# PASSION FRUIT INFUSED GLUTEN FREE NOODLE: OPTIMIZATION OF INGREDIENT LEVELS USING RESPONSE SURFACE METHODOLOGY

By

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A project report submitted to the Department of Agricultural and Food Science Faculty of Science Universiti Tunku Abdul Rahman In partial fulfilment of the requirement for the degree of Bachelor of Science (Hons) Food Science

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#### ABSTRACT

### PASSION FRUIT INFUSED GLUTEN FREE NOODLE: OPTIMIZATION OF INGREDIENTS LEVEL USING RESPONSE SURFACE METHODOLOGY

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Passion fruit, rich in vitamins and minerals, is beneficial to the health of human beings. For this study, purple passion fruit (P. edulis Sim) is infused into the gluten free noodles. Response Surface Methodology (RSM) is then used to optimize the formulation of the ingredients. The independent variables are gluten free flour, passion fruit and egg while the response variables are solid loss and sensory evaluation. Based on the basic formula consisting 2 g of salt and 18 mL of water, the optimum formula obtained were 110 g of gluten free all-purpose flour, 17.5 g of passion fruit and 30 g of egg. Thereafter, the texture (tensile strength and breaking length), color (L\*, a\* and b\*) and potassium content of the optimal formula are compared to the normal wheat noodle. The tensile strength and the breaking length of the optimal formula is lower than that of the normal wheat noodle. This is due to the lack of the gluten protein in the optimal formula. In addition, the optimal formula is more red and yellow than the normal wheat noodle due to the addition of the purple passion fruit. Furthermore, the lightness of both noodles is similar. Lastly, the potassium content of the optimal formula is 8.6 % higher than the normal wheat noodle.

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# DECLARATION

I hereby declare that the project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

LEE MEI YING

# **APPROVAL SHEET**

# This project entitled " <u>PASSION FRUIT INFUSED GLUTEN FREE</u> <u>NOODLE: OPTIMIZATION OF INGREDIENTS LEVEL USING</u> <u>RESPONSE SURFACE METHODOLOGY</u>" was prepared by LEE MEI YING and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Food Science at Universiti Tunku Abdul Rahman.

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#### **PERMISSION SHEET**

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I hereby give permission to the University to upload the softcopy of my final year project in PDF format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

(LEE MEI YING)

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# LIST OF ABBREVIATIONS

CD	Celiac Disease
CsCl	Cesium chloride
HCl	Hydrochloric acid
L*	Lightness of the color
М	Molar concentration
R <sup>2</sup>	Multiple correction coefficients
ppm	Parts per million
K	Potassium
a*	Redness of color
RSM	Response Surface Methodology
b*	Yellowness of color

#### **CHAPTER 1**

#### **INTRODUCTION**

Pasta products and Asian noodles were originated from thousands of years ago and have become a staple food in many countries (Li, et al., 2014). In 2012, 101,420 million packets of noodles were produced globally and noodles' production have experienced a steady increase by 3 % annually since 2010 (Purwandari, et al., 2014). Particularly, Asians are fond of noodles due to its versatility and noodles can be commonly seen in Korea as ramen, Japan as soba or udon and South East Asia as Hokkien noodles, rice noodles or Cantonese noodles (Li, et al., 2014).

The basic ingredients in making Asian noodles are wheat flour, salt and egg. Wheat flour is used due to its elasticity and hardness, thus giving noodles a good texture (Dewi, 2011). In addition, wheat flour also contains gluten, which is an important source of protein (Dewi, 2011). Gluten consists of glutenin and gliadin and they form gluten network in the noodles to increase its elasticity and reduce the loss of soluble matter during cooking (Li, et al., 2014).

However, people diagnosed with Celiac disease (CD) are allergic to food products containing gluten (Purwandari, et al., 2014). In the United States, at least three million people are diagnosed with CD and the only treatment to this disease is to adhere to a gluten free diet (Omari, 2013). Gluten free diet should not contain wheat, barley and rye as they are the source of gluten (Sabbatini, et al., 2014). Globally, the prevalence of CD is increasing (Susanna and Prabhasankar, 2013). Hence, the gluten free food products are in high demand and the sales of the gluten free food products have increased and reached \$2.9 billion in 2010 (Omari, 2013).

In recent years, a lot of research and development have been put into exploring gluten free noodles. For example, wheat can be replaced with another type of flour or gluten in noodles can be replaced with another food additive (Susanna and Prabhasankar, 2013). Normally, the noodles and pasta products are made with the wheat flour and water, with or without salt which caused the noodle lack of nutrients such as dietary fiber, minerals or vitamins (Li, et al., 2014). The ingredients like high protein flour and cereals are common added into the noodle to increase the nutritional value (Li, et al., 2014). A possible food additive could be passion fruit. Passion fruit is a kind of tropical fruit belonging to the family of *Passifloraceae* (Pongener, et al., 2014). There are more than 450 species of Passiflora genus and two of the most common ones are purple passion fruit also called as P. edulis Sim and yellow passion fruit called P. edulis f. flavicarpa (Das, et al., 2013). Passion fruits are usually consumed directly or used as an ingredient in desserts, such as cake, ice cream or fruit juice because they have attractive flavor, nutritional benefits and medicinal properties (Pongener, et al., 2014). Passion fruit contains a high amount of vitamin, minerals and is extremely rich in potassium (K) (Phamiwon and Sheila, 2015). Potassium is regarded as a source to reduce the blood pressure of hypertension patients and thus the risk of stroke. In 2011, hypertension in Malaysia which involved adults aged 18 and above was 32.7 % and aged 30 and above was 43.5 % (MSH, 2013). Hence, the prevalence of hypertension in Malaysia requires more attention. Besides, other health benefits of the passion fruit such as prevent colon cancer, improve digestive system, controls the muscle function of our body, reduce the heart disease and so on (Phamiwon and Sheila, 2015). Whereby, passion fruit is used to increase the potassium content in the noodles which would increase the nutritional value of the noodle.

The major aim of this study is to produce gluten free noodles with the infusion of purple passion fruit. The formulation of raw ingredients in gluten free noodle will be optimized via the response surface methodology (RSM). RSM is a powerful statistical technique which used to develop improve or optimize processes or formula (Šumić, et al., 2016). For example, RSM was used to enhance bread's recipe and shelf life of the gluten free bread (Sabanis, et al., 2009).

The specific objectives of this present study are to develop a purple passion fruit infused gluten free noodle, to optimize the formulation of raw ingredients in gluten free noodle making by using RSM and to elucidate some physicochemical properties (texture, color and potassium content) of the optimized noodle against normal wheat noodle.

### **CHAPTER 2**

## LITERATURE REVIEW

### 2.1 Asian Noodles versus Pasta

Nowadays, most people is living in a face-paced lifestyle. Hence, convenient food products are becoming more and more popular as they can save the food preparation time. Asian noodles and pasta products are one of the convenient food products which have a high acceptance worldwide due to its inexpensiveness and convenience (Li, et al., 2014). Asian noodles and pasta products are different as different types of ingredients and method manufacturing are used. The differences between pasta products and Asian noodles are showed in the table below (Hou and Kruk, 1998).

	Pasta products	Asian noodles	
Ingredients	semolina which milled	Wheat flour + salt + egg	
	from durum wheat +		
	water		
Method manufacturing	Extrude through a metal	The dough of the noodle	
	die under pressure	is pass through sheeting	
		and cutting by machine	
Drying or without	Drying	Without drying	
drying			
Consumption pattern	Served with sauces	Served with soup	
Common in countries	Italy, Europe, etc.	Asian countries such as	
		China, Malaysia, Korea,	
		Japan, Thailand,	
		Indonesia, etc.	
Examples	Fusilli, Spaghetti,	Hokkien noodles,	
	Fettuccine, Penne,	Cantonese noodles,	
	Cannelloni, Farfalle, etc.	udon, soba, rice noodles,	
		wonton noodles, etc.	
		(Hou and Kruk, 1998)	

 Table 2.1: The differences between pasta products and Asian noodles

#### 2.2 Gluten

A good quality noodle must have an acceptable color, texture and flavor. Wheat flour is the main ingredient of the Asian noodles. The gluten in the wheat flour can provide the elastic characteristic and hard texture to the noodle which is also a source of protein in the wheat noodle (Dewi, 2011). The gluten is development during mixing the dough while the gluten network is formed in the sheeting process (Fu, 2008). The gluten network can decrease the soluble matter loss into the cooking water during cooking and also prevent the noodle from breaking down easily (Li, et al., 2014). Gluten contains two types of proteins, i.e. glutenin and gliadin. Glutenin is a high molecular weight protein and the largest protein in nature (Smith and Hui, 2008). The characteristics of the glutenin are high viscoelastic, high elastic and insoluble in alcohol (Kulp, 2000). Besides, glutenin can produce a strength and stability dough (Smith and Hui, 2008). Gliadin is a low molecular weight protein which causes the dough high extensibility, low elastic and soluble in the alcohol (Kulp, 2000). When fully hydrated, the glutenin protein will form a crude rubbery mass while the gliadin protein will form a liquid mass (Susanna and Prabhasankar, 2013). Therefore, the wheat flour can provide the hard and elastic texture to the noodle.

#### 2.3 Celiac Disease (CD)

Some people will develop an allergy or gluten hypersensitivity after they consume wheat products, this is called Celiac disease (CD). CD is a chronic enteropathy and caused by an improper immune response to a protein called gluten (Stauble, 2013). A protein called prolamines cause atrophy of intestinal villi and malabsorption (Sabbatini, et al., 2014). This may happen in childhood and adulthood. Gluten is a mixture wheat storage proteins, there are other similar proteins found from cereals which have a toxin effect to the CD patient which includes gliadin in wheat, secalin in rye and hordein in barley (Niewinski, 2008).

In the United States, at least three million people are diagnosed with CD was estimated by the University of Chicago Celiac Disease Center in 2011 (Omari, 2013). Besides, CD is also very common in Europe, Germany, Finland and some developing countries such as North Africa, Middle East, India and China (Fasano and Catassi, 2012). The symptoms of CD include weight loss, diarrhea, ataxia, steatorrhea, anemia, lethargy and constipation (Omari, 2013). Currently, the only treatment available for CD is adhere to a gluten free diet (Stauble, 2013).

#### 2.4 Gluten Free Diet

A gluten free diet is defined as excluding wheat, barley and rye in the diet (Stauble, 2013). The common gluten free foods are vegetables, fruits, milk, fishes and nuts. There are some gluten free processed foods such as gluten free pasta, bread, pizza, biscuit and so on in the market. Because of the specific diet requirement from the diagnose of CD patients, the consumption of gluten free products has been on increasing in recent years (Sabbatini, et al., 2014).

The development of gluten free foods is a big challenge to food technologists. The food scientists usually utilize other types of gluten free ingredients such as corn, tapioca, potato, rice to replace the ingredients which contain gluten. For example to develop a gluten free noodle, some of the studies were found to use pumpkin, breadfruit or konjac flour (Purwandari, et al., 2014); development of a gluten free pasta with green banana flour has been carried out (Zandonadi, et al., 2012); a gluten free pizza dough with tapioca flour was made (Omari, 2013). According to the Codex Alimentarious standard of the gluten free food, the ingredients must not contain wheat, barley and rye or the ingredients that contain the gluten or they have to pass through proper processing to remove the gluten and the gluten level of the product may not exceed 20 mg/kg in total (Saturni, et al., 2010). This is because CD patient should not consume more than 1mg of gluten per day (Susanna and Prabhasankar, 2013).

20-38 % of CD patients faced nutritional deficiencies such as protein, dietary fiber, minerals such as iron, magnesium, calcium and vitamin D, B and so on (Saturni, et al., 2010). Most of the nutrients were absorbed in the small intestinal. Hence, the damage of the villi from gluten diet was identified as the main reason causing the nutritional deficiencies in CD patients due to malabsorption (Saturni, et al., 2010). Some of the previous studies found that a long period of gluten free diets was able to treat the nutritional deficiencies for the CD patients (Saturni, et al., 2010). However, some studies demonstrated that after a long term consumption of gluten free diets, the nutritional deficiencies of some other nutrients still presented. For example, inadequate intake of fiber happened since most of the products consumed were made by

refined flours. The fiber was lost during refining because the outer layer of grain which rich in fiber was removed. Therefore, a balance of gluten free diet coupled with other good sources of dietary fiber, minerals and vitamins are vital in order can get the adequate nutrients.

#### 2.4.1 Gluten Free Noodle

Gluten free noodle is the noodle which do not contains any gluten. In Asia, noodle is normally made with wheat flour as it can provide a good texture to the noodle. However, the noodle made with wheat flour was not suitable to the CD patient because of the gluten allergy. Hence, the gluten free noodle is important to the CD patient who is noodle lover. Nowadays, the starches and some of the gluten free flours are commonly used in noodle making such as corn starch of flour, rice flour, sweet potato starch and so on (Purwandari, et al., 2014). The lack of gluten in the gluten free noodle will lose the good texture of the noodle. Gluten free noodle has poor extensibility and is more easily to crack than wheat noodle (Wang and Zhang, 2015). Besides, when different types of flour were used, different characteristics of noodle were presented. Hence, it is a common practice to add food additives into the noodle to improve the texture and the noodle quality.

#### 2.5 Food Additive

Food additives are used to improve the quality of the noodle and to diverse the functionality of noodle in order to meet consumer requirements (Li,, et al., 2014). The common food additives use in noodle are salt, egg, edible gum,

starch, hydrocolloids, emulsifiers, antioxidants, preservatives and so on. For example, antioxidants and preservatives can be used to increase the shelf life of the noodles and prevent deterioration of color; edible gums and emulsifiers can be used to enhance the surface properties and the quality of the noodles.

Some of the chemically synthesized food additives can cause digestive problems, cancer, heart disease, obesity and so on. For example, benzoyl peroxide and potassium bromate are commonly used as dough strengthener in wheat flour to enhance the structure of the dough and extend the chewy texture to the noodles and bleaching agent which are able to improve the appearance to the white salt noodles. Sodium polyacrylate is used as a thickening agent to improve the stability of the texture due to improve the texture of the noodles (Li, et al., 2014). Some of the common food additive used in noodle and its details were showed in the below table:

Food	Information	Reference
Additive		
Natural	- fortify gluten and improve or maintain	(Li, et al.,
Proteins	dough structure	2014)
	- improve the chewiness texture to the	
	noodles	
	- improve the nutritional value	
	- E.g.:	
	Soybean protein	
	<ul> <li>Increase the gluten network</li> </ul>	
	and firmness	
	• Enhance the dough	
	properties	
	<ul> <li>Improve color</li> </ul>	
	• Egg albumin	
	<ul> <li>Prevent the easy break</li> </ul>	
	noodle	
	<ul> <li>Good cooking tolerance</li> </ul>	
	• High water absorption	
	<ul> <li>Low solid loss</li> </ul>	
	• Casein, etc.	
	• Can reduce the oil	
	absorption (in the instant	
	fried noodle)	

**Table 2.2**: Examples and the information of the food additives.

Food	ood Information		Reference	
Additive				
Starches	- normally used in the industry	(Li, et a	al.,	
	- starch gelatinization can improve the	2014), (I	Fu,	
	appearance, smoothness of the surface and	2008)		
	increase the eating quality of the final			
	products			
	- improve noodle texture			
	- too much of starch in the noodle will			
	reduce the gluten network thus increase			
	the breaking rate, solid loss and stickiness			
	of the noodle			
	- 2 types:			
	• Native starches			
	<ul> <li>Improve the quality of the</li> </ul>			
	final products			
	• E.g.: potato starch, tapioca			
	starch, corn starch, Mung			
	bean starch, edible canna			
	starch, etc.			

**Table 2.2**: Examples and the information of the food additives continued.

Food	Information	Reference	
Additive			
Starches	Modified starches	(Li, et al.,	
	<ul> <li>Improve the weakness of</li> </ul>	2014), (Fu,	
	the native starch such as	2008)	
	lack of viscosity and		
	thickening power, etc.		
	<ul> <li>Storage stability, good in</li> </ul>		
	thickening power and		
	gelatinization properties,		
	<ul> <li>Common use in noodle</li> </ul>		
	making industry		
	• E.g.: Esterified starches,		
	cross-linked starches		
Edible	- as a gluten enhance to maintain the dough	(Li, et al.,	
gums	structure	2014)	
	- enhance mouthfeel and texture to the		
	noodle		
	- E.g.: guar gum, xanthan gum, locust bean		
	gum, etc.		

**Table 2.2**: Examples and the information of the food additives continued.

Food	Information	Reference
Additive		
Salt	- improve the dough forming the network	(Li, et al.,
	- prolong the shelf life of the noodles as salt	2014), (Hou
	can control the growth of the microbial and	and Kruk,
	the enzyme actives in the fresh noodles	1998), (Fu,
	- flavor enhance	2008)
	- improve texture	
	- Two common types of salt added into the	
	noodles:	
	• regular table salt	
	<ul> <li>use to produce white salt</li> </ul>	
	noodles	
	• E.g.: Japanese noodles,	
	Chinese raw noodles or dry	
	noodles, etc.	

**Table 2.2**: Examples and the information of the food additives continued.

Additive		
Salt	• alkaline salts	(Li, et al.,
	<ul> <li>use to produce yellow</li> </ul>	2014), (Hou
	alkaline noodles	and Kruk,
	<ul> <li>normally used sodium</li> </ul>	1998), (Fu,
	carbonate (Na <sub>2</sub> CO <sub>3</sub> ),	2008)
	potassium carbonate	
	$(K_2CO_3)$ , or the	
	combination	
	• E.g.: Cantonese noodles,	
	Hokkien noodles, Thai	
	bamee, etc.	

**Table 2.2**: Examples and the information of the food additives continued.

Information

Food

A study used four different types of high protein flour (Soya flour, Channa flour, Sorghum flour and Whey Protein Concentrate) with gum to develop the gluten free pasta (Susanna and Prabhasankar, 2013). This study used high protein flours to increase the nutrition value of the gluten free pasta and the addition of gum was able to reduce the solid loss of the pasta.

# 2.6 Purple and Yellow Passion Fruit

Passion fruit is a kind of tropical fruits which has a yellow of purple color of tough peel, oval shape and the orange-yellow color of the flesh are surrounded

Reference

by small and edible black seed. Passion fruit grows well in tropics or subtropics areas while mainly planted in Brazil, Colombia, Peru and Ecuador while others countries such as Malaysia, India, Hawaii, Mexico, Australia and Zimbabwe (Gill, et al., 2015). Passion fruits are from the family of *Passifloraceae* which includes purple passion fruit (*P. edulis* Sim) and yellow passion fruit (*P. edulis* f. *flavicarpa*) (Pongener, et al., 2014). Yellow passion fruit is suitable to grow in tropical lowland areas such as Guyana while the tropical highland areas are more suitable for purple passion fruits growth (NARI, 2004). Besides, yellow passion normally sell in domestic market and a small amount are distributed to export and purple passion fruits are mostly exported to other countries. This may because purple passion fruits have a better aroma and flavor, less acidic and higher juice content than the yellow passion fruits (Paull and Chen, 2014).

Optimum storage condition is important to keep the quality and increase the shelf life of the foods. There is a slight different between yellow and purple passion in their optimum storage conditions. The yellow passion fruit storage is at 7 to 10  $\$  (45 to 50  $\$ F) with 90 to 95 % relative humidity while the optimum storage condition of the purple passion fruit is at 3 to 5  $\$ C (37 to 41  $\$ F) for 3 to 5 weeks (Paull and Chen, 2014). Storage in a correct condition is very important to prevent postharvest diseases. The most common disease is the brown spot (*Alternaria passiflorae*) which are circular, sunken, light-brown spots on ripening fruit (Paull and Chen, 2014). Moreover, the uneven ripening of the skin is caused by septoria spot (*Septoria passiflorae*) and phytophthora

fruit rot (*Phytophthora spp.*) is led to water-soaked dark green patches on the peel (Paull and Chen, 2014).

### 2.6.1 Maturity Index of Passion Fruit

When the passion fruit is mature, the color of the outer skin will turn to purple or yellow color and have good taste and flavor. A research based on the physiological and quality changes during postharvest ripening of purple passion fruit (*P. edulis* Sim) were reported by Pongener, et al. (2014). They harvested passion fruits from four different maturity stages as their samples, they are stage I (0 % color turning), stage II (25 % color turning), stage III (50 % color turning) and stage IV (75 % color turning). The result showed that the optimum color was presented in stage III and stage IV. In addition, the fruits harvested in stage III and stage IV had a lower acidity and better flavor in fruits than the stage I and stage II. After a few tests, they concluded that purple passion fruits must harvest in stage III with 50 % of color turning, this finding is also supported by the study done by NARI (2004). Besides, passion fruit at this stage is also suitable for long-distance transportation to make sure the optimum fruit quality are sales to the consumer.

In addition, the quantity and the quality of the pulp will directly affect the edible economic part and the sales in the market. Pongener, et al. (2014) found that there was no quality and quantity changed when the fruit shriveling. Shriveling is due to the loss of moisture from the peel while the quality of pulp yield and the flavor still maintain. The shriveling fruits are more suitable to the short-term consumption rather than distance transport.

#### 2.6.2 Health Benefits of Passion Fruit

Passion fruit is high in nutrients. Hence, there are a lot of health benefits such as to regulate blood pressure, prevent cancer, improve digestive system and so on. According to Phamiwon and Sheila (2015), the potassium content of 100 g of passion fruit is 348 mg. They also reported that high potassium content of the passion fruit effective in the protection of blood pressure, manage the electrolyte balance and controls the muscle function of our body. Potassium is one of the four deficient nutrients in the American diet, with only 3 % of the Americans meeting the adequate intake of potassium in 2003 to 2006 (Weaver, 2013). The Dietary Guidelines for Americans 2010 Advisory Committee identified that there is a relationship between the potassium intake and blood pressure reduction in adults (Weaver, 2013). The increasing intake of potassium is able to reduce the blood pressure of the hypertension patients and also reduce the risk of stroke (Aburto, et al., 2013). Hence, the sufficient intake of potassium is able to prevent and control the risk of stroke and the increasing of the blood pressure.

In addition, passion fruit is a rich source of dietary fiber in which the fiber content is found to be 10.4 g per 100 g of passion fruit (Phamiwon and Sheila, 2015). Dietary fiber can improve the digestive system and also prevent colon cancer. This is because the soluble fiber is able to clean the toxin in the colon and regulate bowel movement. Also, dietary fiber can help to reduce the LDL (low-density lipoprotein) cholesterol and increase the HDL (high-density lipoprotein) cholesterol levels in the blood. Passion fruit is strong in antioxidants because the high content of vitamin C (1274 IU per 100 g passion fruit) and vitamin A (30 mg per 100 g passion fruit) thus can prevent cancer by neutralizing free radicals and also beneficial for eyes (Phamiwon and Sheila, 2015). Moreover, a study revealed that eating passion fruit can induce relaxation feeling and promote sleep because it contains medicinal alkaloids which have mild sedative properties (Phamiwon and Sheila, 2015). On the other hand, the oil found in the edible black seed of the passion fruit contains high level of unsaturated fatty acid, 87.59 % (Phamiwon and Sheila, 2015). Unsaturated fatty acid is able to reduce the heart disease, lower the LDL cholesterol and also blood pressure and so on.

### 2.7 Response Surface Methodology (RSM)

Response surface methodology (RSM) is a group of mathematical and statistical techniques which is able to determine the interaction between independent variables and the response (dependent) variables (Baş and Boyacı, 2007). RSM is suitable for developing, improving or optimizing the formulation for a new product. For example, RSM is used to optimize the ingredients level were usually highlighted by different studies such as optimize the formulation on a steamed bread which enriched with Tartary buckwheat (Wang and Zhang, 2015); to optimize the formulation of the raw ingredients in the sweet potato-based pasta; to optimize the ingredients level of bread which develop with the barnyard millet bran (Nazni and Gracia, 2007) and so on. RSM is not only used to optimize the formulation of the food product, but it also can be applied to other field of studies such as given a minimum surface roughness of the paper based on the optimum cutting condition (Öktem, et al.,

2005), to optimize the oxygen pressure, temperature and time on the degradation of azo dye solution (Demirel and Kayan, 2012) and so on.

The two commons models used in RSM are first-degree model (Equation 2.1) and second-degree model (Equation 2.2) (Khuri and Mukhopadhyay, 2010).

**Equation 2.1**: First-degree model (d=1).

$$y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \epsilon$$

Equation 2.2: Second-degree model (d=2).

$$y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i < j} \beta_{ij} x_i x_j + \sum_{i=1}^k \beta_{ii} x_i^2 + \epsilon$$

The purpose of the model is to build the relationship between the response of interest and the input whereby to predict the response values (Khuri & Mukhopadhyay, 2010). Besides, according to the Khuri and Mukhopadhyay (2010), the model is also used to determine the optimum settings of the input by using the hypothesis testing to determine the significant of the factors. In RSM, both the equations above are frequently used to determine the polynomial models. For the first-degree model, it is applied to the first-order design, however, the design for fitting the second-degree model is called second-order design (Khuri & Mukhopadhyay, 2010). Moreover, the 2 factorial design, Plackett-Burman design and simplex design are the most frequently used first-order design while the second-order design is commonly used in 3

factorial design, central composite design (CCD) and Box-Behnken design (BBD) (Khuri and Mukhopadhyay, 2010).

The Box-Behnken design is one of the methods in the response surface methodology (RSM) which devised by Box and Behnken in 1980 (Tekindal, et al., 2012). Box-Behnken design is not suitable for the experiment which favors the results under an extreme situation due to the fact that Box-Behnken design does not contain combinations since all factors are at their highest and lowest levels. This, in turn, can help in preventing experiment which is under extreme condition and lead to an undesirable result (Bruns, 2016). Furthermore, this design only require three level (-1, 0 and 1) for each level. Thus, the design runs a minimum number of experiments to optimize the formula which can reduce the cost of the materials and save the time (Khuri and Mukhopadhyay, 2010). Equation 2.3 is used to calculate the number of experiments (N), in which the number of factors is represented by k and the replicate number of central points represented by  $C_0$  (Ferreira, et al., 2007).

**Equation 2.3**: Calculation of the number of experiments in Box-Behnken design.

$$N = 2k(k-1) + C_0$$

Based on the observation of the experiment data, RSM is able to build the polynomial regression model (also called second-order model, polynomial model or quadratic model) (Tekindal, et al., 2012). The polynomial model is able to present the relationship between the factors and responses through a 3D

response surface plot or contour plot in order to assume the optimum level (Sun, et al., 2010). After the statistical analysis, the optimal formulation can be obtained.

#### **CHAPTER 3**

### **MATERIALS AND METHODS**

#### 3.1 Materials

Gluten free all-purpose flour is purchased from Jaya Grocer at Ipoh, Malaysia. Purple passion fruits, eggs, salt are purchased from the local market. The maturity index of the passion fruits in stage IV (75 % of color turning) is used to make the noodles. Besides, Knorr Chicken Cubes purchased from the local market are used to make the soup that are required during sensory evaluation. Furthermore, potassium standard solution, 1000 mg/L K (Merck Millipore, USA), Cesium chloride, CsCl (Merck Millipore, USA) and hydrochloric acid, 37 %, ca. 12 mol/L HCl (Fisher Scientific, United Kingdom) are used in the potassium measurement.
# **3.2** General Plan of the Experimental Work



Figure 3.1: Overall Experiment Flow Chart.

#### **3.3** Experiment Design

The Box-Behnken design in the response surface methodology (RSM) is chosen by using JMP statistical discovery software from SAS (version 13.0). The design matrix of the experiments which involved three independent variables is showed in Table 3.1, namely, gluten free flour (A), purple passion fruit (B), and egg (C). The gluten free all-purpose flour levels from 90-110 g, passion fruit levels from 15-25 g and egg levels from 20-30 g are the upper and lower limits of the independent variables. Three experiments of the center point (0) are included to get the expected satisfactory experimental results (Wang and Zhang, 2015). According to the Equation 2.3, the total number of the experiments is 15. Table 3.2 is showed the information of the 15 experiments and the experiments are randomized to reduce the bias. The response variables are the solid loss (%) and the sensory evaluation of the noodles. Based on the result of the analysis of variance, the experiment data of the response variables are fitted to the polynomial regression model:

Equation 3.1: Polynomial regression models for three factors.

$$Y = \beta_0 + \beta_a A + \beta_b B + \beta_c C + \beta_{ab} AB + \beta_{ac} AC + \beta_{bc} BC + \beta_{aa} A^2 + \beta_{bb} B^2 + \beta_{cc} C^2$$

Note: Y = Response Variables

 $\beta_x$  = Regression Coefficients

A, B, C = Independent Variables

The basis of model selection is based on the P value is significant at 0.05, the lack-of-fit test is not significant different (p>0.05) and the multiple correlation coefficient ( $\mathbb{R}^2$ ) is close to 1 which range between 0.85 to 0.98 is considered as the good model (Sabanis, et al., 2009). If there are any contradictions between these three requirements, the solution that has the overall best performance is selected. Moreover, desirability is a multiple response method which used to calculate the optimal formulation. The desirability score is from 0.0 (undesirability) to 1.0 (very desirability). Then the response surface plot is constructed to show the relationship between the independent and response variables. After the optimum ingredients level has been determined, the color, texture and the potassium content of the optimal formula are compared with the normal wheat noodle in which ingredients formulation is showed in Table 3.3.

Factor	Code	Level					
		-1	0	1			
Gluten free flour/g	А	90	100	110			
Passion fruit/g	В	15	20	25			
Egg/g	С	15	25	30			

**Table 3.1**: Experiment design with three factors and three levels.

Number	А	В	С	So	lid loss	(%)	Sensory evaluation
				1	2	3	
1.	1	0	1				
2.	0	-1	-1				
3.	0	0	0				
4.	1	-1	0				
5.	-1	-1	0				
6.	0	1	-1				
7.	1	0	-1				
8.	0	1	1				
9.	-1	0	-1				
10.	0	0	0				
11.	1	1	0				
12.	0	-1	1				
13.	-1	0	1				
14.	0	0	0				
15.	-1	1	0				

 Table 3.2: Box-Behnken design arrangement.

Ingredients	Weight
All-purpose wheat flour	100 g
Egg	25 g
Salt	2 g
Water	18 mL

**Table 3.3**: The formulation of the normal wheat noodle.

#### 3.4 Noodle Making

The basic ingredients used for making gluten free noodle samples are 2 g of salt and 18 mL of water (Table 3.4), while the amount of the flour, the flesh of the passion fruit and egg are according to the Table 3.1 and Table 3.2. Moreover, the normal wheat noodle making followed the formula in Table 3.3.

 Table 3.4: Gluten free noodle formulation

Ingredients	Weight
Gluten free all-purpose flour	Variable *
Passion fruit	Variable *
Egg	Variable *
Salt	2 g
Water	18 mL

\*Amount varied according to the experiment design (Table 3.1 and Table

First, the pulp of the passion fruit is manually scooped out with a spoon, then blended with the blender and filter with a sieve to remove the bigger size of the seeds. Besides, the egg is slightly stirred to mix the egg white and egg yolk and then salt is dissolved in the water. For noodle manufacturing, all the ingredients are mixed together and kneading to become a dough. Next, the dough is allowed to rest at the room temperature for 20 minutes. After 20 minutes, the dough is steamed in the steamer for 2 minutes and then it is kneaded again before sheeting and cutting to cool down and prevent the dough to absorb water vapor. A pair of sheeting rolls in the noodle making machine are used to sheet the dough for a few times to become a smooth noodle sheet (Hou and Kruk, 1998). After few times of sheeting, the thickness of the sheet is reduced to 1 mm. The noodle sheet is cut into standard 6 mm of width by using a cutting roll which attached to the machine.

#### 3.5 Solid Loss

According to the Ritthiruangdej, et al. (2011), a 10 g of noodles are cooked in 300 mL of distilled boiling water in a container until the optimal cooking time, two minutes. After that, the cooking water is collected into a clean beaker. The cooking water is evaporated in an air oven at 105  $^{\circ}$ C to determine the residue weight. All the samples are determined in triplicate to obtain the mean values. According to the AACC (American Association of Cereal Chemists) method (1999), the calculation of the solid loss is modified and calculated by the following equation:

Equation 3.2: Solid loss (%).

$$Solid Loss(\%) = \frac{weight of total solid}{weight of the noodle before cooking} \times 100\%$$

#### **3.6** Sensory Evaluation

According to the (Wang and Zhang, 2015), the method of the sensory test of the cooked products are done by twenty-five untrained panelists from Universiti Tunku Abdul Rahman, Kampar Campus. The optimally cooked noodles with soup are evaluated for appearance, aroma, taste, texture and overall acceptance by using nine-point hedonic scales, where ranging from 1 = dislike extremely to 9 = like extremely. The evaluation is carried out in a sensory evaluation laboratory equipped with separately divided booths. All the samples are served at room temperature (25 ± 1 °C) and performed the analyses under normal lighting conditions. Besides, how the panelists should perform during the evaluation are briefed before start.

#### 3.7 Noodle Texture Measurement

The two texture parameters tested in this study are tensile strength and the breaking length of the samples. A TA-XT Plus Texture Analyzer (Stable Micro Systems, London) with *Exponent* software is used to test the tensile strength and breaking length. Both 10 cm optimal formula and the normal wheat noodle are optimally cooked in the boiling water. According to the Ritthiruangdej, et al. (2011), the optimally cooked noodles are cooled for 1 minute under the running distilled water and drained in the room temperature for 10 minutes.

Calibration settings used are the 5 kg load cell with a return distance at 20 mm while the instrument settings are showed in the table below. All the tests are performed in triplicate to get the average.

Setting	extension mode
trigger type	auto-0.5 g
pre-test speed	2.0 mm/s
post-test speed	10 mm/s
test speed	3.0 mm/s
trigger distance	80 mm

 Table 3.5: Instrument setting of the texture measurement.

#### 3.8 Noodle Color Measurement

The reading of the raw sheet optimized gluten free noodle and wheat flour noodle are measured by using a CM-600d Spectrophotometer (Konica Minolta, Japan) (Ritthiruangdej, et al., 2011). The color is represented in CIE-Lab parameters as lightness L\* (100 = white; 0 = black), redness a\*(+, red; -, green) and the yellowness b\* (+, yellow; -, blue). The sample is placed in a clean Petri dish and measure the L\*, a\* and b\* value. All the samples are performed in triplicate to get the mean values. The total color difference ( $\Delta E^*$ ) of two samples are determined by the following equation: Equation 3.3: Total color different.

$$\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$$

Note:  $\Delta E = \text{total color difference}$ 

 $\Delta L$  = difference in lightness and darkness

 $\Delta a = difference in red and green$ 

 $\Delta b$  = difference in yellow and blue

#### 3.9 Noodle Potassium Measurement

The ash sample is used in the potassium analysis. The raw sheet samples are heated in an air oven at 105  $\,^{\circ}$ C for overnight. For the preparation of ash, first, the crucible is labeled and preheat in the muffle furnace with 425  $\,^{\circ}$ C for 1 hour. Then, the crucible is cooled in the desiccator and the weight of the crucible is recorded. Five grams of dried samples are weighed and put into the crucible (Siong, et al., 1989). Next, the crucible is heated on the hot plate without cover a lid for 2 hours to make the samples charred. After that, the crucible is cover with a lid and placed into the muffle furnace at 550  $\,^{\circ}$ C for 8 hours to get the ash sample.

The ash is treated with 0.2 M HCl in a volumetric flask and made up to 50 mL. Then, a syringe and syringe filter (pore size:  $0.45 \ \mu m$ ) are used to filter the ash which could not dissolved in the solution. The dilution of the sample is showed in Table 3.6 and the 10 mL of the volumetric flask is used. Moreover, the preparation of the calibration standards is showed in Table 3.7. The 100 mL volumetric flasks are used for calibration blank while 50 mL volumetric flasks are diluted for the individual standard. Then, all the solutions are diluted

again in the clean volumetric flask with deionized water which 1 mL of the solution diluted in 9 mL deionized water. The final concentration of potassium in the blank, standard 1, 2 and 3 solutions are 0 ppm, 0.5 ppm, 1.0 ppm and 2.0 ppm, respectively. The atomic absorption spectrometry (AAS) method is used to determine the amount of potassium in the samples. Hence, an AAnalyst 200 Flame Atomic Absorption Spectrometer (PerkinElmer, USA) with Syngistix<sup>™</sup> software is used and the wavelength is set to 766.5 nm.

All the glassware must leach in a mix solution in which distilled water and diluted nitric acid with 1:10 ration for at least 24 hours before used. Then the glassware is rinsed with the deionized water and dry in the room temperature. Furthermore, to prepare the 1 % CsCl solution, 1.27 g of CsCl (Cesium chloride) is weighed on a weighing boat and 98.73 g of deionized water is added and mixed well in a clean bottle with a screw cap.

**Table 3.6**: Preparation of sample dilution.

	Volume	
Sample solution	2 mL	
CsCl solution (1% CsCl)	2 mL	
1M HCl	0.6 mL	
Deionized water	Up to 10 mL	

	Blank	Standard 1	Standard 2	Standard 3	
1MHCl	10 mL	5 mL	5 mL	5 mL	
CsCl solution (1%	20 mL	10 mL	10 mL	10 mL	
CsCl)					
K stock	0 mL	0.50 mL	1.00 mL	2.00 mL	
<pre>solution(500mg/L)</pre>					
Deionized water	Up to 100	Up to 50 mL	Up to 50 mL	Up to 50 mL	
	mL				

**Table 3.7**: Preparation of calibration standards.

# 3.10 Statistic Analyses

All the data of the color, texture and potassium measurement are analyzed using IBM SPSS Statistics, version 20. All the triplicates of reading are used and presented as the mean  $\pm$  standard deviation (S.D.). The significant different (p≤0.05) of mean values is assessed with Mann-Whitney u test.

## **CHAPTER 4**

#### RESULTS

### 4.1 Experiment Design

Table 4.1 is the results of the dependent variables based on 15 experiments conducted. According to the results, the average solid loss was 3.28 % and in the meanwhile, the highest and lowest solid loss in this experiment was 5.52 and 1.64 respectively. For the sensory evaluation test, panelists were evaluated based on taste, appearance, aroma, texture and overall acceptance. From the results obtained, appearance achieved the highest acceptance level 70.10 %, followed by aroma 63.88 %, texture 61.69 % and taste 61.51 % while the average of overall acceptance was 64.64 %.

No	А	B	С	Solid	Sensory evaluation					
				loss	Taste	Taste Appearance		Texture	Overall	
				(%)	(%)	(%)	(%)	(%)	Acceptance (%)	
1.	1	0	1	1.64	65.33	69.78	66.67	66.67	70.67	
2.	0	-1	-1	5.06	61.78	72.44	65.78	62.67	64.44	
3.	0	0	0	2.39	61.78	70.67	60.89	59.56	65.33	
4.	1	-1	0	3.20	60.89	70.67	64.44	58.67	60.44	
5.	-1	-1	0	3.13	54.67	71.11	60.89	57.78	59.11	
6.	0	1	-1	3.46	62.22	69.78	60.44	58.22	65.78	
7.	1	0	-1	2.87	60.89	71.56	69.33	64.00	67.56	

**Table 4.1**: Result of the response variables.

No	A	B	С	Solid	Sensory evaluation					
				loss	Taste	Appearance	Aroma	Texture	Overall	
				(%)	(%)	(%)	(%)	(%)	Acceptance (%)	
8.	0	1	1	5.52	64.44	71.11	68.89	64.00	64.89	
9.	-1	0	-1	3.16	60.89	66.22	64.44	56.89	61.33	
10.	0	0	0	3.82	62.67	72.89	66.67 67.56		69.78	
11.	1	1	0	2.57	60.44	69.78	57.33	64.89	67.11	
12.	0	-1	1	2.40	61.78	71.11	63.11	62.22	63.11	
13.	-1	0	1	4.50	62.22	67.11	61.33	61.78	63.11	
14.	0	0	0	3.27	60.89	66.67	64.44	58.67	63.56	
15.	-1	1	0	2.21	61.78	70.67	63.56	61.78	64.00	
		Mea	n	3.28	61.51	70.10	63.88	61.69	64.68	

**Table 4.1**: Results of the response variables continued.

\*NOTE: A = Gluten Free – All Purpose Flour, B = Passion Fruit, C = Egg

The regression output of the solid loss and the sensory evaluation were showed in Table 4.2. Based on the analysis of variance (ANOVA), the solid loss (%) and the acceptance level of taste (%) were significantly different at 95 % ( $p\leq0.05$ ) while appearance, aroma, texture and overall acceptance were not significantly different (p>0.05). Besides, the multiple correlation coefficient ( $R^2$ ) of the solid loss (%), taste, appearance, aroma, texture and overall acceptance were 0.76, 0.93, 0.44, 0.63, 0.50 and 0.68, respectively. The lack of fit for all the response variables were not significantly i.e. 0.38 (solid loss), 0.42 (taste), 0.78 (appearance), 0.41 (aroma), 0.77 (texture) and 0.58 (overall acceptance). Finally, the solid loss (%) and the taste (%) were chosen to establish the polynomial regression models and the response surface plots due to the significant differences in the ANOVA, the high  $R^2$  and the not significantly differences in the lack of fit.

Regression	Solid	Taste	Appearance	Aroma	Texture	Overall
Coefficients	loss	(%)	(%)	(%)	(%)	Acceptance
	(%)					(%)
$\beta_0$	3.16*	61.78*	70.07*	64*	61.93*	66.22*
$\beta_a$	-0.34	1.00*	0.83	0.94	2.00	2.28
$\beta_b$	-0.0033	1.22*	-0.50	-0.50	0.94	1.83
$\boldsymbol{\beta}_{c}$	-0.06	1.00*	-0.11	1.25e-9	1.61	0.33
$\beta_{ab}$	0.07	-1.89*	-0.11	-2.45	0.56	0.44
$\beta_{ac}$	-0.65	0.78	-0.67	0.11	-0.56	0.33
$\beta_{bc}$	1.18*	0.56	0.67	2.78	1.56	0.11
$\beta_{aa}$	-0.73	-1.28	-0.98	-0.78	-0.30	-1.22
$\beta_{bb}$	0.34	-1.06	1.46	-1.60	-0.85	-2.33
$\beta_{cc}$	0.61	1.83*	-0.43	2.22	0.70	0.67
R <sup>2</sup>	0.76	0.93	0.44	0.63	0.50	0.68
Lack of fit	0.38	0.42	0.78	0.41	0.77	0.58

**Table 4.2**: Regression output from JMP software of the response variable.

\*Significant differences at p≤0.05

**4.1.1** Response Surface Plot: Effects of interaction among gluten free allpurpose flour (A) and passion fruit (B) on the solid loss (%)

Figure 4.1 was showed the interaction amount of the flour and the passion fruit on the solid loss. The 3D plot showed that the increased amount of flour was found to decrease the solid loss when the amount of passion fruit was at the center point (0). Moreover, when the amount of flour is at the center point (0), increasing or decreasing the amount of passion fruit will induce solid loss



**Figure 4.1**: Effects of interaction among gluten free all-purpose flour (A) and passion fruit (B) on the solid loss (%) of the cooked samples.

**4.1.2** Response Surface Plot: Effects of interaction among gluten free allpurpose flour (A) and egg (C) on the solid loss (%)

The plot showed below (Figure 4.2) was the relationship between the amount of flour and egg. Based on the plot, increasing the level of flour and egg was found to reduce the solid loss in the noodle samples. Besides, increasing the amount of egg and decreasing the amount of flour wound induce solid loss. Conversely, high amount of flour with a low amount of egg also caused increased solid loss.



**Figure 4.2**: Effects of interaction among gluten free all-purpose flour (A) and egg (C) on the solid loss (%) of the cooked samples.

# 4.1.3 Response Surface Plot: Effects of interaction among passion fruit(B) and egg (C) on the solid loss (%)

Based on Figure 4.3, the interaction amount passion fruit and egg on the solid loss were known. By decreasing the amount of passion fruit and increasing the amount of egg or increasing the passion fruit level and decreasing the egg level would reduced the solid loss. In addition, when both the ingredients are at the extreme ends simultaneously, the solid loss were increased.



**Figure 4.3**: Effects of interaction among passion fruit (B) and egg (C) on the solid loss (%) of the cooked samples.

**4.1.4** Response Surface Plot: Effects of interaction among gluten free allpurpose flour (A) and passion fruit (B) on the taste (%)

Effects of interaction among flour and passion fruit on the taste (%) based on 15 experiments were showed in Figure 4.4. When flour and passion fruit were at the center point (0), it provided good taste to the noodle while if the noodle consist of low amount of flour and passion fruit, it was given the lowest acceptance level.



**Figure 4.4**: Effects of interaction among gluten free all-purpose flour (A) and passion fruit (B) on the taste (%) of the cooked samples.

4.1.5 Response Surface Plot: Effects of interaction among gluten free allpurpose flour (A) and egg (C) on the taste (%)

According to the Figure 4.5, the taste improved when the amount of flour and egg increased. When the egg was at the center point (0), decreasing of flour Would resulted in a reduction in taste.



**Figure 4.5**: Effects of interaction among gluten free all-purpose flour (A) and egg (C) on the taste (%) of the cooked samples.

# 4.1.6 Response Surface Plot: Effects of interaction among passion fruit(B) and egg (C) on the taste (%)

The taste was increased when the amount of egg and passion fruit was increased (Figure 4.6). The lowest taste presented when the amount of passion fruit was low and the amount of egg at the center point (0).



**Figure 4.6**: Effects of interaction among passion fruit (B) and egg (C) on the taste (%) of the cooked noodle.

#### 4.2 Formula Optimization

Based on the results obtained, the optimum level of independent variables for the optimal formula was gluten free all-purpose flour of 110 g, passion fruits of 17.5 g and egg of 30 g with predicted minimum solid loss as 1.46 % and maximum acceptance level of taste 64.90 %. This formulation showed a high desirability, 0.94 which represented the formulation was very desirable.

	Optimum			Predicted		
Solution	level					Desirability
	А	В	С	Solid	Taste	-
				loss (%)	(%)	
1.	110 g	17.5 g	30 g	1.46	64.90	0.94

 Table 4.3: Optimum level and predicted value.

### 4.3 Noodle Texture Measurement

The optimal formula and the normal wheat noodle showed significantly difference ( $p \le 0.05$ ) in the tensile strength and breaking length. The mean of the tensile strength of the normal wheat noodle was 2874 N which was higher than optimal formula (4.40 N). In addition, average breaking length of the normal wheat noodle was 125.48 mm and 89.90 for the optimal formula.

Table	<b>4.4</b> :	Texture	measurement	of	the	normal	wheat	noodle	and	optimal
formul	a.									

Samples	Texture	
	Tensile strength (g)	Breaking length (mm)
Normal wheat noodle	2874 ±473.14 <sup>a</sup>	$125.48 \pm 10.91^{a}$
Optimal formula	$4.40\pm 0.75^{b}$	$89.90 \pm 7.27^{b}$

The significant differences which  $p \le 0.05$  within one column was represented by differences superscript letters, <sup>a</sup> and <sup>b</sup>. The values were showed the mean value of the triplicates  $\pm$  standard error, n=3.

#### 4.4 **Noodle Color Measurement**

The lightness (L\*), redness (a\*) and the yellowness (\*) of the normal wheat noodle and optimal formula were showed in Table 4.5. There was no significant difference (p>0.05) in the lightness for the samples as the average of the L\* in three trials were 79.52 for normal wheat noodle and 76.58 for optimal formula. Furthermore, there was a significant effect ( $p \le 0.05$ ) in the redness and yellowness. The table below also recorded that a\* and b\* value of the optimal formula were 1.46 and 23.58 which higher than the normal wheat noodle, 0.60 and 18.25, respectively. Therefore, the optimal formula was found to be more red and yellow than normal wheat noodle.

Samples	Colour			
	L*	a*	b*	ΔΕ
Normal wheat noodle	75.92±0.51 <sup>a</sup>	0.60±0.08 <sup>a</sup>	18.25±0.11 <sup>a</sup>	
Optimal formula	76.58±0.60 <sup>a</sup>	1.46±0.06 <sup>b</sup>	23.58±0.35 <sup>b</sup>	5.45±0.39

**Table 4.5**: Color characteristic of the normal wheat noodle and optimal formula.

The significant differences which  $p \le 0.05$  within one column was represented by different superscript letters, <sup>a</sup> and <sup>b</sup>. The values were showed the mean value of the triplicates  $\pm$  standard error, n=3.

#### 4.5 Noodle Potassium Measurement

The result of the potassium measurement showed in Table 4.6 indicated no significant difference,  $p \le 0.05$ , between the normal wheat noodle and the optimal formula. The means of the absorbance of the normal wheat noodle was 0.117 while for the optimal noodle was 0.124.

 Table 4.6: Potassium measurement of the normal wheat noodle and optimal formula.

 Samples
 Absorbance

-	
Normal wheat noodle	$0.117 \pm 0.006^{a}$
Optimal formula	$0.124 \pm 0.004^{a}$

The significant differences which  $p \le 0.05$  within one column was represented by different superscript letters, <sup>a</sup> and <sup>b</sup>. The values were showed the mean value of the triplicates  $\pm$  standard error, n=3.

#### **CHAPTER 5**

#### DISCUSSION

#### 5.1 Experiment Design

Based on the Box-Behnken experiment design, three factors, including gluten free all-purpose flour, passion fruit and egg, and three levels (-1, 0 and 1) was used to obtain the optimum formulation of the noodle. The minimum solid loss (%) and the maximum sensory evaluation were the response variables. Multiple regression analysis were performed to analyze the response in order to obtain the estimated regression coefficients for the response variables.

The results of ANOVA (analysis of variance), multiple correlation coefficient  $(R^2)$  and the lack of fit, the solid loss (%) and the acceptance level of the taste (%) were selected to establish the polynomial regression model. Based on the results of ANOVA, only solid loss and taste were significant (p<0.05). Besides, the multiple correlation coefficient ( $R^2$ ) of the solid loss and the taste were closed to 1 which were 0.76 and 0.93 respectively (Table 4.2). The  $R^2$  coefficient showed how well the regression model fitted the experiment data and the  $R^2$  value close to 1 indicated that the model was perfected (Sabanis, et al., 2009). The  $R^2$  coefficient of the taste showed that the model was accurate enough for predicting the relevant responses. Furthermore, the lack of fit for both solid loss and taste were not significantly differences at 95 % which meet the conditions for model selection as explained in Chapter 3. Therefore, the polynomial regression model of the solid loss and taste were showed below:

**Equation 5.1**: Polynomial regression model of the solid loss (%).

(Solid Loss) $Y_1$ 

$$= 3.16 - 0.34A - 0.0033B - 0.06C + 0.07AB - 0.65AC$$
$$+ 1.18BC - 0.73A^{2} + 0.34B^{2} + 0.61C^{2}$$

Equation 5.2: Polynomial regression model of the taste (%).

$$(Taste)Y_2 = 61.78 + 1A + 1.22B + 1C - 1.89AB + 0.78AC + 0.56BC$$
$$- 1.28A^2 - 1.06B^2 + 1.83C^2$$

The regression model was used to predict the responses variables and also generated the response surface plot. The 3D response surface plots were used to represent the interactions between solid loss and taste and the response variable which gluten free all-purpose flour and passion fruit (Figure 4.1 and 4.4), gluten free all-purpose flour and egg (Figure 4.2 and 4.5) and passion fruit and egg (Figure 4.3 and 4.6).

#### 5.2 Solid Loss

The amount of matter that soluble into the cooking water of the optimally cook noodle called solid loss (Ritthiruangdej, et al., 2011). A good quality noodle must have a low solid loss, a high solid loss will cause cloudy cooking water and loss of the matter of the noodle such as nutrients into the cooking water. This is due to the poor gluten network and high level of the solubilized starch in the noodle. The solubilized starch in cooking water can also contribute to sticky mouthfeel of the noodle (Thomas, et al., 2014). Normally, the high solid

loss of the noodle is found in the gluten free noodle as weak gluten network lead to more soluble matter to leach out.

For this study, an increasing volume of the flour and egg (Figure 4.2) and the increasing of the amount of egg and decreasing the amount of passion fruit (Figure 4.3) would decrease the solid loss of the noodle. This was because egg protein, especially egg white has an important influence to then. The egg white can give a good cooking tolerance, not easy breakdown and reduce the solid loss of the noodle (Li, et al., 2014). Besides, egg white can be used to substitute the gluten structure although not entirely so (Zandonadi, et al., 2012).

In Figure 4.3, the solid loss was reduced when increased the amount of passion fruit and decreased the amount of egg. According to Hui (2008), the passion fruit juice was high in starch content. Being so, the starch content in the passion fruit juice could reduce the solid loss in the noodle because of the starch gelatinization. However, too much of starch content in the noodle will increased the solid loss (Fu, 2008). In Figure 4.1, the amount of flour at the center point (0) with the increasing or decreasing the amount of passion fruit would increase the solid loss. However, by far, there is no study on the relationship between passion fruit and solid loss. In figure 4.1, the increased amount of flour would decrease the solid loss when the amount of passion fruit was at the center point (0).

The results of the solid loss in this study were compared with other studies. For example, a study used different amount of the unripe banana flour to substitute wheat noodle (Ritthiruangdej, et al. 2011). Their results showed that the high amount of unripe banana flour had a high solid loss which was between 9.35 to 11.49 %. The high level of the banana flour in the wheat noodle has reduced the level of the protein or gluten and caused the weak gluten network hence increased the solid loss. In addition, a study was done to developed a gluten free pasta with green banana flour (Zandonadi, et al., 2012). The pasta was compared with the whole wheat pasta. They found that the solid loss of the green banana pasta was higher than the whole wheat pasta, 12.75 % and 4.48 %, respectively. Moreover, gluten free pasta and noodle have a higher solid loss than wheat pasta and noodle (Susanna and Prabhasankar, 2013). This was due to the devoid of the gluten in the flour which affected the solid loss of the noodle. Besides, another study showed the high level of the starch content in the gluten free noodle also increased the solid loss (Bilgi di, 2008). Based on the results of the solid loss of all the samples in Table 4.1, the range of solid loss was between 1.64 to 5.52 % while this result was better than other studies. For this study, the dough resting for 20 to 30 minutes during the noodle manufacturing to promote the hydration of the dough particles and result in a smoother and less striped dough after sheeting (Hou and Kruk, 1998). Steaming the dough before sheeting was a different method for noodle manufacturing which never implemented in other studies. The aim of the steaming was to expand the starch and also gelatinized it to a greater extent (Hou, 2010). The starch gelatinization can increase the viscoelastic texture and also shorten the rehydration time of the noodle (Hou, 2010). The steam dough would help in withstanding sheeting than the raw dough and reduced the

cooking time. Therefore, the lower solid loss presented in the study is probably due to the shorter cooking time than other studies.

#### 5.3 Sensory Evaluation

Based on the results of the sensory evaluation, the acceptance level of the overall acceptance, texture, aroma and appearance of the samples were at the moderate level where they were no significant difference in the samples statistically. According to the results in Table 4.1, the acceptance level of overall acceptance was 64.68 %. This represented that more than half of the panelists were able to accept the purple passion fruit infused gluten free noodle.

Furthermore, consumer would normally purchase the noodle products with better visual appearance, color and brightness (Susanna and Prabhasankar, 2013). The appearance of the samples has attracted the panelists as it scored the highest acceptance level. This was because the passion fruit increased the color of the noodle and the embellishment of the seeds. Besides, more than 60 % of the panelists liked the aroma of the passion fruit as passion fruit has a unique flavor. For the acceptance level of the texture, there was 61.69 % which scored lower than other attributes as most of the Asian noodles were produced by the wheat flour and people is used to that texture. Wheat flour can provide a hard and elastic texture to the noodle which is preferred by consumers (Dewi, 2011). For the samples in this present study, the noodle was made by gluten free flour whereas the texture of gluten free noodle was less elastic than wheat flour noodle.

According to the plot in Figure 4.5 and 4.6, increased amount of egg and the flour or passion fruit has increased the taste. Therefore, the egg plays an important flavor enhancer in the taste of noodle. Moreover, the addition of the seeds had reduced the sour taste of the noodle as too much of the passion fruit juice would cause the noodle to taste sour. Asian noodle is commonly consumed with different soups (Hou and Kruk, 1998). Therefore, a strong flavor of the noodle such as sour taste is hard to match with different kind of soup and led to reduce liking by the consumers.

There also have some of the comments from the panelists such as the sensory test was let them know what was a gluten free noodle. Gluten free products only begin to gain popularity in recent years. Therefore, some of the panelists were lack of the information about the gluten free products. After the sensory evaluation, they had some basic information for gluten free products. Besides, some of the panelists feel surprised on development of noodle with purple passion fruit and said that the passion fruit seeds enhanced the mouthfeel and also the taste of the noodle.

#### 5.4 Formula Optimization

According to the target to get maximum desirability, the predictive values of the response was showed in Table 4.3. The optimum ingredients level of gluten free all-purpose flour was 110 g, passion fruits 17.5 g and egg 30 g with the predicted value in minimum solid loss as 1.46 % and maximum taste 64.90 %. The desirability was 0.94, mean that the optimal formulation was very desirable.

#### 5.5 Noodle Texture Measurement

The tensile strength and breaking length of the optimally cooked normal wheat noodle and the optimal formula were compared in Table 4.4. Both the tensile strength and the breaking length of the normal wheat noodle were higher than the optimal formula whereas both of them were significantly different ( $p \le 0.05$ ). The mean value of the tensile strength of the normal wheat noodle (2874 g) was higher than the optimal formula (4.4 g). The high score of the normal wheat noodle showed that it has a firm texture and elasticity (springy bite) than the optimal formula (Dewi, 2011). The matrix structural network which will affect the texture of the noodle is affected by the starches, gluten or substitution proteins, edible gums and so on. (Dewi, 2011). The tensile strength of the optimal formula decreased as it was gluten free. In wheat flour, there was 80 % of gluten which contains gliadins and glutenins. Gliadins are responsible for the extensibility or viscosity while glutenins are responsible for strength or elasticity of the dough (Ritthiruangdej, et al., 2011). The breaking length of the optimal formula was similar with the normal wheat noodle. This might caused by the high amount of the egg in the optimal formula. This also in agreement with another study which found that egg protein formed a matrix structure that led to increasing the breaking length of the noodle (Dewi, 2011).

#### 5.6 Noodle Color Measurement

The color is one of the important quality factors that attracts the consumers and also parameter use to evaluate visual quality of the noodle by the consumer. For this study, the L\* (lightness), a\* (redness) and b\* (yellowness) value of the

normal wheat noodle and the optimal formula were measured by colorimeter. The results showed the total color difference ( $\Delta E^*$ ) between two samples was 5.44 (Table 4.5). The lightness of the normal wheat noodle and the optimal formula were not significantly different (p>0.05), the mean value of the normal wheat noodle and optimal formula were 75.92 and 76.58, respectively. Besides, the redness and yellowness were significantly different (p≤0.05). There found that the optimal formula was more red and yellow than the normal wheat noodle. This was contributed by the carotenoids which is the natural pigment present in the purple passion fruit (Phamiwon and Sheila, 2015). Carotenoids also presence in carrot, papaya, orange and some other orange, yellow and red color fruits and vegetables.

#### 5.7 Noodle Potassium Measurement

The result showed in Table 4.6 was no significant difference between both samples while the potassium content in the optimal formula was 8.6 % higher than normal wheat noodle. This might because the addition amount of the passion fruit in the optimal formula was not high enough. Besides, this result proved that the potassium content in the optimal formula was retained and showed higher value than normal wheat noodle. The concentration of potassium in the normal wheat noodle was 0.86 ppm and the optimal formula was 0.94 ppm.

### 5.8 Further Recommendation

There were some tests that could not be done in this project due to time limitation. For example, the shelf life study of the noodle, crube fiber determination, mineral determination or different drying method. Therefore, the future research is very important to collect more information about this study. Besides, different drying method also can be used to increase the shelf life of the noodle. Some of the studies have showed that passion fruit is rich in fiber and minerals such as iron. Therefore, the crube fiber and minerals analysis can be tested in the future. Lastly, the texture measurement can be included into the experiment design to optimize the ingredients level.

#### CHAPTER 6

#### CONCLUSION

In this project, the purple passion fruit was infused into the gluten free noodle. Response Surface Methodology (RSM) was used to optimize the ingredients level. The Box-Behnken design was used to design the experiment as this design can avoid conducting the experiment under extreme conditions. Hence, saving time and cost. Three independent variables (gluten free all-purpose flour, passion fruit and egg) with three levels were used in Box-Behnken design. Based on the results, the optimum formula was 110 g of gluten free all-purpose flour, 17.5 g of passion fruit and 30 g of egg with the basic formula where salt (2 g) and water (18 mL). According to the sensory evaluation results, the overall acceptance level of the noodle was in moderate level. Consequently, the purple passion fruit infused gluten free noodle was successfully developed. The texture, color and potassium content of the normal wheat noodle and the optimal formula were compared. The results showed that the tensile strength of the normal wheat noodle was higher than the optimal formula one while the breaking length of both noodles was similar. This was because the absence of gluten in the optimal noodle. Besides, the optimal formula was more red and yellow than the normal wheat noodle due to the nature pigment in the purple passion fruit. Although the passion fruit was high in potassium, there was no significant difference (p>0.05) between normal wheat noodle and optimal noodle. However, the optimal formula was 8.6 % higher than normal wheat noodle.

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#### **APPENDICES**

#### Appendix A

#### **Questionnaire for Hedonic Scaling Test**

#### **Product: Gluten Free Noodle**

Panel No.:Date:Occupation:Gender: M / F

#### **Instruction:**

You are given three coded samples. Please taste the sample and use the number scale below to rate how much you LIKE or DISLIKE the sample in terms of appearance, aroma, taste, texture and overall acceptance.

(9) Like extremely
(8) Like very much
(7)Like moderately
(6) Like slightly
(5) Neither like nor dislike
(4)Dislike slightly
(3) Dislike moderately
(2) Dislike very much
(1) Dislike extremely

Please rinse your mouth with water before tasting each sample. Evaluate both products starting from the left.

Sample codes		
Attributes		
Appearance		
Aroma		
Taste		
Texture		
Overall Acceptance		

Comments:

Thank you.

#### Appendix **B**

#### **Output of Response Surface Methodology**

Prediction Profiler with Maximum Desirability Set for optimize ingredients level.



# Appendix C

# Output of Mann-Whitney Test

# **Tensile Strength**

	type	Ν	Mean Rank	Sum of Ranks
	normal wheat noodle	3	5.00	15.00
Tensile Strength	optimal formula	3	2.00	6.00
Suongin	Total	6		

Ranks

## **Test Statistics**<sup>a</sup>

	tensile
Mann-Whitney U	.000
Wilcoxon W	6.000
Z	-1.964
Asymp. Sig. (2-tailed)	.050
Exact Sig. [2*(1-tailed Sig.)]	.100 <sup>b</sup>

a. Grouping Variable: type

b. Not corrected for ties.

# **Breaking Length**

Ranks

	type	Ν	Mean Rank	Sum of Ranks
	normal wheat noodle	3	5.00	15.00
Breaking Length	optimal formula	3	2.00	6.00
_	Total	6		

## Test Statistics<sup>a</sup>

	breaking
Mann-Whitney U	.000
Wilcoxon W	6.000
Z	-1.964
Asymp. Sig. (2-tailed)	.050
Exact Sig. [2*(1-tailed Sig.)]	.100 <sup>b</sup>

a. Grouping Variable: type

b. Not corrected for ties.

# Lightness (L\*)

## Ranks

	type	Ν	Mean Rank	Sum of Ranks
	normal wheat noodle	3	2.50	7.50
L*	optimal formula	3	4.50	13.50
	Total	6		

#### **Test Statistics**<sup>a</sup>

	L
Mann-Whitney U	1.500
Wilcoxon W	7.500
Z	-1.328
Asymp. Sig. (2-tailed)	.184
Exact Sig. [2*(1-tailed Sig.)]	.200 <sup>b</sup>

- a. Grouping Variable: type
- b. Not corrected for ties.

# Redness (a\*)

#### Ranks

	type	Ν	Mean Rank	Sum of Ranks
	normal wheat noodle	3	2.00	6.00
a*	optimal formula	3	5.00	15.00
	Total	6		

## **Test Statistics**<sup>a</sup>

	а
Mann-Whitney U	.000
Wilcoxon W	6.000
Z	-1.964
Asymp. Sig. (2-tailed)	.050
Exact Sig. [2*(1-tailed Sig.)]	.100 <sup>b</sup>

- a. Grouping Variable: type
- b. Not corrected for ties.

# Yellowness (b\*)

# Ranks

	type	Ν	Mean Rank	Sum of Ranks
	normal wheat noodle	3	2.00	6.00
b*	optimal formula	3	5.00	15.00
	Total	6		

## **Test Statistics**<sup>a</sup>

	b
Mann-Whitney U	.000
Wilcoxon W	6.000
Z	-1.964
Asymp. Sig. (2-tailed)	.050
Exact Sig. [2*(1-tailed Sig.)]	.100 <sup>b</sup>

a. Grouping Variable: type

b. Not corrected for ties.

# Appendix D

Tensile strength and breaking length of the optimally cooked normal

	Tensile Strength		Breaking Length	
Samples	Normal	Optimal	Normal	Optimal
	Wheat Noodle	Formula (g)	Wheat Noodle	Formula (mm)
	(g)		( <b>mm</b> )	
a. 1 <sup>st</sup> Trial	3182.90	5.10	131.92	81.51
b. 2 <sup>nd</sup> Trial	2329.30	4.50	112.88	94.31
c. 3 <sup>rd</sup> Trial	3109.80	3.60	131.64	93.87
Mean ±	2874 ±473.14	$4.40 \pm 0.75$	$125.48 \pm 10.91$	89.90 ±7.27
Standard Error				

wheat noodle and Optimal formula.

	L	*		a <sup>*</sup>		b*						
Types of	Normal	Gluten		Normal	Gluten		Normal	Gluten				
Noodle	wheat	free	$\Delta \mathbf{L}$	wheat	free	$\Delta \mathbf{a}$	wheat	free	$\Delta \mathbf{b}$	$\Delta \mathbf{E}$	Mean of	Standard
	noodle	noodle		noodle	noodle		noodle	noodle			$\Delta \mathbf{E}$	Error
1st trial	75.82	76.04	0.22	0.58	1.52	0.94	18.16	23.48	5.32	5.41		
2nd trial	76.47	77.23	0.76	0.54	1.4	0.86	18.22	23.97	5.75	5.86		
3rd trial	75.46	76.47	1.01	0.69	1.47	0.78	18.38	23.3	4.92	5.08	5.45	0.39

Color characteristics of fresh normal wheat noodle and optimal formula.

#### **Calibration Curve for Potassium Measurement**

