of the noodles strand. Thus, the retrograded outer part of the noodle strand might act as a barrier that keep noodles apart and not sticking to each other. It is hypothesized that GDL dipping coupled with in-pack pasteurization are not only able to improve microbial quality of the fresh rice noodles, but also able to promote the handling and textural properties of rice noodles over storage.

In this study, the effects of GDL towards pasting and textural quality of rice flour and its gel were investigated. This aims to predict the behaviour of the rice noodles when it is being dipped in GDL solution followed by heat processing. Moisture content and the textural qualities of the acid dipped rice noodles were determined as well to test on the hypothesis. Microbial qualities of the treated and untreated noodles were monitored for a month under room temperature (25 ^{o}C) storage.

To validate the hurdles protocol, a challenge study using *Bacillus cereus* was carried out with an inoculation of 10^8~10^9 cells. The pathogen was intended to challenge the previously developed hurdles (Byrne *et al.*, 2010). However, samples were subjected to a higher pasteurization temperature (85, 90 and 95 ^{o}C) instead of 62 ^{o}C/ 1 hr due to the insufficiency of the thermal processing. The composition dependent microbial inactivation kinetic data (*D*-value and *z*-value) of this pathogen in rice noodles can be determined. At the end of the project, a three-month storage study was carried out to determine the shelf stability of the noodle products treated with 1.0 % w/v GDL dipping and in-pack pasteurized at 95 ^{o}C for 0, 10, 20 and 30 mins.

1.4 Objectives

The general objective of this project is to improve the safety and quality of packed fresh rice noodles. The specific objectives are listed as follows:

a) To determine the effects of GDL on the properties of rice flour and the quality of rice noodles.

b) To determine the effects of GDL dipping and mild heating treatment on the microbial and textural quality of rice noodles.

c) To determine the thermal death time of challenged pathogen, *Bacillus cereus* (*B. cereus*) affected by GDL dipping and different pasteurization temperature.

d) To determine the microbial and textural quality of rice noodles pasteurized with different pasteurization time (0, 10, 20 and 30 mins) for three months storage.

CHAPTER 2

LITERATURE REVIEW

2.1 Rice starch

2.1.1 General properties

Rice (*Oryza sativa L.*) is one of the major sources of staple food consumed by half of the population in the world. Rice is normally classified as long, medium- and short grain rice. Rice starch is unique because it has a broad range of amylose to amylopectin ratio (Mitchell, 2009). Rice starch is also known for its broad range of granule size distribution and rice granules are the smallest (2 – 7 µm) when compared to other starches (Vandeputte and Delcour, 2004). Rice starch granules are polygonal in shape that facilitates a compact packing among rice granules.

When starch granules are heated in excess of water, the swelling of granules and the leaching of amylose leads to gelatinization. Once the swollen granules reached its maximum swelling capacity, the granules will rupture and release all its amylose and amylopectin molecules. The resulted paste will form soft gel or hard gel upon cooling depending on the concentration used. The quality of rice starch gel strength is influenced by factors such as starch varieties, rice ageing, grain milling and fermentation. For instance, different amylose and amylopectin composition in rice starch granules of different rice cultivars, determines the structural network strength of the noodle's product.

2.1.2 Rice grain variety

Short grain rice has 0% amylose content, it is known as sticky, waxy or glutinous rice. On the other hand, long grain rice contains 20-25% amylose and the highest amylose content recorded can reached up to 35% (Corke, 2015). Generally, long-grained rice with intermediate to high amylose content (> 22% amylose) are preferred in the rice noodles production (Kohlwey *et al.*, 1995). According to Li and Luh (1980), in order to produce good quality rice noodles, the rice flour should be derived from rice varieties that is high in amylose content and can be gelatinised at a relatively low temperature.

Lu *et al.* (2009b) used two types of rice, namely non-waxy *Indica* and waxy *Japonica* to produce rice starch gel and the information acquired was used to relate molecular weight distribution of the rice starch to the rice noodle texture. The study showed that besides amylose contents and rice varieties, chain length distribution of the amylopectin fraction plays a significant role in determining the dynamic viscoelastic properties of the rice gel. It was reported that, the super long chains in amylopectin is accountable for the slower retrogradation observed in *Japonica* rice starch gels; thus *Japonica* rice paste is too sticky for production of rice noodles.

Han *et al.* (2011) have further investigated the suitability of long grained rice variety in rice noodles production by comparing different Korean rice varieties. The two high amylose line of Korean rice varieties were found to be most suitable for making extruded rice noodles with excellent cooking and textural properties. Similar findings were supported by Bhattacharya *et al.* (1999) and Yoenyongbuddhagal and Noomhorm (2002) who reported that rice noodles quality is significantly correlated with starch swelling power, pasting properties and cohesiveness of the gel network formed. All these properties are also found correlated well with the starch amylose content which affected by rice grain variety.

2.1.3 Rice grain ageing

It is a common practice to use aged rice to produce rice noodles, which is normally about 9 months. Rice ageing happens before harvesting and continues to happen post-harvest in the post-harvest period. This process of aging is found to be dependent on time, temperature and moisture content (Okabe, 1979; Perdon *et al.*, 1997). When rice grains are stored for a certain period of time, the chemical composition remains unchanged, but a number of physicochemical and physiological changes occur (Zhou *et al.*, 2002). These changes influence pasting properties, flavour, colour, and the texture of cooked rice.

Moritaka and Yasumatsu (1972) have suggested that free fatty acids formed during the ageing process will form a complex with amylose molecule, and carbonyl compounds and hydroperoxides formed during the ageing process will accelerate protein oxidation and condensation, as well as causing further accumulation of carbonyl compounds. Disulphite linkages formed during protein oxidation and increased in the strength of micelle binding of starch were found to inhibit starch granules swelling and thus affecting cooked rice texture. Mod *et al.* (1983) reported that ferulate esters (found in hemicellulose) oxidation contributes to cross-linking and resulted in enhanced cell wall strength. It was found that this enhancement prevents a high degree of starch damage during the rice milling process. Rice flour with high degree of starch damage will produce rice noodles with a high degree of cooking loss, which could be attributed to a greater water solubility of the damaged starch (Heo *et al.*, 2013).

2.1.4 Rice grain milling

Rice flour can be prepared by dry-milling or wet-milling, but it must be noted that the physicochemical properties of the flour produced differ significantly. Dry-milled flour can be prepared by subjecting the rice grains to direct grinding using roller mill whereas to prepare wet-milled flour, the rice grains need to be steeped in water for hours before grinding and then dried to target moisture content. It has been found that wet-milled rice flour is preferred in rice noodles production. During the milling process, the rice flour is exposed to mechanical and heat energy and this is of course more intensive in dry milling than wet milling. As a result, more damage to the starch granules is found in dry-milled flour than the wet-milled counterpart (Chiang and Yeh, 2002; Heo *et al.*, 2013). As dry milling produces a higher amount of broken rice starch granules, the resultant flour exhibits higher water absorption and increased cooking loss (Heo *et al.*, 2013; Yoenyongbuddhagal and Noomhorm, 2002).

2.1.5 Rice grain fermentation

In China and South Asia, fermentation of rice grains prior to milling is an essential traditional processing step in the production of rice noodles (Lu *et al.*, 2009a). Starch granules of fermented rice flour have been reported to show decreased gelatinization temperature, able to swell to a greater extent and has higher resistance towards breakdown (Yang and Tao, 2008). The fermented rice flour gel was able to form with a fine structure with a slower retrogradation rate, which would be a desired texture feature of rice noodles (Lu *et al.*, 2009a). Lu *et al.* (2003) also provided evidence that fermented rice noodles have a more favourable chewy mouthfeel compared to the unfermented ones. Lu *et al.* (2005) suggested that fermentation changed the starch granules crystalline region and its chemical composition with a decreased protein, lipid and ash content, all these were found to favour the formation of hydrogen bonding between and among starch molecules in the rice flour as well as the rice noodles. The study conducted by Yang and Tao (2008) authenticated this result who reported that during fermentation, the crystalline structure of starch granules was moderately damaged and collapsed, thus causing a decline in breakdown and setback of the rice starch granules. This accounts for the lower disintegration and retrogradation rate seen on fermented rice noodles.

2.2 Modifications on rice starch

The gelatinization and retrogradation behaviour of starch are largely influenced by the physical and chemical modifications. During gelatinization, starch granule absorbs water and get swollen, the maximum viscosity is reached when all the granules swell, and amylose leached out from the granule, which the process is called pasting. Whereas, the realignment and reassociation of amylose amylopectin chains in the gelatinized paste back to a more structured order is known as retrogradation. To understand the changes in behaviour of native starch before modification, some general characteristic of the pasting properties of native rice starch is showed in Table 2.1.

Table 2.1 General gelatinization and pasting properties of native rice starch.

Properties	Native rice starch
Gelatinization temperature (^{o}C)	68 to 74 to 78
Brabender peak viscosity (BU at 8% starch concentration)	500
Swelling power at 95 ^{o}C (Weight of sedimented swollen granules per gram of dry starch)	19
Paste viscosity	Medium-low
Cold paste texture	Short
Paste clarity	Opaque
Resistance to shear	Medium
Retrogradation rate	High

Source: Biliaderis (2009)

2.2.1 Acid modifications

Acid treatment on starch is a very common way of starch modification. By suspending starch in 1 – 3 % of hydrochloric acid medium for 12- 14 hr at 25 – 55 ^{o}C is the most common method of producing acid modified starch (Joye, 2019). Acid modification happened at two distinct stages whereby both amylose and amylopectin were attacked during the early stage of treatment but mainly happened to the armorphous region, in the later stage the crystalline region which mainly composed of amylopectin molecules were attacked at a slower rate, depending on the extent of hydrolysis commenced (Thirathumthavorn and Charoenrein, 2005; Wang and Wang, 2001). During the treatment, the glycosidic bonds of the starch granule were cleaved (Hoover, 2000). Common characteristics of acid treated starch are lower gelatinization temperature, lower hot -paste viscosity and more soluble as a result from the molecular weight reduction (Rohwer and Klem, 1984). To the best of our knowledge, there is no literature works reported on improving fresh rice noodles safety and quality via acid treatment coupled with in-pack pasteurization.

2.2.2 Heat modifications

Heat modifications include annealing and hydrothermal treatments on starch. Annealing on starch is defined as hydrothermal treatment on starch in excess water (> 60 % w/w) or at intermediate water content (40 % - 55 % w/w) (Jacobs and Delcour, 1998). This hydrothermal treatment occur at above the glass transition temperature (T_g) but below gelatinization temperature which can modify the physicochemical properties of a starch without destroying its network structure (Adebowale *et al.*, 2005). There are several physicochemical changes induced by annealing and the principal changes are: (1) decrease in the swelling power and solubility (Eerlingen *et al.*, 1997; Gomes *et al.*, 2004; Hoove and Vasanthan, 1993), (2) reduce the potential and extent of amylose leaching; an increase of gelatinization temperature and enthalpy, a narrower range of the gelatinization temperature (Gough and Pybus, 1971; Jacobs *et al.*, 1997; Sekine *et al.*, 2000); and (3) a decline in peak viscosity and retrogradation trend (Gomes *et al.*, 2004; Stute, 1992). This treatment will cause an increase in starch crystallinity.

Hormdok and Noomhorm (2007) studied the effect of substituting a certain percentage of rice flour with annealed (55 ^{o}C, 24 hours) or heat-moisture treated (110 ^{o}C, 1.5 hours) rice starches on the quality of rice noodles. The noodles made from the composite flour were found to exhibit good quality as those of commercial noodles. The rice gel hardness and the gelatinization endotherms increased after the treatment and the rice noodles produced from the composite flour were found to have a reduced paste viscosity which was attributed to the restricted starch swelling power. Collado *et* *al.* (2001) proposed that an ideal starch gel for noodles production should resist to swelling and demonstrate a good paste stability. Similar rice noodles quality was observed when aged rice starch was used in noodles making.

2.3 Rice noodles

2.3.1 History, variation and market

Rice noodles have been consumed for more than 2,000 years in China, since the Qin dynasty (259-210 B.C.). Historical records suggested that the internal migratory pattern of the Chinese from the northern China to the southern and the eastern regions of China, especially the coastal areas, have given rise to the culture of eating rice noodles, because the migrants were not used to eating rice as a staple food. Ever since then rice noodles have been introduced worldwide and have been popularized in Southeast Asia (Li *et al.*, 2015).

Many forms and shapes of rice noodles may be found in the market (Table 2.2). Rice vermicelli or known as the dried form of rice noodles is called *bihun* in Malay, *mi fen* in Mandarin, *sen mee* in Thai and *bahn hoi* in Vietnamese. Sticky rice noodles that is called *pad Thai* (available in Thailand) and *banh pho* (available in Vietnam) come in different widths, and it is usually sold in the dried form. The other types of large, broad, and flat rice noodles which are called *shahe fun*, *ho fun*, and *kuay teow* (available in Malaysia and Singapore), are sold in fresh wet form and known for their soft and slicky texture. *Chee cheong fun* (available in Malaysia and Singapore) is another type of scroll-like rice noodle roll which is made fresh and sold in specialty stores such as dim sum restaurants. Silver needle rice noodles called *Lau shu fun*, another type of fresh wet rice noodles, are moulded in short (2 inches long and 1/4-

inch-wide) strands with pointed tips on each end, presented like a little white pointy tail of a rat. *Lai fun* (in Cantonese), laksa (in Malay) or *banh canh* (in Vietnamese) is similar to *lau shu fun* but they are longer, about six to eight inches with an extremely slippery but firm, gummy and springy texture. In Malaysia, *lai fun* and laksa noodles are used interchangeably, but typically they differ in various aspects. These noodles are served in Penang Asam Laksa dishes that have been ranked the 7th the most delicious food in the world by CNN in 2011. Figure 2.1 shows the typical rice noodles products found in local market of Malaysia.

Table 2.2 Classification and variation of rice noodles in Asia local market.

Rice Noodles	Other name	Description
Rice Vermicelli	*Bihun* (Malay), *mifen* (Chinese), *sen mee* (Thai), *bahn hoi* (Vietnam)	Very thin, round shape snow-white noodles up to 18 inches in length.
Rice Stick Noodles	*pad Thai* (Thai), *banh pho* (Vietnam)	Long (10 to 12 inches), straight, flat, and opaque, like bleached fettuccini.
Kuay Teow	*shahe fun, ho fun* (Chinese)	Flat, bright and wide up to two inches
Steamed Rice Roll	*Chee cheong fun* (Chinese)	Scroll-like rolls with six until eight inches long, thin and floppy squares when unrolled.
Silver Needle Noodles	*Lau shu fun* (Chinese)	Round in shape, short and fat, about two inches long and up to 1/4-inch-wide, with pointed tips on each end.
Lai fun/ Laksa Noodles	*Lai fun* (Cantonese), Laksa (Malay) *banh canh* (Vietnam)	Similar to *lau shu fun*, six to eight inches with extremely slippery, firm, and springy texture.

Source: Adopted from Cox (2014) and personal observation from local market.

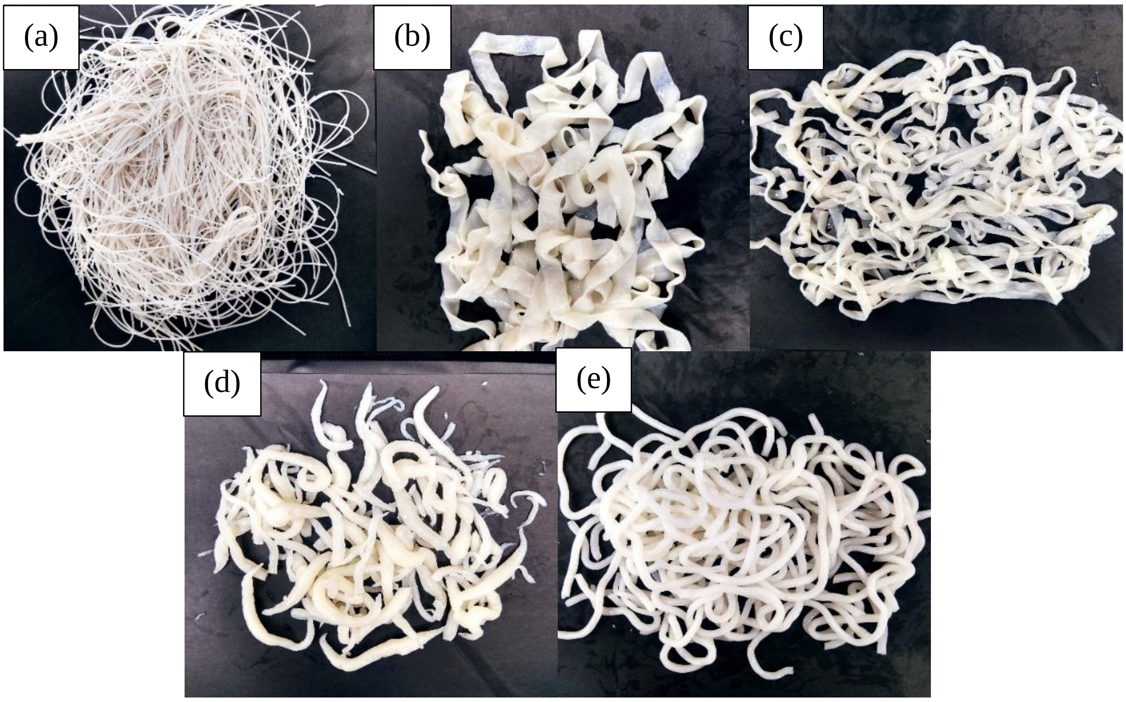

Figure 2.1 Typical fresh rice noodles found in local market.
(a) Rice vermicelli (*Bihun)*; (b) Kuay Teow (thick type); (c) Kuay Teow (thin type); (d) Silver Needle Noodles (*Lau shu fun)*; (e) Laksa noodles *(Lai fun)*

According to market research published by Grand View Research Inc. (Grand View Research Inc, 2016), rice noodles market size in Europe and Asia Pacific was estimated to be USD 1.69 billion in 2014, and this figure is forecasted to increase steadily through year 2022 to reach USD 3.6 billion. It is interesting to note that China and India are expected to propel the expansion of the market on a worldwide basis. The key factors influencing the expansion of this rice noodles market are attributable to consumer awareness of maintaining a healthy lifestyle. To ensure a sustainable market growth, rice noodles manufacturers are striving for technologies to support and enable expansion of distribution channels to reach a broader target market. It is challenging to distribute fresh rice noodles because their shelf life is relatively short (2 – 3 days) due to its high moisture content (62.51 %) and high water activity (aw, 0.91) (Rachtanapun and Tangnonthaphat, 2011). Naturally, fresh rice noodles are susceptible to microbial attack, in addition it is easily retrogradable and as a result the noodles strands become weak and show poor handling properties. It is a commonplace fact that the fresh rice noodles' market is confined to the geographical proximity of the noodles' producers, and many a time alluring fresh rice noodles dishes become a tourist attraction for the place at where the noodles are produced.

2.3.2 Raw ingredients and processing method

The basic ingredients to produce rice noodles are non-glutinous rice flour and water, along with additional additives such as salt to taste, or blending in other type of starches such as tapioca and corn starch in order to enhance the structural integrity. Rice starch gels network happens to be weaker than other types of starch gel, especially the wheat-based type. This is because rice starch lacks of gluten and the only way for amylose and amylopectin crystallites to create a continuous network is by linking each other strongly at the junction zones (Mestres *et al.*, 1988). Generally, native rice flour has poor elastic gel forming properties, poor resistance to shear force, as well as low thickening power and viscosifying properties (Cham and Suwannaporn, 2010). This leads to an unsatisfactory eating quality of rice noodles.

Bhattacharya *et al.* (1999) pointed out that the high cooking loss and low degree of swelling are examples of rice noodles quality defects. High cooking loss is commonly attributed to high solubility of starches during cooking, which resulted in turbid cooking water, weak cooking tolerance and sticky mouthfeel. The stickiness problem of rice noodles is believed to be caused the leaching of the smaller molecular weight starch fractions during cooking. Charutigon *et al.* (2008) have reported that the application of modified starch and monoglyceride may help in reducing the stickiness of rice vermicelli. The complex formed between monoglyceride and amylose molecules inhibit swelling of starch granules, and hence lower the water-binding capacity of starch and leachate of small molecular weight starch fractions. As a result, stickiness and cooking weight of the cooked noodles reduced substantially. These shortcomings of rice-based noodles justify for the massive research works conducted aiming of improving rice noodles quality through various modifications.

A typical fresh rice noodles processing protocol (Figure 2.2) usually starts with preparing the raw rice grains by cleaning and soaking, before subjecting it to grinding, heating (steaming or boiling), moulding (extruding or cutting), and cooling (and drying) (Li *et al.*, 2015). Fresh rice noodles are usually produced at night and then distributed to markets before dawn, to keep the noodles fresh and good in quality (Lu and Collado, 2010).

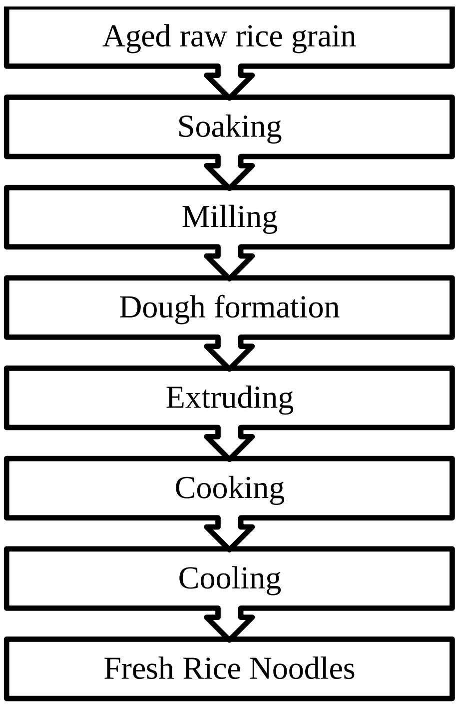

Figure 2.2 Primary processing steps in the production of fresh rice noodles. Adopted from Li *et al.* (2015)

Traditionally, rice noodles are made from long grain Indica rice and the process starts with overnight soaking of the rice grains followed by wet milling and filtering. Normally, rice grains that have been aged for more than 9 months will be used. The soaking process can last from several hours to several days depending on the desired end product characteristics. During fermentation, the growth of lactic acid bacteria and yeasts will cause the pH of the suspension to drop from pH 7 to pH 4 (Li *et al.*, 2015). The starch slurry will be pressed, and the starch cake recovered will be kneaded into a weak "dough". Since rice flour is gluten-free, thus it is lack of dough forming properties. Therefore, in order to form a 'standalone' dough, the weak "dough" is steamed partially. The freed amylose and amylopectin molecules will then play the role of a network extender to hold the other non-gelatinized starch granules together to form a 'standalone' "dough" during the subsequent mixing process. Alternatively, one portion of the rice cake can be pre-gelatinized using hot boiling water or steam to serve as binder, prior to being re-mixed with the remaining flour cake to form a dough by kneading (Bhattacharya *et al.*, 1999). The degree of pre-gelatinization achieved at this stage is vital in providing the desirable texture of the noodle strands. Too much gelatinization may cause the "dough" difficult to handle, and noodle strands produced will be rigid. On the other hand, insufficient gelatinisation will form friable "dough" and brittle noodle strands.

To produce a better quality rice noodles in terms of greater elasticity and chewier texture with a shinier and smoother appearance, tapioca and/or corn starch are added at 5-25% flour basis (Fu, 2008). With a lower pasting temperature, rapid granules swelling and high peak viscosity, the presence of tapioca and corn starch, would provide immediate structuring effects to rice noodle strands during subsequent extrusion or moulding process (Saeleaw and Schleining, 2010).

The rice dough formed will be extruded or sheeted into a boiling water bath or a steam bath for cooking. Extrusion produces vermicelli such as *bihun* whereas sheeting is used to produce flat noodles or sheets such as *kuay teow*. During cooking, gelatinization of the rice noodles strands occurs and swelling capacity of starch is one of the major factors affecting rice noodles quality. Bhattacharya *et al.* (1999) reported that the higher the amylose content of rice flour, the lower the swelling power of the starch granules. This will help to limit the amount of starch exudation during cooking. Ideally, starch noodles must not be overcooked during this stage otherwise granules on the noodle strands will absorb excessive water that will cause stickiness and reduce firmness to the final product (Kim and Wiesenborn, 1996).

Cooling is carried out right after cooking to decrease temperature of the noodles. At this step, first stage retrogradation among amylose molecules will be initiated causing the formation of a strong gel. Before packing the noodles to be sold, rice noodle strands are normally washed thoroughly using water to eliminate those gelatinized starch molecules which adhere to the surface of the noodle strands. This will reduce stickiness among the noodle strands and make them have a shinier and smoother appearance. Alternatively, stickiness can be overcome by applying oil to split the noodle strands to facilitate handling and storage.

2.4 Hurdle technology

2.4.1 Principles

Food products can be preserved by various means and the application of these preservative factors in combination is called hurdle technology. Hurdle technology has been widely used in the food industry in securing food safety and quality during the storage process (Leistner and Gould, 2012). Preservation technologies such as modification of temperature, pH, water activity, redox potential, microstructure or addition of preservatives (natural or chemical) as well as other physical preservation techniques (irradiation, high pressure processing, pulse electric field, ultrasonic, ozonation, ohmic heating, cold plasma etc.) have been widely used in the food processing industry. Each type of technology has its own pros and cons when being adopted to solve a particular issue pertaining to safety and quality of a particular category of food.

Microorganisms that are naturally present in food or present due to contamination during handling or processing can be destroyed with appropriate hurdles to ensure food safety and quality. Their growth are determined by the intrinsic (pH, moisture, nutrients, redox potential, antimicrobial resistance, biological structure) and extrinsic factors (relative humidity, oxygen or gases in surrounding environment, temperature), (Tucker and Featherstone, 2011), which could be manipulated by researchers and food industry to produce safe and quality food products. For example, in order to keep a high water activity food such as fresh starchy noodles for long term storage, mild heat treatment may not be adequate to suppress the growth of microorganisms, additional preservative measures such as addition of chemical or natural preservatives, and or modifying packaging atmosphere can be effective in prolonging the shelf life of the food product.

2.4.2 Selective preservative measures

Naturally, microorganisms present everywhere and good manufacturing practice is essential to ensure a low initial microbial load before the product enters any critical control processing point. Washing, trimming, filtration and centrifugation are the basic physical methods to reduce the nature microbial populations on raw ingredients of food product (Tucker and Featherstone, 2011). Washing off soil and dirt as well as pesticide residues and microorganisms on surface of raw ingredients are vital to reduce microbial load and quality loss (Zagory, 1999). However, the utilization of water in washing must be clean to avoid the contamination of pathogenic microorganisms. Moreover, water might increase moisture content of the food product and expose it to a higher risk of spoilage.

In the processing of fresh rice noodles, washing the noodles after cooking is an essential step with two purposes: (1) to cool down the noodles strands and induce first stage of retrogradation and (2) wash off the starch exudate on the surface of noodles to prevent stickiness. This step is crucial for rice noodles production, nevertheless the washing water that comes from the tap might carry contaminants that may reduce shelf life of the noodles product. It can be foreseen that the shelf life of fresh noodles can be enhanced if the washing steps can be improved by various means, in particular acid bath dipping.

2.4.2(a) Control of pH

Acidification of food to a pH value lower than 4.6 is the most common ways of food preservation. Most microorganisms grow well at pH value near to neutral (6.5–7.5) and only a few are able to grow at pH < 4.0. Under normal condition, bacteria spores that survives from heat treatment are less likely to germinate under acidic environment. Organic acid such as acetic acid, sorbic acid, lactic acid, propanoic acid and benzoic acid are commonly used in food industry to inhibit growth of bacteria, yeast and mould depending on types of food and characteristic of the acids. Their action of mechanisms are similar by disrupting cell membrane and denaturing protein or enzyme of the microorganisms (Tucker and Featherstone, 2011).