

**DEVELOPMENT AND OPTIMIZATION OF PUMPKIN (*Cucurbita  
moschata*)-CARROT (*Daucus carota*) PASTA SAUCE FORMULATIONS  
USING RESPONSE SURFACE METHODOLOGY**

By

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## ABSTRACT

### DEVELOPMENT AND OPTIMIZATION OF PUMPKIN (*Cucurbita moschata*)-CARROT (*Daucus carota*) PASTA SAUCE FORMULATIONS USING RESPONSE SURFACE METHODOLOGY

**Kang Kah How**

Sauce is a popular complementary food in the community. A sauce containing pumpkin and carrot as an antioxidant combined with the habits of the people who often use it for seasoning food, will be very beneficial in improving public health and prevention from many diseases. The study aimed to develop a pumpkin-carrot pasta sauce product using response surface methodology (RSM). RSM with three-factor and three-level mixture design was used to optimize the formula of the product. The effects of three independent variables namely pumpkin puree, carrot puree and sugar on sensory evaluation (colour, appearance, viscosity, aroma, taste, and overall acceptability) and colour analysis (difference in yellowness,  $\Delta b^*$ ) of pumpkin-carrot pasta sauce were investigated. The response surface plot was generated which is a helpful tool for a better understanding of the relationships between each factor and response. The optimum formulation of pumpkin-carrot pasta sauce consisted of 500 g pumpkin puree, 200 g carrot puree, 0.96 g sugar, 30 g water, 15 g corn oil, 15 g cooking cream, 8 g unsalted butter, 3 g vinegar, 3 g corn starch, 3 g salt, 2 g garlic puree, 0.4 g black pepper powder and 0.4 g mixed herbs. Thereafter, the

moisture content test, total soluble solid test, pH measurement, and viscosity measurement were carried out for optimized pumpkin-carrot pasta sauce. The moisture content, total soluble solid and pH were  $86.67 \pm 0.03$  %,  $24.93 \pm 0.65$  °Brix and  $5.09 \pm 0.01$ , respectively. Rheological properties (yield stress, consistency index and flow behaviour index) of sauce were measured at selected temperatures (5 °C, 25 °C and 85 °C). The flow behaviour of the sauce was adequately described by the Herschel Bulkley model. The optimized pumpkin-carrot pasta sauce exhibited pseudoplastic behaviour and consistency index or viscosity decreased with increase in temperature.

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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 acknowledge. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

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**KANG KAH HOW**

## APPROVAL SHEET

This project entitled "**DEVELOPMENT AND OPTIMIZATION OF PUMPKIN (*Cucurbita moschata*)-CARROT (*Daucus carota*) PASTA SAUCE FORMULATIONS USING RESPONSE SURFACE METHODOLOGY**" was prepared by KANG KAH HOW 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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It is hereby certified that **KANG KAH HOW** (ID No: **13ADB03494**) has completed this final year project entitled “**DEVELOPMENT AND OPTIMIZATION OF PUMPKIN (*Cucurbita moschata*)-CARROT (*Daucus carota*) PASTA SAUCE FORMULATIONS USING RESPONSE SURFACE METHODOLOGY**” under the supervision of Mr. Chung Kok Heung from the Department of Agricultural and Food Science, Faculty of Science.

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## LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
a*	Redness
BBD	Box-Behnken design
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
b*	Yellowness
CCD	Central composite design
C.	<i>Cucurbita.</i>
DNA	Deoxyribonucleic acid
FDA	Food and Drug Administration
FAO	Food and Agriculture Organization
G	Gram
IFIC	International Food Information Council
Kcal	Kilocalorie
L*	Lightness
MSG	Monosodium glutamate
Mg	Milligram
µg	Microgram
Pa	Pascal
Pa.s	Pascal-second
pH	Potential hydrogen
RSM	Response surface methodology

$R^2$	Multiple correlation coefficient
SD	Standard deviation
S	Seconds
TSS	Total soluble solid
USDA	United States Department of Agriculture
wt	Weight
3D	3-dimensional
2D	2-dimensional
%	Percentage
$^{\circ}\text{C}$	Degree in Celsius
$^{\circ}\text{F}$	Degree in Fahrenheit
$^{\circ}\text{Brix}$	Degree brix
$\Delta E^*$	Total colour differences
$\Delta b^*$	Differences in yellowness

# CHAPTER 1

## INTRODUCTION

### 1.1 Background

In the century of advanced science and technology, white-collar and blue-collar workers are living in a fast-paced environment. Hence, they are looking for quick, easy and convenient foods on-the-go. Therefore, fast foods are in high demand due to the short preparation time needed. Based on studies, sauces had turned out to be one of the popular food product and they are often used in fast food as dipping or used as a condiment to enhance the eating experience (Perera and Pavitha, 2017).

Sauces and seasonings category is ranked as one of the top 10 ethnic products from 2007 to 2011 and is expected to continue its strong growth, since the usage of sauce increased due to conventional home cooking all around the world (Meszaros, 2012). According to Chicago-based Mintel International's Cooking and Pasta Sauces, Marinades – US report in December 2015, the sales data of cooking sauces, pasta sauces and marinades grew 12 % between 2010 and 2015 and is expected to continue increase up to 13 % between 2015 and 2020, reaching \$6.2 billion (Fuhrman, 2016). Besides that, sales of sauces, dressings and condiments in Agri-Food retail sales in Malaysia are increasing progressively since 2009 to 2013 by a compound annual growth rate (CAGR) of 3.4 % and is expected to grow until 2018 by a compound annual growth rate (CAGR) of 3.5 % (Agriculture and Agri Food Canada, 2014).

In Malaysia, pumpkin is still underutilization. According to the Department of Agriculture in Malaysia, the productions of tomatoes are the highest among other vegetables. Whereas, production of pumpkin increased from 2009 of 11,185 tonne up to 2014 of 111.377 tonne. The production of pumpkin was ranked number 2 after the tomatoes (Shahirah, 2015). Since the production of pumpkin is high in Malaysia, they are widely used in Malaysia cuisines like ‘pengat labu’ for its flesh and ‘kuaci’ production. Other than that, pumpkins can be preserved with sugars and serve as dessert (Norshazila, et al., 2013). Pumpkin can be used as food ingredients that can support food security and processed into food that is economical and healthy through the diversification process due to its readily available, low cost, and high durability (Khamidah, 2013).

Many people do not aware of the health benefits of pumpkin and its ability to diversify into a variety of products, including sauce. Moreover, the consumer communities have opted old-fashioned way of using tomato as sauce-based. Alternatively, pumpkin can be used as a substitute for tomatoes, especially when sudden factors affecting the tomatoes market. A study also claimed that the nutritional value of the pumpkin is not less than the tomatoes (Khamidah, 2013).

In this project, pumpkin (*Cucurbita moschata*) was chosen as the main ingredient of the sauce with carrot (*Daucus carota*) added to enhance the nutritional values of the product. The major aim of this study was to produce pumpkin-carrot pasta sauce and the formulation of the three ingredients

(pumpkin puree, carrot puree and sugar) was optimized *via* the response surface methodology (RSM) through Box-Behnken design.

## **1.2 Objectives**

The objectives of this project were:

- To develop and determine an optimum pumpkin-carrot pasta sauce formulation by using RSM based on colour analysis and sensory evaluation.
- To evaluate the moisture content, total soluble solid, pH and viscosity of optimized pumpkin-carrot pasta sauce.

## **CHAPTER 2**

### **LITERATURE REVIEW**

#### **2.1 Sauce**

##### **2.1.1 Definition of Sauce**

The word “sauce” is a French word taken from feminine word form of the Latin word “salsa”, meaning salted (Grimsdale, 1986). According to Food Act 1983 and Food Regulations 1985, sauce shall be a fluid, semi-fluid and sometimes semi-solid food product that containing with or without different spices and having desired flowability and consistency (Akram, et al., 2012). The applications of sauces were heavily diversified around the world. Often, they were used in preparing other foods such as barbecue sauce, chili sauce, tomato sauce, black pepper sauce, soy sauce and etc. Table 2.1 shows the research studies on the sauce products.

##### **2.1.2 Applications of Sauce**

Sauce is a beloved complementary food in the community and is one of the flavouring ingredients often used in various foods. The main functions of a sauce are to enrich food, improve its flavor and aroma, as well as the taste. Besides, adding moisture, texture and improving visual appeal of the food (Krystyjan, et al., 2012).

**Table 2.1.** The research studies on the sauce products.

<b>Title</b>	<b>References</b>
Development of a sauce using <i>Gymnema sylvestre</i> Leaves.	(Zhong, et al., 2015)
The development of functional Black bean soy sauce.	(Wan, et al 2013)
Effect of added ingredients on water status and physico-chemical properties of tomato sauce.	(Diantom, et al., 2017)
Application of lactic acid bacteria and yeasts as starter cultures for reduced-salt soy sauce (moromi) fermentation.	(Singracha, et al., 2017)
Antioxidant activity of fermented meat sauce and isolation of an associated antioxidant peptide.	(Ohata, et al., 2016)
Comparison of non-volatile taste-active components in fish sauce produced from lizardfish <i>Saurida wanieso</i> viscera under different conditions.	(Zhong, et al., 2015)
Effect of thermally inhibited starches on the freezing and thermal stability of white sauces: Rheological and sensory properties.	(Sanz, Tarrega and Salvador, 2016)



### **2.1.3 The Five Mother Sauces**

In the 19<sup>th</sup> century, the four original mother sauces were Allemande, Béchamel, Velouté and Espagnole, which created by Chef Marie-Antoine Carême. In the 20<sup>th</sup> century, Chef Auguste Escoffier refined Carême's list of basic sauces by demoted Allemande to a secondary sauce of Velouté, and added sauce Tomate and Hollandaise. The five mother sauces are different based on their main ingredient and thickening agent (Moncel, 2016). These five mother sauces act as the starting points and it can be made into various secondary sauces or “small sauces” by adding herbs, spices, or other ingredients (Alfaro, 2017).

Four out of five mother sauces initiate with a roux. Roux is a cooked mixture of fat and flour that generally used to thicken sauces. The fat used is either butter or oil. When the liquid is added to a roux, the flour thickens the liquid and end up with sauce (Gallary, 2014). The details information about five mother sauces were listed in Table 2.2.

**Table 2.2.** The information about five French mother sauces.

<b>Sauce</b>	<b>Base</b>	<b>Classically Served With</b>	<b>Classically Flavouring</b>	<b>Common Secondary Sauce</b>
<b>Béchamel</b>	White roux (flour and butter), Milk	Eggs, Fish, Steamed Poultry, Pasta, Vegetable	White Onion, Clove, Bay Leaf, Salt, White Pepper, Nutmeg	Cream Sauce, Mornay Sauce, Cheddar Cheese Sauce
<b>Tomate</b>	Tomatoes and Roux	Pasta, Fish, Vegetable, Polenta, Veal, Poultry, Breads, Pizza	Salt Pork, Mirepoix, Garlic, White Veal Stock, Salt, Pepper, Sugar	Spanish Sauce, Creole Sauce, Portuguese Sauce
<b>Velouté</b>	White Stock, Roux	Eggs, Fish, Steamed Poultry, Steamed Vegetables, Pasta, Veal	None, used specifically as a base	Mushroom Sauce, Herb Sauce, Normandy Sauce, Sauce Allemande

(Alfaro, 2017)

**Table 2.2.** The information about five French mother sauces (continued).

<b>Sauce</b>	<b>Base</b>	<b>Classically Served With</b>	<b>Classically Flavouring</b>	<b>Common Secondary Sauce</b>
<b>Espagnole</b>	Roasted Veal Stock, Brown Roux	Roasted Meats (Beef, Duck, Veal, Lamb)	Mirepoix, Sachet (Bay Leaf, French Thyme, Parsley), Tomato Puree	Mushroom Sauce, Robert Sauce, Chasseur Sauce, Bercy Sauce
<b>Hollandaise</b>	Egg Yolks, Butter	Eggs, Vegetables, Light Poultry dishes, Fish, Beef	Peppercorns, White Wine Vinegar, Salt, Lemon Juice, Cayenne Pepper	Bearnaise Sauce, Maitaise Sauce, Mousseline Sauce, Choron Sauce

(Alfaro, 2017).

## **2.2 Food Additives**

Nowadays, food additives and advances in technology has provided consumers with high quality food supply. In order to ensure food security and compliances with legislations, all the food additives are carefully regulated by local government and few international associations and foundations.

According to Food and Drug Administration (FDA) and Codex Alimentarius, food additive is any substance that added to food for a technological purpose in the manufacturing, processing, preparation, treatment, packing, packaging, transportation or storage of food (Casal, Rosas and Malavé, 2016). Food additives serve the purpose to improve and maintain the nutritional value of the foods. Such enrichment can help to reduce malnutrition in worldwide. Next, addition of food additives can enhance stability of food. For instance, preservatives retard activity of food pathogens and slow down the product spoilage (IFIC and FDA, 2010). Moreover, food additives improve its organoleptic properties such as taste, texture and appearance of the food and maintain the nature of the food so as to defraud the consumer (Casal, Rosas and Malavé, 2016).

## 2.2.1 Types of Food Ingredients

There is a list of additives for the use in sauces under food category 12.0 that approved in the Codex General Standard for Food Additives by Codex Alimentarius. Table 2.3 shows the information of some food ingredients that commonly used in sauces.

**Table 2.3.** Information of food ingredients.

<b>Types of ingredients</b>	<b>Information and Source</b>
Preservatives	<ul style="list-style-type: none"><li>• Prevent food spoilage.</li><li>• Slow down or prevent the changes in colour, flavor, or texture and delay rancidity.</li><li>• Extend the shelf life of products.</li><li>• Maintain freshness.<ul style="list-style-type: none"><li>✓ Ascorbic acid, citric acid, sodium benzoate, calcium propionate, sodium erythorbate, sodium nitrite, calcium sorbate, potassium sorbate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT).</li></ul></li></ul>
Flavors and Spices	<ul style="list-style-type: none"><li>• Add specific flavors (natural and synthetic).<ul style="list-style-type: none"><li>✓ Natural flavoring, artificial flavor, and spices.</li></ul></li></ul>

(IFIC and FDA, 2010)

**Table 2.3.** The information of food ingredients (continued).

<b>Types of ingredients</b>	<b>Information and Source</b>
Flavor Enhancers	<ul style="list-style-type: none"><li>• Enhance flavors already present in foods (without providing their own separate flavor)<ul style="list-style-type: none"><li>✓ Monosodium glutamate (MSG), hydrolyzed soy protein, autolyzed yeast extract, disodium guanylate or inosinate.</li></ul></li></ul>
Emulsifiers	<ul style="list-style-type: none"><li>• Allow smooth mixing of ingredients, prevent separation.</li><li>• Keep emulsified products stable.</li><li>• Help products dissolve more easily.<ul style="list-style-type: none"><li>✓ Soy lecithin, mono- and diglycerides, egg yolks, polysorbates, sorbitan monostearate.</li></ul></li></ul>
Stabilizers and Thickeners, Binders, Texturizers	<ul style="list-style-type: none"><li>• Produce uniform texture, improve "mouth-feel"<ul style="list-style-type: none"><li>✓ Gelatin, pectin, guar gum, carrageenan, xanthan gum, whey, cornstarch, potato starch.</li></ul></li></ul>

(IFIC and FDA, 2010)

## 2.3 Pumpkin

### 2.3.1 Background of Pumpkin

The word “pumpkin” came from the Greek word “*pepon*”, which carry the meaning of large melon (Ott, 2012). Pumpkin is a member of the “*Cucurbitaceae*” family and genus of “*cucurbita*”, which also includes squash, cantaloupes, cucumbers, melons, and gourds and zucchinis (Orzolek, 2012). There are total 27 species can be found under the branch of genus *Cucurbita*. The term “pumpkin” refers to members of five main species which are, *C. moschata*, *C. maxima*, *C. ficifolia*, *C. pepo* and *C. mixta* (Lakshman and Prasad, 2015). Pumpkin is an important *cucurbitaceous* vegetable, due to the high productivity, low cost of production, good storability and comparatively high content of carotene in fruits, which is grown under wide range of agro- climatic conditions all over the world and have great demand in the market (Shivananda, et al., 2012).

In Malaysia, the two species of pumpkins commonly grown are *Cucurbita moschata* (labu manis) and *Cucurbita moschata* Duchesne (labu loceng). *Cucurbita moschata* (labu manis) can be found throughout Malaysia. Contrastily, *Cucurbita moschata* Duchesne (labu loceng) come solely from Kedah and they are different in size, shape and colour. Next, Labu manis is commonly known for its spherical shape while labu loceng can be recognized by its unique bell shape (Norshazila, et al., 2014). *C. moschata* is the most extensively grown species of *cucurbita*. Most of them are hard-skinned and are originated from either China or Central America (Napier, 2009).

### 2.3.2 Harvest Maturity Indices and Storage of Pumpkin

The maturity of pumpkins depends on cultivar and ready to be plucked when the pumpkins are completely developed. The pumpkin, being very simple and rapid for grow, harvesting at about two to three months (approximately 45 days after flowering) and are able to be stored for as long as six months (Margaret, 2015). Placing pumpkins in a well-ventilated storage area is preferred, because it is protected from the environment factors (dust or rain). Besides, the quality of pumpkin can be retained at the appropriate relative humidity of 50-70 % and temperature of 50-55 °F (Orzolek, 2012).

In order to determine the harvest maturity, there are different measurements, like checking on the hardness of the rind, stem texture, internal colour and calculating the planting time (Ministry of Fisheries, Crops and Livestock, New Guyana Marketing Corporation and National Agricultural Research Institute, 2004). Pumpkins are harvested by bare hand when they are fully matured. When matured, the skin will subtle changes in rind colour. For instance, green stripes of Japanese pumpkins, '*Kabocha*' turn dull green when mature. Other than that, the rind tissue becomes noticeably tougher and harder. When breaking the skin, a 'crisp' sound can be heard (Napier, 2009). The fruit is immature when there is a green actively growing tendril and it is ready for harvest once the tendril completely dries. Immature fruit have a cream-coloured flesh and will affect the eating quality, due to the lower stored carbohydrates. In addition, they tend to lose more weight during storage than mature fruits. During storage, the concentrations of the yellow and orange carotenoids generally increase. In other



words, the intensity of internal pulp orange colour will increase as the fruit matures (Ministry of Fisheries, Crops and Livestock, New Guyana Marketing Corporation and National Agricultural Research Institute, 2004).

### **2.3.3 Nutritional Value of Pumpkin**

Pumpkin is a remarkably nutrient-dense food and is one of the very low calories food, which can bring a lot of beneficial effects to the body. According to United States Department of Agriculture, 100 g of pumpkin provides just 26 calories and contains no saturated fats or cholesterol (USDA, 2017). Table 2.4 summarises the energy and parts of the nutritional constituents of raw pumpkin per 100 g of edible portion.

**Table 2.4.** The energy and parts of the nutritional constituents of raw pumpkin per 100 g of edible portion.

<b>Nutrients</b>	<b>Amount per serving (100.0g)</b>	<b>Nutrients</b>	<b>Amount per serving (100.0g)</b>
Energy (kcal)	26.00	Calcium (mg)	21.00
Water (g)	91.60	Potassium (mg)	340.00
Carbohydrate (g)	6.50	Phosphorus (mg)	44.00
Protein (g)	1.00	Magnesium (mg)	12.00
Lipid (g)	0.10	Sodium (mg)	1.00
Fibre (g)	0.50	Vitamin A (µg)	426.00
Sugars (g)	2.76	Vitamin B1 (mg)	0.05
		Vitamin B2 (mg)	0.11
		Vitamin B3 (mg)	0.60
		Vitamin B6 (mg)	0.06
		Vitamin C (mg)	9.00
		Vitamin E (mg)	1.06

(USDA, 2017)

\*Based on a 2000 kcal reference diet.

\*Nutrients values and weights are for edible portion.

Scientifically, deep colour vegetables (pumpkin) and fruits can be one of the sources of carotenoid, which have the anti-diabetic, antioxidant, anti-carcinogenic and anti-inflammatory activities (Norshazila, et al., 2014). Specifically, the carotenoid compounds that can be found in pumpkin were lutein, lycopene, zeaxanthin,  $\beta$ -cryptoxanthin, and  $\beta$ -carotene (Margaret, et al., 2015). When consumed, beta-carotene turns in to vitamin A in the body (Ware, 2016). Thus, it can enhance the nutritional status of the people (Lakshman and Prasad, 2015).

#### **2.3.4 Health Benefits of Pumpkin**

There are many health benefits associated with the consumption of pumpkins (Ware, 2016). Increasing consumption of pumpkin that are rich in carotenoids can enhance the immune response to degenerative diseases such as cancer, cardiovascular diseases, atherosclerosis, cataracts, and age-related macular degeneration (Noelia, et al., 2011). The carotenoids act as free radical traps. It will protect the cells and organisms against photooxidation and they can deactivate singlet oxygen and react with free radicals found in human body, which also known as mutagenic, able to inactivate enzymes and damage DNA molecules and lipids (Norshazila, et al., 2014).

Besides that, pumpkin also helps stave off diabetes, neurological disease, increase energy, and helps in weight loss. Consuming the potassium rich

pumpkin can lower the sodium level in body. Thus, it can be used as treatment of hypertension (high blood pressure). It is able to reduce the stroke risk, and promote health being (Ware, 2016). Pumpkin is naturally rich in phytosterols that can reduce low-density lipoprotein in the body (Klein, 2014). According to the National Institutes of Health, a cup of cooked pumpkin provides more than 200 percent of the recommended daily intake of Vitamin A and 20 percent of the recommended vitamin C and more potassium than a banana which aids vision or keep eyesight sharp (Naeve, 2015).

## **2.4 Carrot**

### **2.4.1 Description of Carrot**

The word “carrot” originated from the Greek word “*karoton*” under “*Apiaceae*” family and belongs to the genus and species of plant known as *Daucus carota* (Ma, et al., 2015). It is an important vegetable crop that grown worldwide due to its highly nutritious and inexpensive (Jabbar, et al., 2014). According to the FAO, the world production of carrot in 2013 was approximately 37.2 million metric tons. There are different varieties of carrot, categorized by colour. For instance, yellow carrots, red carrots, purple carrots, white carrots and the most widely recognized orange carrots (Ipek, et al., 2016). Carrot can be consumed in raw, cooked or dried. The raw carrot should be stored in the refrigerator or controlled atmosphere condition. Whereas, dried carrot can be stored in room temperature (Demiray and Tulek, 2014).

#### **2.4.2 Nutritional Value and Health Benefits of Carrot**

Carrot is one of the beneficial vegetables which can be served in diversified cuisine and ways. Carrot becomes one of the most popular choice of vegetable due to their crispy texture and sweet taste. Amazingly, vitamin A, B1, B2, B3, B6,  $\beta$ -carotene and dietary fiber were found in carrots (Demiray and Tulek, 2014). According to Sezer and Demirdoven (2015), carrots are known for their nutrient contents, including carotene and carotenoids, natural antioxidants, phytonutrients, phenolics and polyacetylenes. Consuming carrots will provide 210 % of the average adult's needs for the day, 6 % of vitamin C needs, 2 % of calcium needs and 2 % of iron needs per serving (Ware, 2016). Table 2.5 summarises the energy and parts of the nutritional constituents of raw carrot per 100 g of edible portion. Some health benefits of carrot are summarised in Figure 2.1.

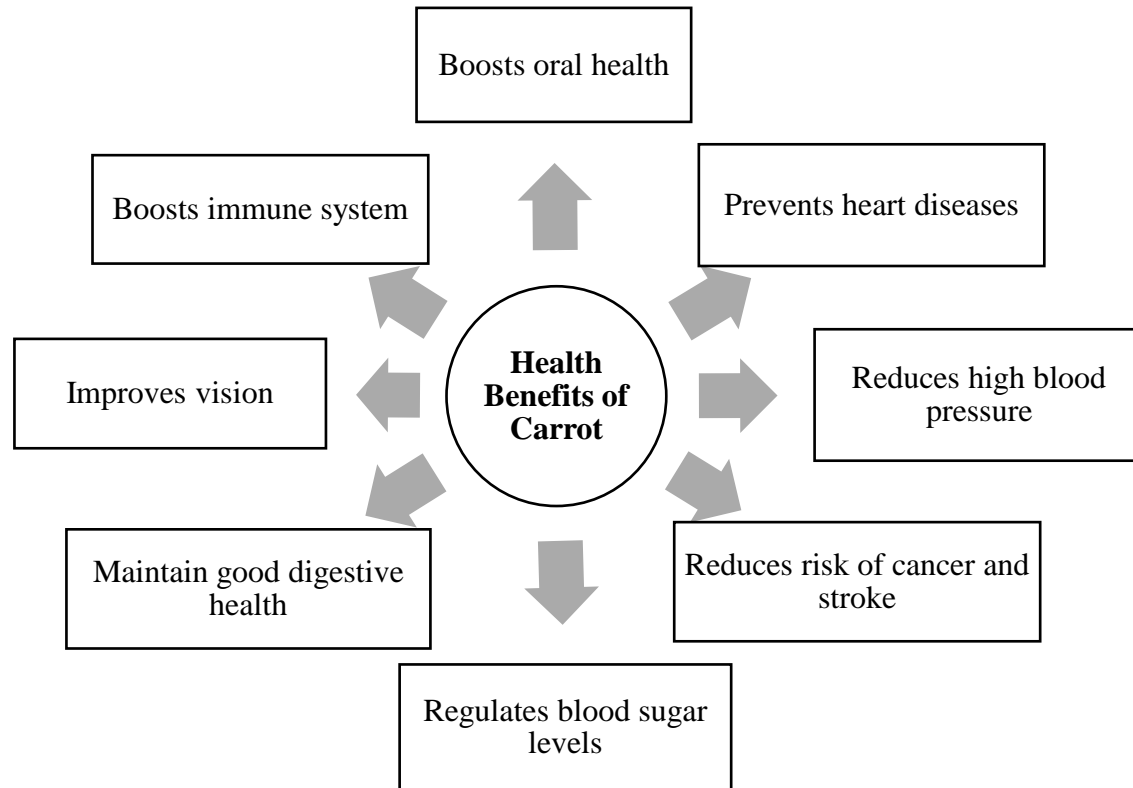
**Table 2.5.** The energy and parts of the nutritional constituents of raw carrot per 100 g of edible portion.

<b>Nutrients</b>	<b>Amount per serving (100.0g)</b>	<b>Nutrients</b>	<b>Amount per serving (100.0g)</b>
Energy (kcal)	41.00	Calcium (mg)	33.00
Water (g)	88.29	Potassium (mg)	320.00
Carbohydrate (g)	9.58	Phosphorus (mg)	35.00
Protein (g)	0.93	Magnesium (mg)	12.00
Lipid (g)	0.24	Sodium (mg)	69.00
Fibre (g)	2.80	Vitamin A ( $\mu$ g)	835.00
Sugars (g)	4.74	Vitamin K ( $\mu$ g)	13.20
		Vitamin B1 (mg)	0.01
		Vitamin B2 (mg)	0.06
		Vitamin B3 (mg)	0.98
		Vitamin B6 (mg)	0.14
		Vitamin C (mg)	5.90

(USDA, 2017)

\*Based on a 2000 kcal reference diet.

\*Nutrients values and weights are for edible portion.



**Figure 2.1.** Health benefits of carrot (Mercola, 2013; Ware, 2016).

## **2.5 Response Surface Methodology (RSM)**

### **2.5.1 Background of RSM**

Response surface methodology (RSM) is a collection of powerful statistical and mathematical techniques which useful for developing, improving, and optimizing processes or formulation (Baş and Boyacı, 2006). It is being used to determine the interaction between independent variables and the dependent variables (response variables). RSM has been widely used and become popular nowadays. There are some examples of the RSM application in the published studies which listed in Table 2.6.

There are two important models normally used in RSM to determine the polynomial models, which is first-degree model and second-degree model (Khuri and Mukhopadhyay, 2010). According to Khuri and Mukhopadhyay, the purpose of the model is to establish the relationship between the response of the interest,  $y$  and the input,  $x_1, x_2 \dots x_k$  that can be used to predict the response values. Next, it is to determine the optimum settings of the input by using the hypothesis testing to determine the significant of the factors and response over a certain region of interest. The design fitting the first-degree models are called first-order designs. Whereas, for the design fitting second-degree models are referred to as second-order designs.

In first-order designs, the most common designs are  $2^k$  factorial, Plackett-Burman and simplex design, while  $3^k$  factorial design, central composite design (CCD) and Box-Behnken design (BBD) are used in second-order designs.



**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).

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

**Table 2.6.** RSM application in the published studies.

Title	References
Optimization on tartary buckwheat enriched steamed bread: a response surface methodology study.	(Wang and Zhang, 2015)
Sweet potato-based pasta product: optimization of ingredients levels using response surface methodology.	(Singh, et al., 2003)
Optimization of ingredients for noodle preparation using response surface methodology.	(Vijayakumar and Boopathy, 2012)
Use of response surface methodology to compare vacuum and atmospheric deep-fat frying of papaya chips impregnated with blackberry juice.	(Wexler, et al., 2016)
Investigation of fusion behaviour in the PVC/XNBR/Nanoclay composites by RSM.	(Moghri and Zanjanijam, 2017)
RSM base study of the effect of argon gas flow rate and annealing temperature on the [Bi]:[Te] ratio and thermoelectric properties of flexible Bi-Te thin film.	(Nuthongkum, Sukulkalavek and Sakdanuphab, 2017)

### **2.5.2 Optimization**

One of the most commonly used in optimization technique in food processing operations is response surface methodology (RSM) due to the high efficiency and simplicity (Ritthiruangdej, Srikamnoy and Amatayakul, 2010). The purpose of the optimization is to enhance the yield of the systems by minimizing cost (Baş and Boyacı, 2006). There are some advantages of RSM in optimization process (Ritthiruangdej, Srikamnoy and Amatayakul, 2010). Firstly, lesser experimental trials are needed and hence a short period of time is required to find the target value. Next, RSM examine the interaction of each independent parameter to the responses. From there, a desired condition can be obtained (Ritthiruangdej, Srikamnoy and Amatayakul, 2010).

There are 3 stages in optimization study by using RSM (Baş and Boyacı, 2006). The first stage is the preliminary work, in which the determination of independent variables and their levels. The second stage is the selection of experimental design, followed by the prediction and verification of model equation. The last stage in the optimization study is the graphical presentation of the model equation and determination of the optimal operating conditions. The polynomial model is able to present the relationship between the response and the independent variable in 3-D display of the response surface plot or in 2-D display of the contour plot (Baş and Boyacı, 2006).

### 2.5.3 The Box-Behnken Design

The Box-Behnken design is one of the method used in RSM, in which the design was developed by Box and Behnken (Khuri and Mukhopadhyay, 2010). The Box-Behnken design is famous in research and development area due to an economical design and requires only three levels ( -1, 0, 1) for each factor. It consists of a particular subset of the factorial combinations from the  $3^k$  factorial design. In other word, Box–Behnken design is a spherical and consists of a central point and the middle points of the edges of the cube circumscribed on the sphere (Maran, et al., 2013). Box-Behnken design does not contain combinations since all factors are at their highest and lowest levels and thus, it is not suitable for the experiment which favors the results under an extreme situation. This design has been applied to study the effect of process variables and the numbers of experiments are decided accordingly. The number of experiments (N) using BBD can be calculated by using the Equation 2.3 (Anwar and Afizal, 2015).

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

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

Where,

$k$  = Factorial Number

$C_0$  = Replicate Number of the Central Point

## CHAPTER 3

### MATERIALS AND METHODS

#### 3.1 Materials

All the ingredient used for this research were purchased from the TESCO, Kampar, Malaysia. The details of the instruments and ingredients used in this study are listed in Table 3.1 and Table 3.2 respectively.

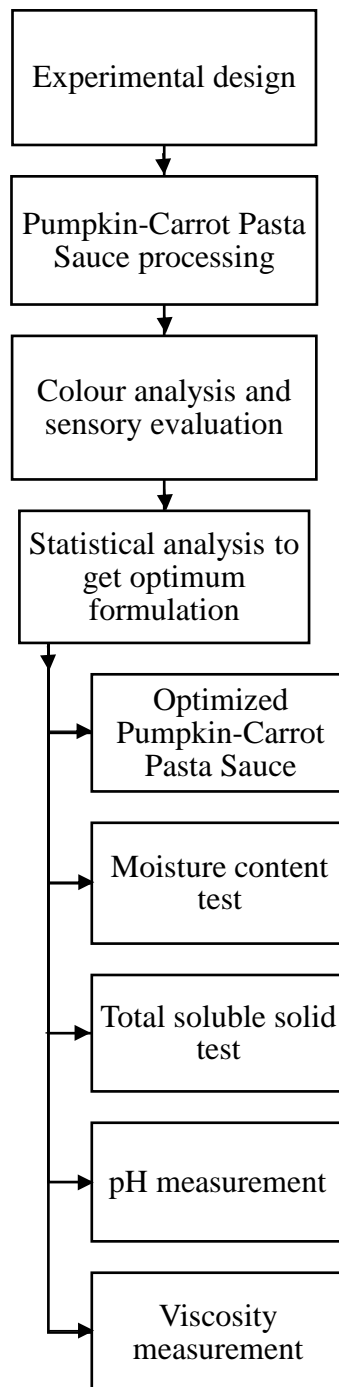
**Table 3.1.** List of instruments used.

<b>Instruments</b>	<b>Model</b>	<b>Brands</b>	<b>Country</b>
Analytical balance	ML304T	Mettler Toledo	Ohio
Kitchen blender	HR 2104/00	Philips	Singapore
Colorimeter	CM-600d	Konica Minolta	Japan
Forced draft oven	FD115	Binder	Germany
PH meter	FiveEasyPlus FP20	Mettler Toledo	Ohio
Refractometer	3830 PAL-3	ATAGO	Japan
Rheometer	DISCOVERY HR-1 hybrid rheometer	TA Instruments	Germany

**Table 3.2.** Ingredients used in this study.

<b>Ingredients</b>	<b>Brands</b>	<b>Country</b>
Pumpkin ( <i>Cucurbita moschata</i> )		Malaysia
Carrot ( <i>Daucus carota</i> )		Malaysia
Garlic		Malaysia
Salt		Malaysia
Sugar		Malaysia
Water		Malaysia
Corn oil	Daisy	Malaysia
Cooking cream	EMBORG	Denmark
Unsalted butter	Anchor	New Zealand
Vinegar	Tamin	Malaysia
Corn starch	Bestari	Malaysia
Black pepper powder	Peace	Malaysia
Mixed herbs	MasterFoods	Australia
Spirals pasta	San Remo	Australia

### 3.2 General Plan of The Experimental Work



**Figure3.1.** Overview experimental flow chart.

### 3.3 Experimental Design

The optimum formulation of pumpkin-carrot pasta sauce can be determined by response surface methodology (RSM) through using JMP statistical discovery software from SAS (version 13.1.0). The Box-Behnken design in RSM with three-level three-factor mixture design was used to optimize the formula of the pumpkin-carrot pasta sauce. The effects of three independent variables which are listed in Table 3.3, namely pumpkin puree, carrot puree and sugar were coded as A, B and C respectively. The upper and lower limits for the independent variables were established. The colour analysis and sensory evaluation were selected as response variables. The design of Box-Behnken response surface analysis is showed in Table 3.4. There were total 15 randomized experiments. Three experiments at the center point which the value of each coded variable 0 were performed to calculate the repeatability of the method to obtain satisfactory experimental results (Wang and Zhang, 2015).

**Table 3.3.** Experiment design with three factors and three levels.

Factors	Code	Levels		
		-1	0	1
Pumpkin puree/g	A	400	450	500
Carrot puree/g	B	100	150	200
Sugar/g	C	5	10	15



**Table 3.4.** Design of Box-Behnken response surface analysis.

<b>Number</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>Colour analysis</b>	<b>Sensory evaluation</b>
<b>1</b>	0	1	-1		
<b>2</b>	1	1	0		
<b>3</b>	0	0	0		
<b>4</b>	0	0	0		
<b>5</b>	-1	-1	0		
<b>6</b>	1	0	-1		
<b>7</b>	-1	0	-1		
<b>8</b>	-1	0	1		
<b>9</b>	0	0	0		
<b>10</b>	1	0	1		
<b>11</b>	-1	1	0		
<b>12</b>	0	-1	-1		
<b>13</b>	0	-1	1		
<b>14</b>	1	-1	0		
<b>15</b>	0	1	1		

\*NOTE: A = Pumpkin puree, B = Carrot puree, C = Sugar

Polynomial regression equation was used to study the interaction and describe the effects of the three factors on the response variables (Anwar and Afizal, 2015). The experimental data for response variables were fitted into the polynomial regression model, based on the result from the analysis of variance (ANOVA). The proposed model was listed in following equation (Vijayakumar and Boopathy, 2012).

**Equation 3.1.** Polynomial regression models for three factors.

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

Where,

**Y** = Response Variables

**$\beta_x$**  = Regression Coefficients

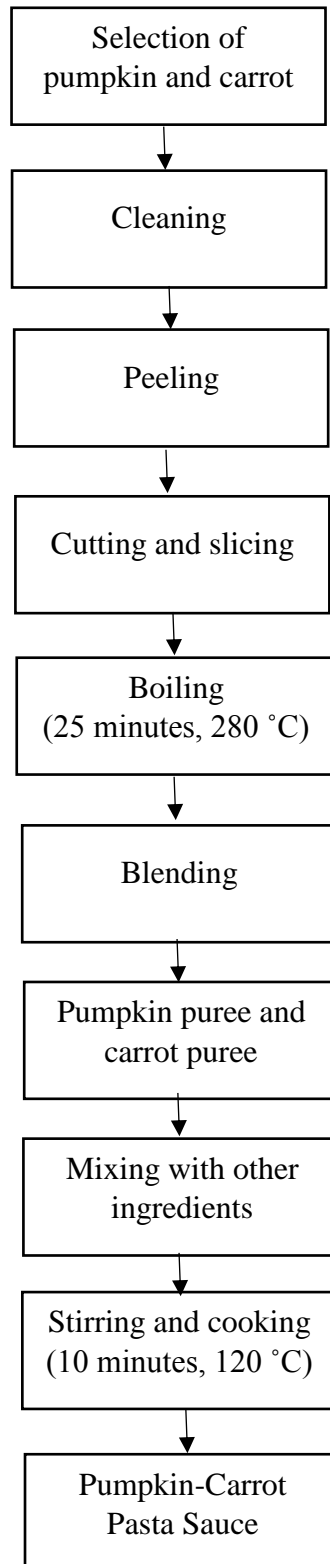
**A, B, C** = Independent Variables

In order to find or select the model that best fitted the response, there are three requirements to be followed. Firstly, the P value in the analysis of variance (ANOVA) should be significant ( $p < 0.05$ ). Secondly, the multiple correlation coefficient ( $R^2$ ) which represent the power of fit is closest to 1. Thirdly, the lack of fit test should be insignificant ( $p > 0.05$ ). The solution that has the overall best performance is selected, if there are any contradictions between these three requirements (Sabanis, Lebesi and Tzia, 2009).

The calculation of the optimum formulation of pumpkin-carrot pasta sauce recipe was performed using a multiple response method called desirability which ranged from 0 (undesirable) to 1 (very desirable). The response surface plot was generated which is a helpful tool for a better understanding of the relationships between each factor and response and it will display in a three-dimensional view (Sabanis, Lebesi and Tzia, 2009).

The moisture content test, total soluble solid test, pH measurement, and viscosity measurement were carried out for optimum formulation of pumpkin-carrot pasta sauce.

### 3.4 Pumpkin-Carrot Pasta Sauce Processing



**Figure 3.2.** Overview Pumpkin-Carrot Pasta Sauce processing.

The pumpkin-carrot pasta sauces were prepared in the kitchen and the flow chart of the sauce processing is shown in Figure 3.2. The ingredients used for making the pumpkin-carrot pasta sauces were listed in Table 3.5.

**Table 3.5.** Pumpkin-Carrot Pasta Sauce formulation.

<b>Ingredients</b>	<b>Weight/g</b>
Pumpkin puree	Variable *
Carrot puree	Variable *
Sugar	Variable *
Water	30.0
Corn oil	15.0
Cooking cream	15.0
Unsalted butter	8.0
Vinegar	3.0
Corn starch	3.0
Salt	3.0
Garlic puree	2.0
Black pepper powder	0.4
Mixed herbs	0.4

\* Amount varied according to the experimental design (Table 3.3 and Table 3.4)

### 3.5 Colour Analysis

Colorimeter (Model CM-600d; Konica Minolta, Tokyo, Japan) was used to determine the colour of pumpkin-carrot pasta sauce. Once the colorimeter was calibrated by using a standard white calibration plate, a small amount of sample was spread consistently then placed on the optical glass cell inside the granular attachment. The colour was reported in CIE-Lab parameters in which L\* (intensity of lightness), a\* (+: redness, -: greenness) and b\* (+: yellowness; -: blueness) value. All samples were run in triplicate and the result was expressed as mean. The total colour difference ( $\Delta E^*$ ) of the samples was calculated by using the equation below.

**Equation 3.2.** Total colour difference.

**Total colour difference ( $\Delta E^*$ )**

$$= \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Where,

$\Delta E^*$  = Total colour difference

$\Delta L^*$  = Difference in lightness

$\Delta a^*$  = Difference in redness or greenness

$\Delta b^*$  = Difference in yellowness or blueness

### **3.6 Sensory Evaluation**

In order to find an optimum formulation of pumpkin-carrot pasta sauce, the sensory evaluation was carried out by a panel of 25 untrained panelists from Universiti Tunku Abdul Rahman, Kampar, Malaysia. San Remo spirals pasta was acts as a carrier which tested with pumpkin-carrot pasta sauce. Panelists were required to evaluate on colour, appearance, viscosity, aroma, taste, and overall acceptability of pumpkin-carrot pasta sauce with nine-point hedonic scaling test, where 9 = like extremely, 5 = neither like nor dislike and 1 = dislike extremely. The evaluation was carried out in a sensory evaluation laboratory room equipped with separately divided booths which located at block D211A, Faculty of Science, Universiti Tunku Abdul Rahman, Kampar, Malaysia.

### **3.7 Moisture Content Test**

The moisture content was carried out by using oven drying method. Firstly, the crucible with lid was pre-dried at temperature of 70°C for approximately 24 hours. Next, the crucible with lid was allowed to cool down in desiccator prior to weighing. The weight of the crucible with lid was measured using analytical balance (Model ML304T; Mettler Toledo, Columbus, Ohio) with four decimal places. Approximately 10.0 g of sample was added into the crucible and the weighing procedure was repeated again. The crucible with lid and sample was then placed into the forced draft oven (Model FD115; Binder, Tuttlingen, Germany) and was maintained at a constant temperature of 105°C. The sample was left in the oven for approximately 12 hours to obtain a constant weight. The

final weight of the crucible with lid together with the sample was weighed after cooling. All the samples are determined in triplicate to obtain the mean values. The percentage of the moisture content (wt %) was calculated using the following equation.

**Equation 3.3.** Percentage of moisture content.

**Percentage of moisture content (%)**

$$= \frac{\text{Weight of sample before drying} - \text{Weight of sample after drying}}{\text{Weight of sample before drying}} \times 100\%$$

### **3.8 Total Soluble Solid (TSS) Test**

Refractometer (Model PAL-3; ATAGO, Tokyo, Japan) was used for the determination of TSS. The refractometer was calibrated using distilled water. Approximately 0.5 mL of the sample was placed on the prism of the refractometer. The measured Brix value (°Brix) of the sample was displayed on the digital display. Total soluble solid content was then expressed as °Brix and average values of three replications were reported.

### **3.9 pH Measurement**

Firstly, the pH meter (Model FiveEasyPlus FP20; Mettler Toledo, Columbus, Ohio) was calibrated using the buffer solutions of pH 4.0, 7.0 and 9.0. After that,



the electrode of the pH meter was submerged into the sample a few seconds. The pH of the sample was shown on the digital display. The constant reading was recorded. All of the samples were run in triplicate.

### 3.10 Viscosity Measurement

Rheometer (DISCOVERY HR-1 hybrid rheometer; TA Instruments, Germany) was used to determine the viscosity of sauce. A spoonful of sample was spread on the stage, then the probe was adjusted to the required height. The excess sample that squeezed out from the probe was removed by using a piece of tissue paper. The analysis was then started. All samples were performed in duplicate and the result was expressed as mean. The instrument settings of the viscosity measurement were listed in Table 3.6.

**Table 3.6.** The instrument settings of the viscosity measurement.

<b>Settings</b>	
Geometry name	40mm parallel plate, Peltier plate Steel - 111229
Temperature	5°C, 25°C and 85°C
Shear rate	0 to 800 s <sup>-1</sup>
Time	180 s

The rheological parameters of sauce were based on the Herschel-Bulkley model.

**Equation 3.4.** The Herschel-Bulkley model.

$$\tau - \tau_0 = \mathbf{K}(\gamma)^n$$

Where,

$\tau$  = Shear stress (Pa)

$\tau_0$  = Yield stress (Pa)

$\mathbf{K}$  = Consistency coefficient (Pa s<sup>n</sup>)

$\gamma$  = Shear rate (s<sup>-1</sup>)

$\mathbf{n}$  = Flow behaviour index

### 3.11 Statistical Analysis

JMP statistical discovery software from SAS (version 13.1.0) was used to determine the optimum formulation of pumpkin-carrot pasta sauce based on the sensory evaluation and colour analysis. The results are considered statistically significant if the P value was <0.05. The remaining analysis for the optimized pumpkin-carrot pasta sauce, such as moisture content test, total soluble solid test, pH measurement were performed in triplicate measurements. Whereas, the viscosity measurement was performed in duplicate. All data obtained were presented as mean ± standard deviation (SD).

## CHAPTER 4

### RESULTS

#### 4.1 Experimental Design

There were 15 experiments in total that have been conducted based on the colour analysis and sensory evaluation as response variables. For the colour analysis, the reference values of the normal pumpkin pasta sauce (without carrot) were  $L^*$  ( $42.93 \pm 0.11$ ),  $a^*$  ( $15.27 \pm 0.22$ ),  $b^*$  ( $20.07 \pm 0.10$ ). The results of the colour analysis were tabulated in Table 4.1. The calculation of total colour difference ( $\Delta E^*$ ) between 15 samples were performed by taking into account the differences in  $L^*$ ,  $a^*$  and  $b^*$  parameters. Only the colour differences in  $b^*$  ( $\Delta b^*$ , yellowness) served as response variable for colour analysis. Whereas for the sensory evaluation test, panelists were then asked to assess the sensory attributes such as colour (%), appearance (%), viscosity (%), aroma (%), taste (%), and overall acceptability (%). The results of the response variables were tabulated in Table 4.2.

Based on the results, the average colour differences in  $b^*$  ( $\Delta b^*$ , yellowness) was 29.16 and in the meanwhile the highest and the lowest values of the differences were 39.45 and 14.97 respectively. Next, from the sensory results obtained, colour achieved the highest acceptance level of 76.75 %, followed by appearance 75.69 %, overall acceptability, 75.58 %, taste 74.34 %, viscosity 72.78 %, and the aroma was 71.07 %.

**Table 4.1.** Results of lightness (**L\***), redness (**a\***), yellowness (**b\***), and total colour differences (**ΔE\***) in 15 samples.

No	A	B	C	Colour Analysis			
				L*	a*	b*	ΔE*
1	0	1	-1	44.49 ± 0.42	17.41 ± 0.66	59.52 ± 0.44	39.54 ± 0.77
2	1	1	0	43.95 ± 0.27	17.54 ± 0.85	58.62 ± 0.65	38.63 ± 0.68
3	0	0	0	44.18 ± 0.44	15.96 ± 0.46	57.40 ± 0.63	37.36 ± 0.08
4	0	0	0	43.92 ± 0.17	16.03 ± 0.18	58.56 ± 0.35	38.51 ± 0.55
5	-1	-1	0	43.79 ± 0.40	15.44 ± 0.43	57.82 ± 0.18	37.76 ± 0.54
6	1	0	-1	44.48 ± 0.36	16.00 ± 0.07	58.02 ± 0.25	37.99 ± 0.76
7	-1	0	-1	45.19 ± 0.24	16.18 ± 0.25	59.09 ± 0.42	39.10 ± 0.44
8	-1	0	1	42.74 ± 0.25	15.05 ± 0.57	55.29 ± 0.07	35.22 ± 0.21
9	0	0	0	43.39 ± 0.58	14.86 ± 0.33	56.58 ± 0.57	36.52 ± 0.54
10	1	0	1	50.34 ± 0.08	13.50 ± 0.34	35.11 ± 0.25	16.86 ± 0.43
11	-1	1	0	51.46 ± 0.21	16.02 ± 0.44	36.82 ± 0.09	18.81 ± 0.22
12	0	-1	-1	52.80 ± 0.53	13.65 ± 0.29	38.23 ± 0.75	20.73 ± 0.41
13	0	-1	1	49.80 ± 0.38	13.06 ± 0.20	35.04 ± 0.44	16.62 ± 0.32
14	1	-1	0	51.36 ± 0.08	13.33 ± 0.67	36.57 ± 0.26	18.63 ± 0.21
15	0	1	1	50.38 ± 0.47	14.86 ± 0.26	35.83 ± 0.45	17.44 ± 0.56

\*NOTE: A = Pumpkin puree, B = Carrot puree, C = Sugar

**Table 4.2.** Results of response variables in 15 samples.

No	A	B	C	Differences in b* ( $\Delta b^*$ )	Sensory evaluation					
					Colour	Appearance	Viscosity	Aroma	Taste	Overall acceptability
					(%)	(%)	(%)	(%)	(%)	(%)
1	0	1	-1	39.45	76.55	77.78	69.78	75.56	77.34	76.44
2	1	1	0	38.55	84.23	77.78	72.44	71.11	83.22	78.67
3	0	0	0	37.33	79.11	72.89	74.22	68.76	80.00	80.44
4	0	0	0	38.49	80.34	72.70	74.32	65.55	81.76	80.30
5	-1	-1	0	37.75	72.22	79.56	76.89	75.11	75.24	80.44
6	1	0	-1	37.95	86.56	80.44	76.88	72.44	71.44	75.56
7	-1	0	-1	39.02	72.40	79.11	71.11	70.67	72.56	71.56
8	-1	0	1	35.22	73.35	73.78	70.67	70.67	62.34	67.11
9	0	0	0	36.51	79.00	72.40	73.87	67.76	76.76	82.38

\*NOTE: A = Pumpkin puree, B = Carrot puree, C = Sugar

**Table 4.2.** Results of response variables in 15 samples (continued).

No	A	B	C	Differences in b* ( $\Delta b^*$ )	Sensory evaluation					
					Colour	Appearance	Viscosity	Aroma	Taste	Overall acceptability
					(%)	(%)	(%)	(%)	(%)	(%)
<b>10</b>	1	0	1	15.04	83.24	77.78	72.44	74.22	62.55	76.40
<b>11</b>	-1	1	0	16.75	73.56	77.33	73.78	70.67	81.70	76.89
<b>12</b>	0	-1	-1	18.16	70.23	68.89	68.89	64.89	72.34	64.89
<b>13</b>	0	-1	1	14.97	70.54	72.89	73.33	74.22	62.93	73.33
<b>14</b>	1	-1	0	16.50	75.60	72.89	70.22	70.67	76.81	72.89
<b>15</b>	0	1	1	15.76	74.32	79.11	72.89	73.78	78.12	76.44
<b>Mean</b>				29.16	76.75	75.69	72.78	71.07	74.34	75.58

\*NOTE: A = Pumpkin puree, B = Carrot puree, C = Sugar

The regression output of the colour differences in  $b^*$  ( $\Delta b^*$ , yellowness) and sensory evaluation were tabulated in Table 4.3. Based on the analysis of variance (ANOVA), the colour difference in  $b^*$  ( $\Delta b^*$ , yellowness), colour (%) and taste (%) in sensory evaluation were significantly different ( $p < 0.05$ ) while the appearance (%), viscosity (%), aroma (%), and overall acceptability (%) were not significantly different ( $p > 0.05$ ). The multiple correlation coefficient ( $R^2$ ) for the colour difference in  $b^*$  ( $\Delta b^*$ , yellowness), colour (%), appearance (%), viscosity (%), aroma (%), taste (%), and overall acceptability (%) were 0.93, 0.96, 0.66, 0.37, 0.70, 0.95, and 0.75 respectively. Besides that, the lack of fit for the colour difference in  $b^*$  ( $\Delta b^*$ , yellowness, 0.23), colour (%), 0.12), aroma (%), 0.17), taste (%), 0.49), and overall acceptability (%), 0.06) were insignificant ( $p > 0.05$ ). Whereas, for the appearance (%), 0.003), and viscosity (%), 0.003), were significantly different ( $p < 0.05$ ), which means that the both order of the regression were not secondary.

Based on the Table 4.3, the colour difference in  $b^*$  ( $\Delta b^*$ , yellowness), colour (%) and taste (%) in sensory evaluation were chosen to establish the polynomial regression models and generate the response surface plots due to P value  $< 0.05$ , lack of fit  $> 0.05$  and  $R^2$  is closest to 1.

**Table 4.3.** Regression output from JMP software of the response variable.

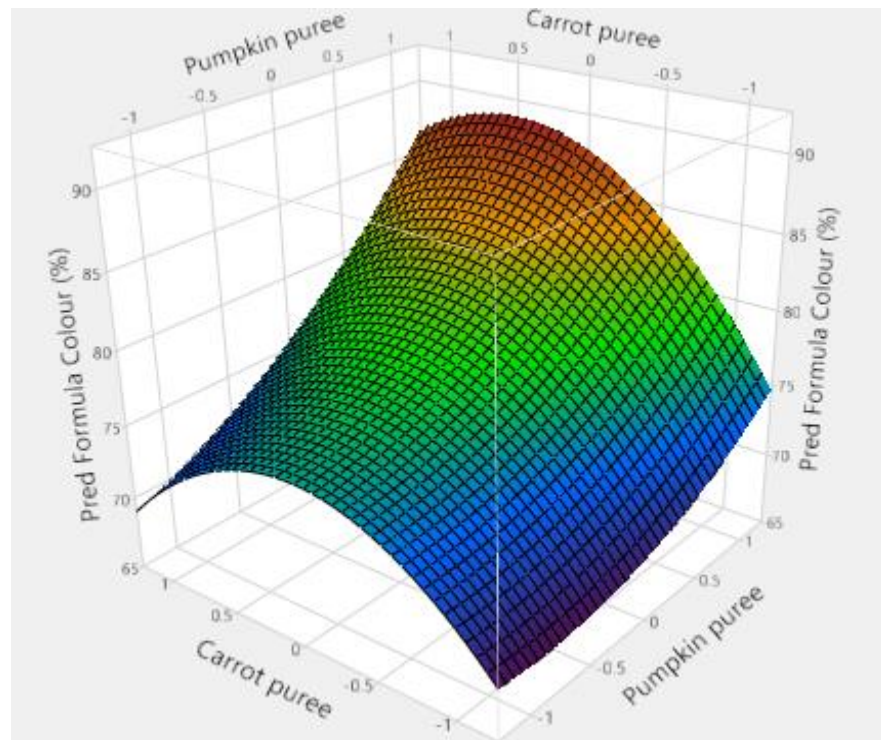
<b>Regression</b>	<b>Differences in b*</b>	<b>Colour (%)</b>	<b>Appearance</b>	<b>Viscosity</b>	<b>Aroma</b>	<b>Taste</b>	<b>Overall acceptability</b>
<b>Coefficients</b>	<b>(<math>\Delta b^*</math>)</b>		<b>(%)</b>	<b>(%)</b>	<b>(%)</b>	<b>(%)</b>	<b>(%)</b>
$\beta_0$	37.44*	79.48*	72.66*	74.14*	67.36*	79.51*	81.04*
$\beta_a$	-2.59	4.76*	-0.11	-0.06	0.17	0.27	0.94
$\beta_b$	2.89	2.51*	2.22	-0.06	0.78	4.13*	2.11
$\beta_c$	-6.70*	-0.54	-0.33	0.33	1.17	-3.47*	0.60
$\beta_{ab}$	10.76*	1.82	1.78	1.33	1.22	-0.01	2.33
$\beta_{ac}$	-4.78	-1.07	0.67	-1.00	0.45	0.33	1.32
$\beta_{bc}$	-5.13	-0.64	-0.67	-0.33	-2.78	2.55	-2.11
$\beta_{aa}$	-0.17	1.45	3.67	0.37	2.21	-2.86	-1.97
$\beta_{bb}$	-9.90*	-4.53*	0.56	-1.18	2.32	2.60	-1.85
$\beta_{cc}$	-5.47	-2.04	1.45	-1.74	2.43	-9.42*	-6.42
<b>R<sup>2</sup></b>	0.93	0.96	0.66	0.37	0.70	0.95	0.75
<b>Lack of fit</b>	0.23	0.12	0.003*	0.003*	0.17	0.49	0.06

\*There was statistically difference ( $p < 0.05$ ).



#### 4.1.1 Response Surface Plot: Effects of Interaction Among Pumpkin Puree and Carrot Puree on The Colour (%)

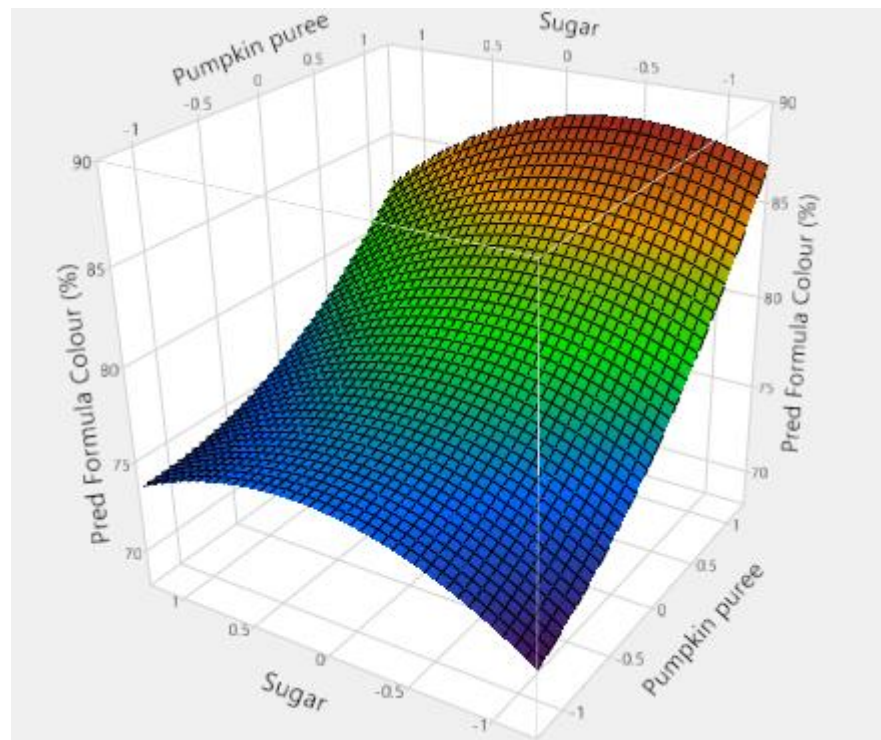
Based on Figure 4.1, addition of more pumpkin puree resulted in better colour profile of sauce. In addition, acceptance of colour profile was increased by increasing amount of carrot puree. However, the colour turned undesirable once the amount of carrot puree exceeds a point.



**Figure 4.1.** Effects of interaction among pumpkin puree and carrot puree on the colour (%).

#### 4.1.2 Response Surface Plot: Effects of Interaction Among Pumpkin Puree and Sugar on The Colour (%)

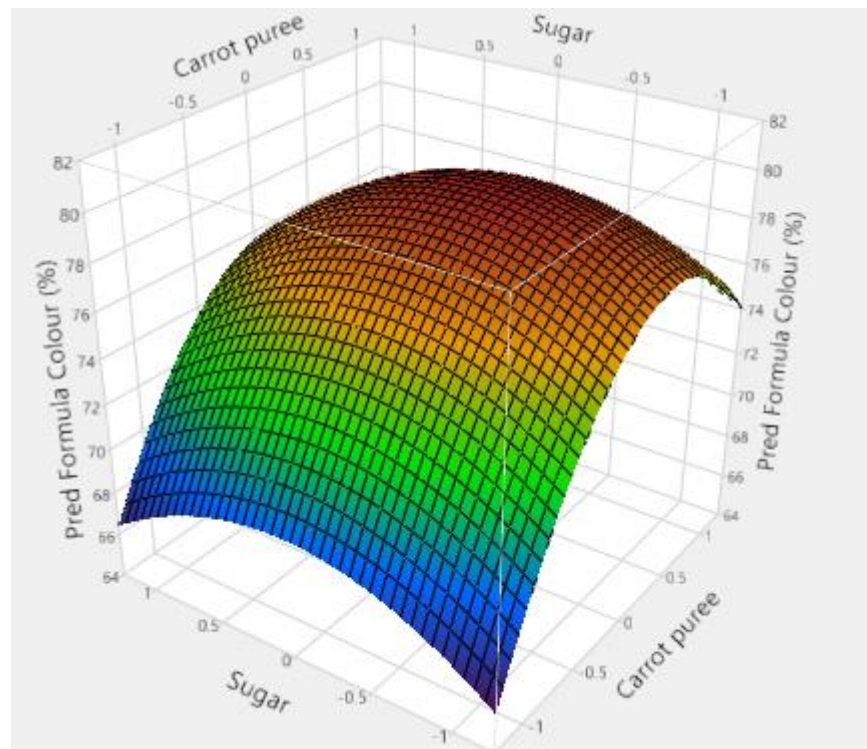
Based on Figure 4.2, high level of pumpkin puree induced better acceptance of colour profile. At level of high pumpkin puree, acceptance of colour profile was raised by adjusting sugar level to -0.5.



**Figure 4.2.** Effects of interaction among pumpkin puree and sugar on the colour (%).

### 4.1.3 Response Surface Plot: Effects of Interaction Among Carrot Puree and Sugar on The Colour (%)

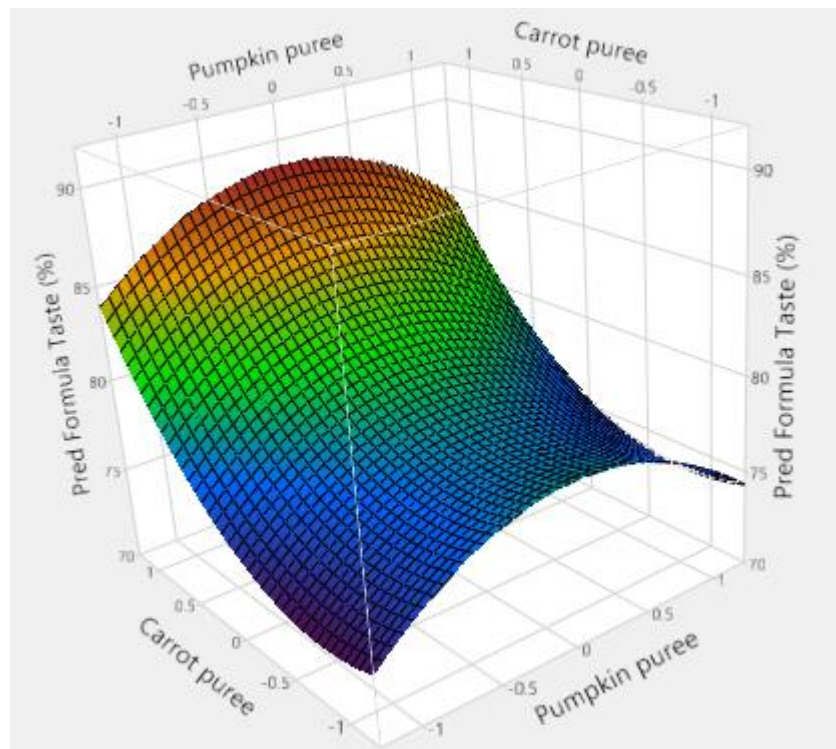
Based on Figure 4.3, acceptance of colour's profile was raised when the levels of carrot puree and sugar were at centre point (0). However, the colour turned less desirable when both of the factors exceeded certain points.



**Figure 4.3.** Effects of interaction among carrot puree and sugar on the colour (%).

#### 4.1.4 Response Surface Plot: Effects of Interaction Among Pumpkin Puree and Carrot Puree on The Taste (%)

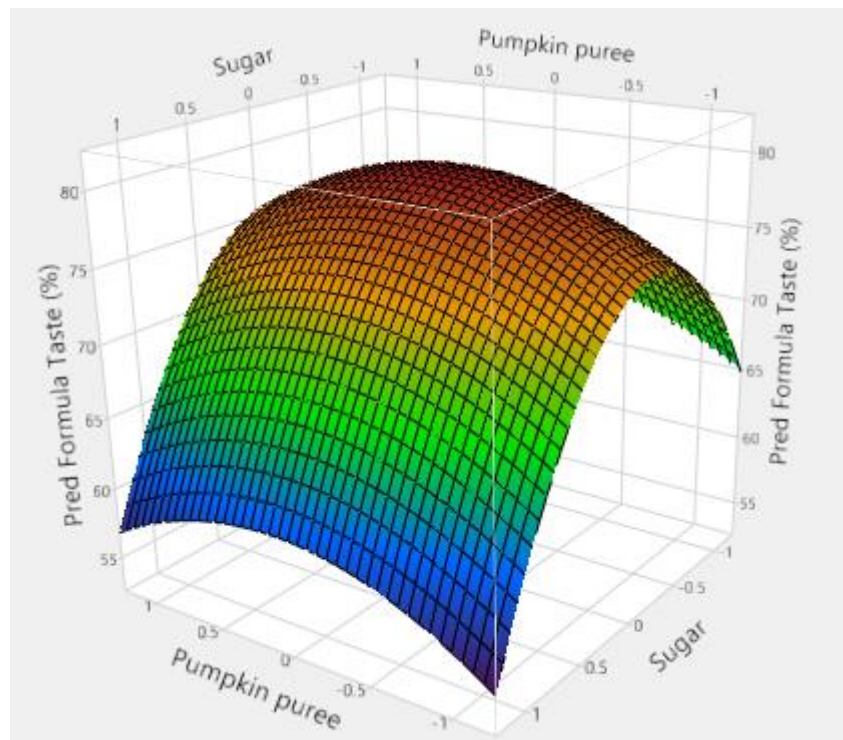
Based on Figure 4.4, taste of sauce was maximized when the large amount of carrot puree was added. At high level of carrot puree, taste of sauce was further enhanced by increasing amount of pumpkin puree. Yet, the taste become less favourable when the level of pumpkin puree exceeded a point.



**Figure 4.4.** Effects of interaction among pumpkin puree and carrot puree on the taste (%).

#### 4.1.5 Response Surface Plot: Effects of Interaction Among Pumpkin Puree and Sugar on The Taste (%)

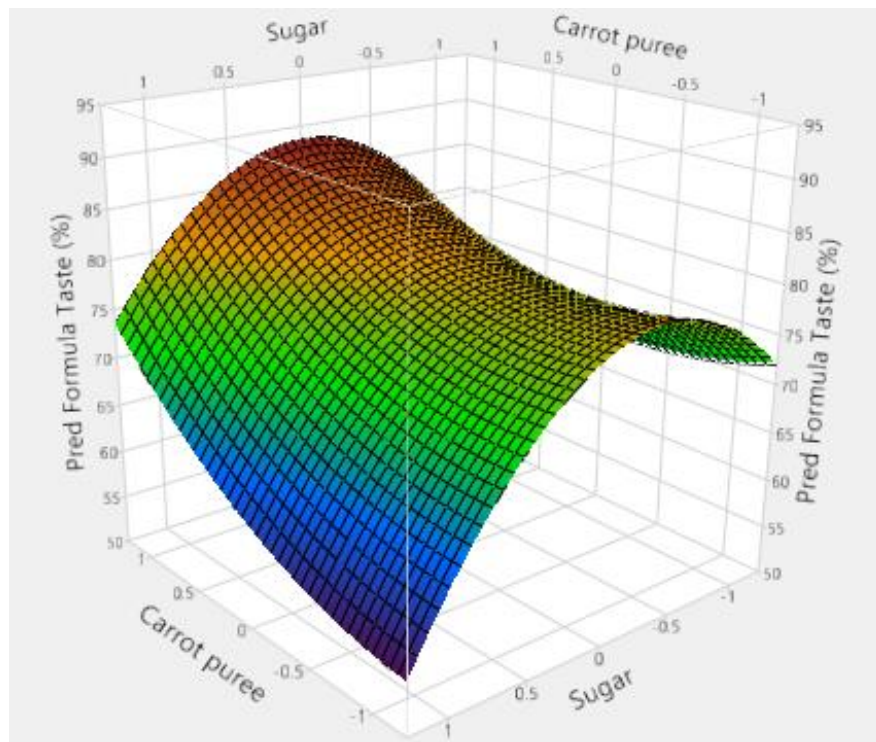
Based on Figure 4.5, taste profile was increased when the levels of pumpkin puree and sugar were at centre point (0). However, when both of the factors exceeded certain point, the taste of sauce turned unfavourable.



**Figure 4.5.** Effects of interaction among pumpkin puree and sugar on the taste (%).

#### 4.1.6 Response Surface Plot: Effects of Interaction Among Carrot Puree and Sugar on The Taste (%)

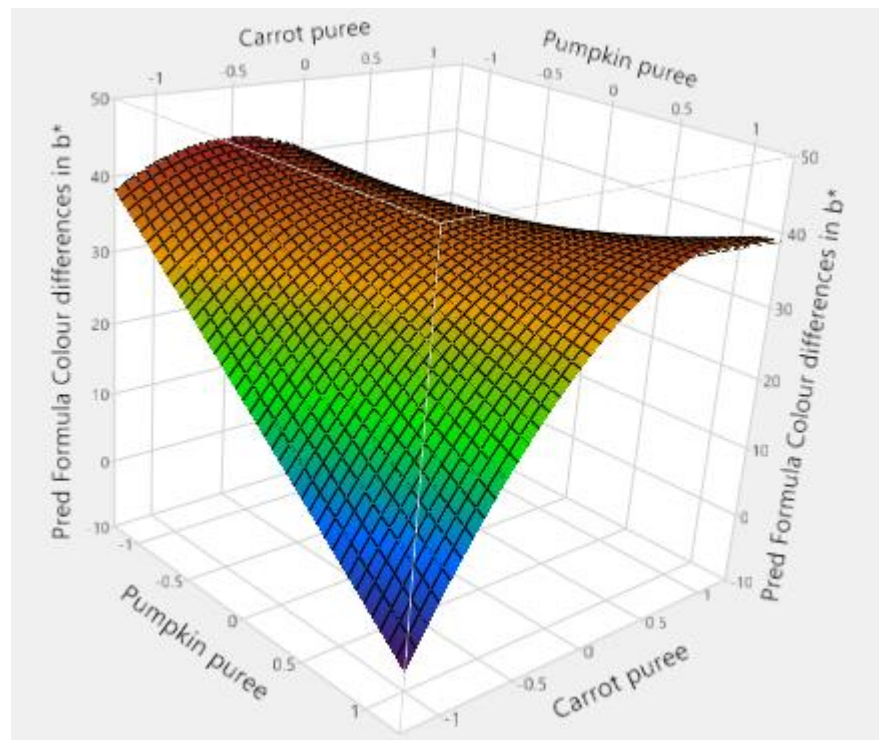
Based on Figure 4.6, taste of sauce was increased when high amount of carrot puree was added. Besides, addition of sugar to certain point further increased the taste profile.



**Figure 4.6.** Effects of interaction among carrot puree and sugar on the taste (%).

#### 4.1.7 Response Surface Plot: Effects of Interaction Among Pumpkin Puree and Carrot puree on The Colour Differences in $b^*$ ( $\Delta b^*$ )

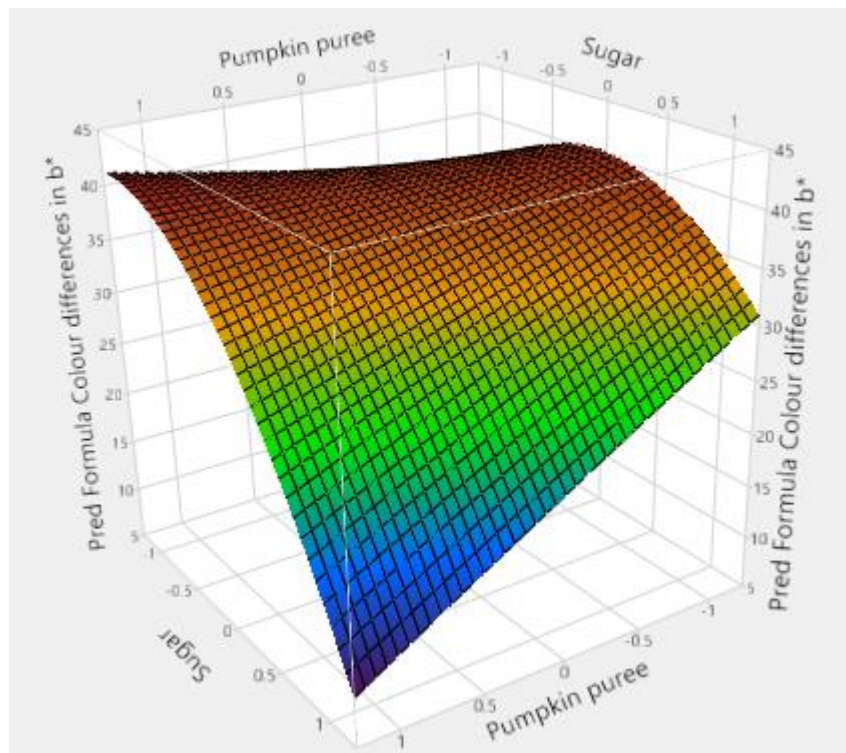
Based on Figure 4.7, decreasing amount of pumpkin puree added into sauce induced large colour difference in term of  $b^*$  (yellowness). In addition, the colour difference of  $b^*$  was further increased by adjusting the carrot puree level to -0.5. The colour difference in  $b^*$  was minimized when level of carrot puree was at -1.



**Figure 4.7.** Effects of interaction among pumpkin puree and carrot puree on the colour differences in  $b^*$  ( $\Delta b^*$ ).

#### 4.1.8 Response Surface Plot: Effects of Interaction Among Pumpkin Puree and Sugar on The Colour Differences in $b^*$ ( $\Delta b^*$ )

Based on Figure 4.8, when sugar level was below certain point, the colour difference of  $b^*$  was maximized regardless of the amount of pumpkin puree added. However, increasing sugar level, lowered the difference of  $b^*$ . If more pumpkin was added, difference of  $b^*$  was minimized.

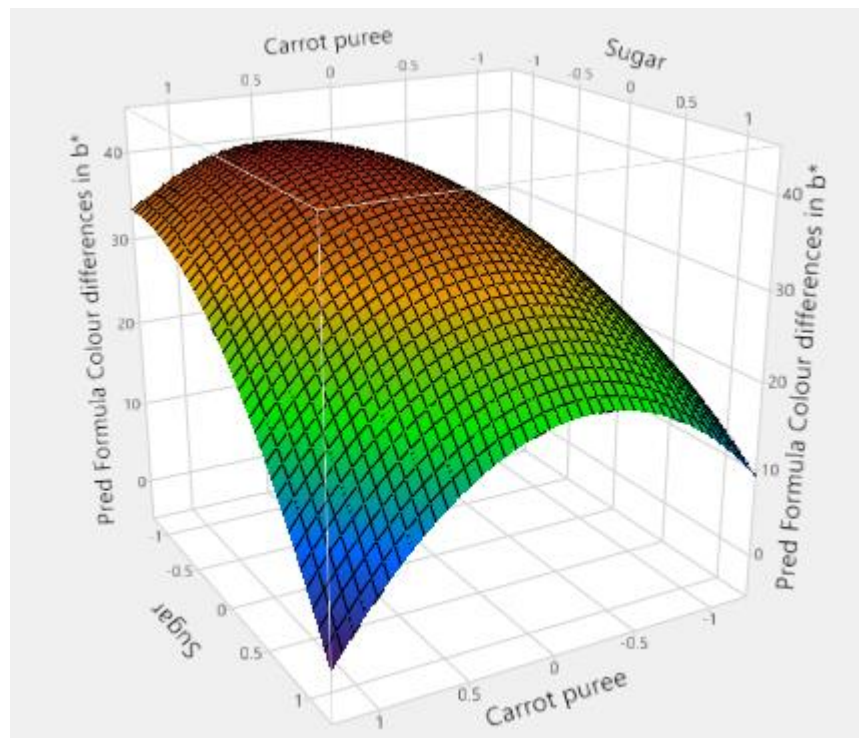


**Figure 4.8.** Effects of interaction among pumpkin puree and sugar on the colour differences in  $b^*$  ( $\Delta b^*$ ).



#### 4.1.9 Response Surface Plot: Effects of Interaction Among Carrot Puree and Sugar on The Colour Differences in $b^*$ ( $\Delta b^*$ )

Based on Figure 4.9, difference of  $b^*$  was maximized when carrot puree was added at level of 0.5. At this condition, decreasing sugar content further increased the difference of  $b^*$ . In contrast, high levels of sugar and carrot puree added in sauce minimized the difference of  $b^*$ .



**Figure 4.9.** Effects of interaction among carrot puree and sugar on the colour differences in  $b^*$  ( $\Delta b^*$ ).

## 4.2 Formula Optimization of Pumpkin-Carrot Pasta Sauce

Based on the results, the obtained optimum formula of pumpkin-carrot pasta sauce was 500 g of pumpkin puree, 200 g of carrot puree and 0.96 g of sugar, with predicted colour difference in  $b^*(\Delta b^*$ , yellowness) as 41.43, colour in sensory evaluation as 85.85 % and taste in sensory evaluation as 83.40 %. The desirability for this formulation was 0.93, which considered as very desirable due to the value is closest to 1.

**Table 4.4.** Optimum formulation of pumpkin-carrot pasta sauce and predicted value.

Solution	Optimum level			Predicted value			Desirability
	A	B	C	Differences in $b^*(\Delta b^*)$	Colour (%)	Taste (%)	
	500g	200g	0.96g	41.43	85.85	83.40	0.93

\*NOTE: A = Pumpkin puree, B = Carrot puree, C = Sugar

### 4.3 Moisture Content Test, Total Soluble Solid Test and pH Measurement

Result presented in Table 4.5 showed the moisture content, total soluble solid and pH of optimized pumpkin-carrot pasta sauce.

**Table 4.5.** The moisture content, total soluble solid and pH of optimized pumpkin-carrot pasta sauce.

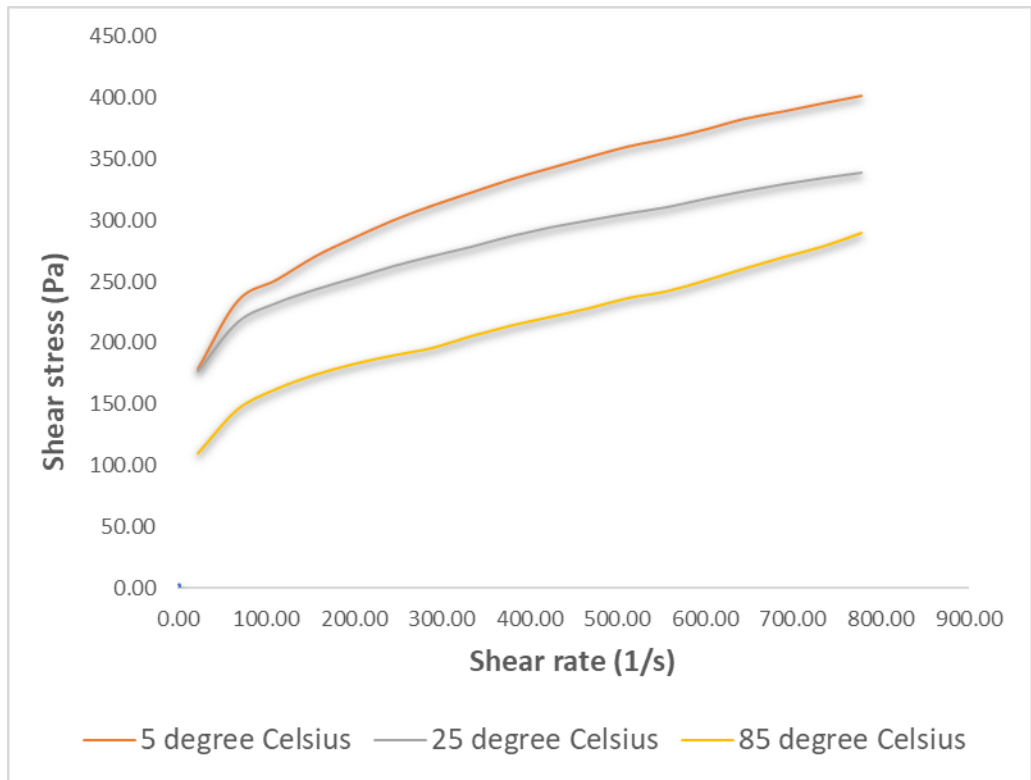
<b>Description</b>	<b>Optimized pumpkin-carrot pasta sauce</b>
<b>Moisture content (%)</b>	86.67 ± 0.03
<b>Total soluble solid (°Brix)</b>	24.93 ± 0.65
<b>pH</b>	5.09 ± 0.01

#### 4.4 Viscosity Measurement

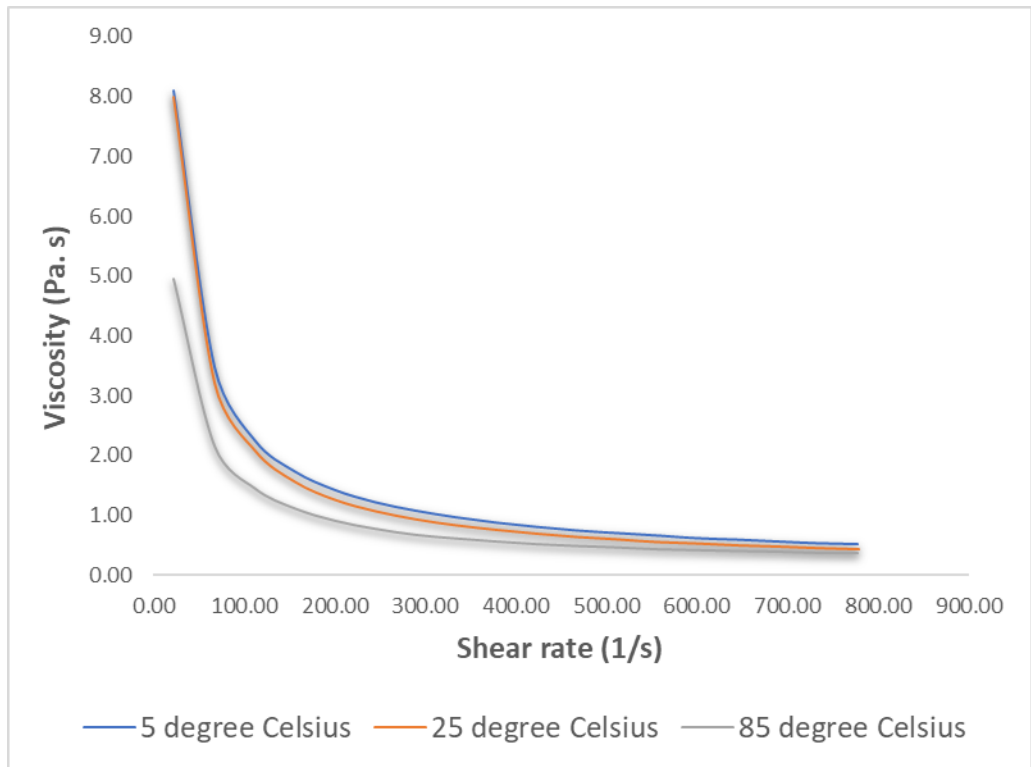
Result presented in Table 4.6 showed the viscosity of optimized pumpkin-carrot pasta sauce at different temperature. The viscosity of optimized pumpkin-carrot pasta sauce at temperature 5 °C, 25 °C and 85 °C was 35.83, 20.23, and 3.47 respectively. In other words, it was noticed that the viscosity decreased as temperature increased. Figure 4.10 and Figure 4.11 showed the shear stress-shear rate relationships at different temperature and the effect of temperature on the viscosity of optimized pumpkin-carrot pasta sauce respectively.

**Table 4.6.** The rheological parameters of optimized pumpkin-carrot pasta sauce based on the Herschel-Bulkley model.

Temperature (°C)	Yield stress (Pa)	Viscosity (Pa.s)	Rate index	R <sup>2</sup>
5	84.26 ± 71.16	35.83 ± 3.25	0.33 ± 0.02	0.9979
25	120.19 ± 6.09	20.23 ± 0.91	0.36 ± 0.02	0.9968
85	97.12 ± 9.23	3.47 ± 1.11	0.60 ± 0.05	0.9878



**Figure 4.10.** The shear stress-shear rate relationships at different temperature of optimized pumpkin-carrot pasta sauce.



**Figure 4.11.** The effect of temperature on the viscosity of optimized pumpkin-carrot pasta sauce.

## CHAPTER 5

### DISCUSSION

#### 5.1 Experimental Design

The Box-Behnken design in RSM with three-level (-1, 0 and 1) three factor (pumpkin puree, carrot puree and sugar) mixture design was used to optimize the formula of the pumpkin-carrot pasta sauce. The maximum colour analysis and maximum sensory evaluation were selected as response variables. There were total 15 randomized experiments. Three experiments at the center point which the value of each coded variable 0 were performed to calculate the repeatability of the method to obtain satisfactory experimental results (Wang and Zhang, 2015).

Multiple regression analysis was performed to analyze the response in order to obtain the estimated regression coefficients for the response variables. Based on the Table 4.3, the colour difference in  $b^*(\Delta b^*, \text{ yellowness})$ , colour (%) and taste (%) in sensory evaluation were chosen to establish the polynomial regression models and generate the response surface plots. Based on the ANOVA, the colour difference in  $b^*(\Delta b^*, \text{ yellowness})$ , colour (%) and taste (%) in sensory evaluation were significantly different at 95 % ( $p < 0.05$ ). Analysis of variance was used to assess how well the model represented the data. Besides, the multiple correlation coefficient ( $R^2$ ) for the colour difference in  $b^*(\Delta b^*, \text{ yellowness})$ , colour (%), and taste (%) were 0.93, 0.96, and 0.95 respectively. The  $R^2$  coefficient showed how well the regression model fit the experiment data and

the  $R^2$  value considered as good if the value is closest to 1 (Sabanis, et al., 2009). The lack of fit for the colour difference in  $b^*$  ( $\Delta b^*$ , yellowness, 0.23), colour (%), 0.12), and taste (%), 0.49), were insignificant at 95% ( $p>0.05$ ).

The polynomial regression model of colour difference in yellowness ( $\Delta b^*$ ), colour and taste in sensory evaluation were listed in Equation 5.1, Equation 5.2 and Equation 5.3 respectively.

**Equation 5.1.** Polynomial regression model of colour difference in yellowness ( $\Delta b^*$ ).

$$\begin{aligned} &\text{Differences in } b^* (\Delta b^*, \text{ yellowness}), Y_1 \\ &= 37.44 - 2.59A + 2.89B - 6.70C + 10.76AB - 4.78AC - 5.13BC - \\ &0.17A^2 - 9.90B^2 - 5.47C^2 \end{aligned}$$

**Equation 5.2.** Polynomial regression model of colour (%) in sensory evaluation.

$$\begin{aligned} &\text{Colour (sensory evaluation)}, Y_2 \\ &= 79.48 + 4.76A + 2.51B - 0.54C + 1.82AB - 1.07AC - 0.64BC + \\ &1.45A^2 - 4.53B^2 - 2.04C^2 \end{aligned}$$



**Equation 5.3.** Polynomial regression model of taste (%) in sensory evaluation.

Taste (sensory evaluation),  $Y_3$

$$= 79.51 + 0.27A + 4.13B - 3.47C - 0.01AB + 0.33AC + 2.55BC - 2.86A^2 + 2.60B^2 - 9.42C^2$$

The 3D response surface plots were generated for a better understanding of the relationships or interactions between each factor (pumpkin puree, carrot puree and sugar) and response (colour (%), taste (%), colour difference in yellowness ( $\Delta b^*$ )). The interaction among pumpkin puree and carrot puree (Figure 4.1, Figure 4.4 and Figure 4.7). The interaction among pumpkin puree and sugar (Figure 4.2, Figure 4.5 and Figure 4.8). The interaction among carrot puree and sugar (Figure 4.3, Figure 4.6 and Figure 4.9).

## 5.2 Sensory Evaluation

Overall, 25 untrained panelists participated in the sensory tests. Panelists were asked to assess the sensory attributes such as colour (%), appearance (%), viscosity (%), aroma (%), taste (%), and overall acceptability (%) of pumpkin-carrot pasta sauce. Based on the sensory results tabulated in Table 4.2, the colour (%) and taste (%) were significantly different at 95 % ( $p < 0.05$ ) while the appearance (%), viscosity (%), aroma (%), and overall acceptability (%) were not significantly different ( $p > 0.05$ ). The overall acceptability was 75.58 % which

represented more than half of the panelists were able to accept pumpkin-carrot pasta sauce.

The colour profile achieved highest acceptance level of 76.75 %, followed by appearance 75.69 %. According to Susanna and Prabhasankar (2013), consumers are more likely to purchase foods with better colour and brightness. Based on Figure 4.1 and Figure 4.2, the addition of more pumpkin puree and carrot puree resulted in the better colour profile of sauce. The deep colour pumpkin and carrot were the sources of carotenoid compound that provided orange vibrant colour (Margaret, et al., 2015).

According to the plot in Figure 4.4, the taste of sauce was maximized when a large amount of carrot puree was added and further enhanced by increasing amount of pumpkin puree. Based on Figure 4.5 and Figure 4.6, the taste profile increased when the levels of sugar were at centre point (0). Therefore, sugar played an important role as flavour enhancer to the taste of sauce. Most of the panelists preferred sugar in moderate level instead of high or low sugar level. If sugar exceeded a certain point, the taste of sauce turned unfavourable.

### 5.3 Colour Analysis

Besides attracting consumers, colour also act as an extensive quality factors used to evaluate quality of the sauce (Khamidah, 2013). Based on the Table 4.1, the colour characteristics for all sauce samples ranged from 42.74 to 52.80 for L\* (lightness), 13.06 to 17.54 for a\* (redness) and 35.04 to 59.52 for b\* (yellowness). The total colour difference ( $\Delta E^*$ ) in 15 samples ranged from 16.62 to 39.54. It was observed that the pumpkin-carrot pasta sauce was characterized as yellow-orange in colour. This was due to carotenoids which is naturally present the pumpkin and carrot. Carotenoids also found in many tropical, citrus fruits and vegetables (Britton, Jensen and Pfander, 2009).

Until now, there was no relevant study that focus on relationships between pumpkin puree, carrot puree, and sugar to colour difference in yellowness. Hence, based on Figure 4.7, decreasing amount of pumpkin puree added into sauce induced large colour difference in term of b\*(yellowness). In addition, the colour difference in yellowness was minimized when level of carrot puree was at -1 and it was maximized when carrot puree was at -0.5. This showed that addition of carrot puree will not result changes in yellowness once the level reached -0.5. According to the Figure 4.8 and 4.9, colour difference in yellowness was maximized when amount of sugar level added was low. In contrast, high level of sugar added in sauce minimized the colour difference in yellowness. To explain this phenomenon, addition of sugar resulted in lower water activity and retard browning activity of sauce. Thus, yellowness of sauce

was found to be slightly lower if more sugar was added (Labuza, Warren and Warmbier, 1977).

#### **5.4 Formula Optimization of Pumpkin-Carrot Pasta Sauce**

The optimum ingredients level of pumpkin-carrot pasta sauce was 500 g of pumpkin puree, 200 g of carrot puree and 0.96 g of sugar, with predicted maximum colour difference in  $b^*(\Delta b^*$ , yellowness) as 41.43, maximum colour in sensory evaluation as 85.85 % and taste in sensory evaluation as 83.40 %. The desirability for this formulation was 0.93, which considered as very desirable due to the value is closest to 1.

In order to verify the optimum formulation, the pumpkin-carrot pasta sauce using the optimal ingredients level was analyzed and the results were statistically compared to the predicted values of the mathematical model. The validation test results of the optimum formula was within the range and found not statistically different at the 95 % confidence level.

## 5.5 Moisture Content Test

Moisture content considered as an important parameter in foodstuffs. It contributes great effect in determining acceptability, freshness, and durability of the materials (Khamidah, 2013).

As shown in the result, the moisture content of optimized pumpkin-carrot pasta sauce ranged from 86.64 % to 86.70 %. According to Khamidah (2013), the moisture content of different materials can affect water levels of sauce. For instance, the water content of pumpkin was 91.60 g /100 g and the water content of carrot was 88.29 g/100 g (USDA, 2017).

A research study carried out by Ritthiruangdej, Srikamnoy, and Amatayakul (2010) on the optimization of jackfruit sauce formulations. It was found that the moisture content of jackfruit sauce ranged from 57.62 % to 66.45 % in six different formulations. Sosa, Sgroppo and Bevilacqua (2011) also reported that the moisture levels of 50:50 (pumpkin : pepper) sauces were higher than 80 : 20 (pumpkin : pepper) proportion during refrigerated storage. These can be explained by the use of the different composition of recipes for sauce, which contribute to different moisture content level.

## 5.6 Total Soluble Solid Test

TSS is a paramount important factor that used to check out the quality of sauce. Basically, TSS is corresponding to the capability to refract light beam, and it is tested by using a refractometer. According to FAO (1995), a high value of TSS signified presence of high sugars level, with other dissolved acids and minerals.

According to Food Regulations 1985, chilli sauce and tomato sauce shall contain not less than 25 per cent of total soluble solids (Bahagian Keselamatan dan Kualiti Makanan, 2014). It was noticed that the total soluble solid of optimized pumpkin-carrot pasta sauce was similar to that of chili and tomato sauce, which ranges from 24.28 °Brix to 25.68 °Brix, and considered as an acceptable quality of the product. A similar observation was reported by Berna, et al. (2013) that the TSS of the rowanberry and rowanberry-pumpkin sauce ranged from 21.05 °Brix to 28.30 °Brix.

Besides different composition of recipes for sauce, factors such as climate, soil type, fertilizer, irrigation, maturity at harvest and postharvest handling can also strongly affect the percentage of TSS (Anton, 2013).

## 5.7 pH Measurement

pH measurement is important as it determines the acidity or alkalinity of the food. In other words, it is a parameter in determining the quality of sauce standards (Khamidah, 2013).

As shown in the result, the pH of optimized pumpkin-carrot pasta sauce ranged from values 5.08 to 5.10, which considered as a medium acid food product. In the present study, the pH of the pumpkin was in value 5.3, whereas the pH value of carrot was within the range of 5.1 to 5.3 (Smith, et al., 1997). It can be concluded that the pumpkin and carrot were under alkaline foods category. A study reported by Khamidah (2013) showed that tomato was found capable in decreasing the pH value of other foods. To be specific, pumpkin-tomato sauce was found to have relatively lower pH level of 4.30 to 4.34. It was because tomatoes (pH 3.7 – 4.9) contain more acid than pumpkin.

Ultimately, acidity of food will influence the thermal processing conditions that are required for producing safe products. In high acid food products (pH < 4.5) are considered relatively stable. Hence, the heat treatment to control microbial spoilage and enzyme inactivation is less severe if compared to low acid food products (pH > 4.5) (Berna, et al., 2013).

## 5.8 Viscosity Measurement

The viscosity of fluid foods is strongly associated with the texture as it determines to a great extent of the overall mouthfeel, as well as the appearance of the resulting sauce, ease of flow and packaging when sauce gets poured (Ritthiruangdej, Srikamnoy, and Amatayakul, 2010). The viscosity of sauce will influence the preference of consumers. For instance, very high viscosity (thick) sauce would convolute consumers when pouring sauce because it required a greater force in order to let the fluid flow (Toledo, 2007).

The relationship between shear rate and shear stress at different temperature of optimized pumpkin-carrot pasta sauce are shown in Figure 4.10. The results show that shear rate increases with shear stress for all temperatures (5 °C, 25 °C and 85 °C). The result presented in Figure 4.11 showed similar trends for the relationship between viscosity and shear rate for all temperatures (5 °C, 25 °C and 85 °C) of optimized pumpkin-carrot pasta sauce. It was noticed that the viscosity decreases with shear rate. During application, the sauce can be easily poured at 25 °C without being too watery. Therefore, the optimum temperature for serving was at 25 °C.

The flow curves were analysed using Herschel-Bulkley model. Based on Table 4.6, flow behaviour index ( $n$ ) increases slightly with temperature. Yet, the consistency index or viscosity ( $K$ ) decreases as the temperature raises. The viscosity function data indicated optimized pumpkin-carrot pasta sauce as non-Newtonian fluid and poses pseudoplasticity (shear thinning) behaviour. This can



be explained by the values for flow behaviour index (n) were below 1 at all temperatures (5 °C, 25 °C and 85 °C) (Omoregbe and Bushi, 2008). The correlation coefficients for Herschel-Bulkley model has fit the data satisfactorily since  $R^2 > 0.98$  for all temperatures (5 °C, 25 °C and 85 °C).

The levels of pectin in fruits and vegetables will influence the viscosity of sauce. Based on a research carried out by Sigit (2007) about the sauce technology from chilli, papaya and tomatoes substitution. It was found that, higher concentration of papaya resulted in a higher viscosity of sauce, as papaya contains pectin which serves as a stabilizer and increases the viscosity of the product. Other than that, viscosity of sauce can also be affected by moisture content. For instance, a high moisture content sauce is low in viscosity.

## **5.9 Further Recommendation**

In the present study, physicochemical and rheological properties of optimized pumpkin-carrot pasta sauce were studied. For the further studies, it is recommended to involve the microbiological analysis such as shelf-life study of the sauce. Other than that, proximate analysis such as ash, protein, fat, carbohydrate, fibre and calorie content of sauce can be carried out. Lastly, bioactive compounds analysis of sauce such as total phenolic content, total flavonoid content, total carotenoids content, and total antioxidant capacity can be tested, since the pumpkin and carrot were high in carotenoid compounds.

## CHAPTER 6

### CONCLUSION

Response Surface Methodology (RSM) with a three factor, three level mixture design was successfully used to optimize the formula of pumpkin-carrot pasta sauce. In other words, pumpkin-carrot pasta sauce was successfully developed. The optimum formulation of pumpkin-carrot pasta sauce consisted of 500 g pumpkin puree, 200 g carrot puree, 0.96 g sugar, 30 g water, 15 g corn oil, 15 g cooking cream, 8 g unsalted butter, 3 g vinegar, 3 g corn starch, 3 g salt, 2 g garlic puree, 0.4 g black pepper powder and 0.4 g mixed herbs. The overall acceptability of the sauce in sensory results were in moderate-high level. As for profiling purpose, the moisture content, total soluble solid and pH of optimized pumpkin-carrot pasta sauce were  $86.67 \pm 0.03$  %,  $24.93 \pm 0.65$  °Brix and  $5.09 \pm 0.01$ , respectively. The optimized pumpkin-carrot pasta sauce exhibited pseudoplastic behaviour and consistency index or viscosity decreased with increase in temperature. The optimum temperature for serving was at 25 °C.

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## APPENDICES

### APPENDIX A

#### QUESTIONNAIRE FOR HEDONIC SCALING TEST

Panel No. : Date :

Product : **Pumpkin-Carrot Pasta Sauce** Age :

Gender : ( F / M ) Occupation :

**Instruction:**

You are given three coded samples. Please evaluate these samples and rate how much you like or dislike each other according to the scale below for the respective sensory attribute.

Please rinse your mouth with water before tasting each sample. Taste the samples and rate it using the scale below:

- 9 Like extremely
- 8 Like strongly / very much
- 7 Like very well / moderately
- 6 Like fairly well / slightly
- 5 Neither like nor dislike
- 4 Dislike slightly
- 3 Dislike moderately
- 2 Dislike strongly / very much
- 1 Dislike extremely

Sensory attribute	Sample code		
Colour			
Appearance			
Viscosity			
Aroma			
Taste			
Overall acceptability			

Comment:

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Thank you.

## APPENDIX B

### OUTPUT OF RESPONSE SURFACE METHODOLOGY

Prediction Profiler with Maximum Desirability Set for optimize ingredients level

