FORMULA OPTIMIZATION ON MATCHA BUTTER:

A RESPONSE SURFACE METHODOLOGY STUDY

By

YAP KAH SHING

A project report submitted to the Department of Agricultural and Food Science

Faculty of Science

University Tunku Abdul Rahman

In partial fulfilment of the requirements for the degree of

Bachelor of Science (Hons) Food Science

September 2017

ABSTRACT

FORMULA OPTIMIZATION ON MATCHA BUTTER: A RESPONSE SURFACE METHODOLOGY STUDY

Yap Kah Shing

Response surface methodology (RSM) combined with three variables (matcha powder, salt, sugar) at three levels (-1, 0, +1) under Box-Behnken design was used to optimize the matcha butter based on color matching and sensory evaluation. Butter was churned from 36 % milk fat at 15.6 ℃ to ensure its high fat and low moisture level. The amount of matcha powder, salt and sugar were added according to the design of Box Behnken model in order to produce 15 matcha butters. The color profile of each matcha butter (L*,a*,b*) was determined using colorimeter, while the differences in lightness (ΔL*) between the lightness of matcha butters (L*) and lightness of premium matcha ($L_{standard}$*) were calculated. The lightness difference (ΔL*) was set to minimum during optimization to match the lightness profile of premium matcha powder. Sensory evaluations (color, appearance, taste, mouthfeel, overall acceptability) of 15 matcha butters were done using 9 points hedonic scale on 25 untrained panelists. JMP software was used in analysing the significance of responds. In addition, determination of optimum matcha butter was done by maximizing sensory scores and minimizing the difference in lightness (ΔL*). Based on the investigation, formula of optimized matcha butter was 10, 3.6 and 7 g respective to matcha powder, salt and sugar. Besides, differences in lightness (ΔL*), color

and taste had significant impact on the color matching and sensory evaluation. Therefore, three dimensional response surface graphs were created to study the interactive effects between two variables simultaneously on differences in lightness (ΔL^*), color, and taste. As for profiling purpose, optimized butter contains 12.42 % ± 0.035 moisture and 78.12 ± 0.75 % fat. Temperature sweep was done by heating the matcha butter from 5 to 40 ℃ and then cooled to 5 ℃. Storage and loss modules showed elastic behavior and indicated an overall melting of matcha butter at 18 ℃.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my very great appreciation to my project supervisor, Mr. Chung Kok Heung for giving me freedom to decide the project title. His expertise in product optimization has helped me a lot throughout the project. I am also grateful to Dr. Phuah Eng Tong, lecturer, in the Department of Agriculture and Food Science. I am extremly thankful and indebted to him for sharing expertise, and sincere and valuable guidance and encouragement extended to me.

I place on record, my sincere thank you to Kang Kah How, for his helps and continuous encouragement. This work would not have been possible without his support. Also, I would like to take this opportunity to express gratitude to all of the Department faculty members for their help and support.

Finally, I thank my parents and siblings for the unceasing encouragement, support and attention. To them I say “No matter how old I get, I always want my family when I don’t feel good. Thank you for being there when I need you the most”.

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.

____

(YAP KAH SHING)

APPROVAL SHEET

The project report entitled "**FORMULA OPTIMIZATION ON MATCH BUTTER: A RESPONSE SURFACE METHODOLOGY STUDY**" was prepared by YAP KAH SHING and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Food Science at Universiti Tunku Abdul Rahman.

Approved by:

____

Date:

Mr. Chung Kok Heung

Supervisor

Department of Agricultural and Food Science

Faculty of Science

Universiti Tunku Abdul Rahman

FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

Date: ______________

PERMISSION SHEET

It is hereby certified that **YAP KAH SHING** (ID No: **13ADB03520**) has completed this final year project entitled "**FORMULA OPTIMIZATION ON MATCH BUTTER: A RESPONSE SURFACE METHODOLOGY STUDY**" under the supervision of Mr. Chung Kok Heung from the Department of Agricultural and Food Science, Faculty of Science.

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,

____

(YAP KAH SHING)

TABLE OF CONTENTS

CHAPTER **PAGE**

LIST OF TABLES

LIST OF FIGURES

LIST OF EQUATIONS

LIST OF ABBREVIATIONS

a*	Redness
Å	Angstroms
b*	Yellowness
°C	Degree celsius
COMT	Cytosolic catechol-O-methyltransferase
EGCG	Epigallocatechin gallate
G’	Storage modulus
G’’	Loss modulus
L*	Lightness
∆L*	Difference in lightness
Lstandard*	Lightness profile of premium matcha
OECD	Organisation for economic cooperation and development
ORAC	Oxygen radical absorbing capacity
R^2	Coefficient of determination
RSM	Respond surface methodology
TAG	Triacylglycerol
UV	Ultraviolet
µm	Micrometer

CHAPTER 1

INTRODUCTION

1.1 Background

As you stand at the diary department of any local supermarket, you may be amazed by looking at the refrigerators where butters are kept. It is prodigious that food as simple as butter can be diversified in so many variations. It is common to see raw cream butter, salted butter, lightly salted butter, unsalted butter, blended butter with vegetable oil, canola oil, olive oil and even butter with omega-3 fatty acid that can reduce the risk of cardiovascular disease (Helweg, 2010). Although, there are so many variety of butters in the market, but flavoured butter rarely appear.

Statistical studies from OECD Agricultural Outlook (2017) showed that butter consumptions in Malaysia were 9650 tons unit in year 2013 and it rocketed significantly to 12500 tons unit in year 2014 with an increase of 29.5 %. The trend will not stop until year 2017 as the expected consumption to be 13530 tons unit. The amount of butter consumption is expected to increase deliberately until the year of 2024 with the forecast consumption of 15870 tones unit butters nationwide (OECD Agricultural Outlook, 2017).

Meanwhile, Global Food Forum reported flavored butter as one of the food and beverage trends in the year 2017 (Global Food Forum, 2017). Besides, matcha green tea was chosen as top 7 trendy foods based on the national food trend survey (Global Food Forum, 2017). Matcha green tea is the super food that carry numerous health effects on human body. It was honoured as “Health Elixir” during the past due to its extraordinary antioxidant effect and health benefits (Snyder, et al., 2015).

The matcha green tea trends had caused huge production of matcha-related products in local and international food companies. There are few giant food companies such as Nestle (Switzerland), Northern Tea Merchant (U.K.), Unilever Group (U.K.) and Tetley (England) found involving themselves in the matcha market (Market research future, 2016). As a result, there are many Matcha flavored commercial products in the market, for example, Pocky green tea flavored confectionery, Nestle green tea kit kat, BOH green tea latte, etc. Besides, there are even restaurants, an example of Nana’s Green Tea serving diversified matcha related drinks and dessert.

Currently, the matcha green tea trend is off global interest. Studies also showed that matcha market is expanding rapidly in asia pacific region (Market research future, 2016). Hence, incorporation of matcha green tea into butter is definitely a worth trying idea to produce a new product: Matcha Butter. In this project, the amount of matcha powder, salt and sugars are adjusted according to response surface methodology (RSM) through Box-Behnken design and the optimization of product are based on consumer preference and colour matching.

Besides, rheological properties are one of the important aspect in developing a new product. Rheological properties of food emulsion in butter play an essential role in understanding the food structural organization and can be related to stability, melting point and spreadability of the final product. Hence, temperature sweep will be carry out on the optimized product to examine the melting behaviour.

1.2 Objectives

The objectives of this study were:

- To develop an optimised Matcha butter using Respond Surface Methodology (RSM) based on colour matching and sensory evaluation.
- To determine the water and fat content of the optimized matcha butter.
- To evaluate meltability of optimized matcha green tea butter.

CHAPTER 2

LITERATURE REVIEW

2.1 Butter

2.1.1 Definition of Butter

Butter can be defined as solid product derived exclusively from milk or cream, or both, and shall be free from rancidity. According to FOOD ACT 1983 and FOOD REGULATIONS 1985, Butter shall contain not less than 80 per cent of milk fat; shall not contain more than 16 per cent of water; and may contain salt. Besides, butter may contain permitted colouring substances of vegetable origin and permitted antioxidant (Kementerian Kesihatan Malaysia, 2014).

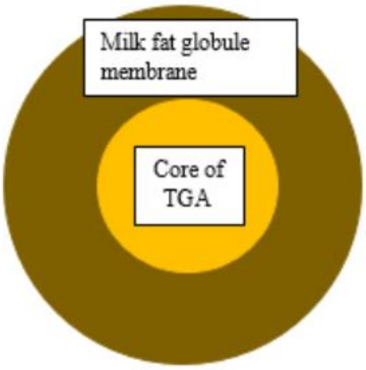
2.1.2 History of Butter

Butter was present for more than 4000 years and it was cited in the Old Testament (First part of Christian Bible): “She brought forth butter in lordly dish". Besides, the word "butter" originated from Greek word *bouturo,* which carried the meaning of "cow cheese". According to the literature, butter was first discovered when warm milk was unintentionally churned in saddle bag as it was being transported from place to place (Helweg, 2010).

During the past, butter was made by inflating goat skin with air and filled with some milk before being sealed. Then, the skin was hung with ropes on a tripod of sticks, and swung until the formation of butter. In late nineteenth centuries, the mechanical cream separator was invented. Initially, churns were made from woods. The cream was subjected to shear and mild aeration by spinning the vessel. Buttermilk was drained when fats clumped into butter. However, this process barely met the hygiene standard. Often, cream turns sour before turning into butter. In addition, the wooden churn was very hard to keep clean. The absence of refrigerator causes rapid growth of microorganism. Slowly, wooden churns were replaced by aluminium and stainless steel. Eventually, the technology was overtaken by the development of continuous butter making processes (Deosakar, Khedkar and Kalyankar, 2016).

2.1.3 Milk Fat Globule Composition

Bovine milk fat is one of the popular choice as main ingredient of butter. When look in to nanostructure, bovine milk fat is spherical globules in shape and have the diameter between 2 and 8 um. It is surrounded by membrane that is made up of lipids, proteins and phospholipid (Deosakar, Khedkar and Kalyankar, 2016). The milk composition may differ among cow species, season and food fed (Marrero, 2017). This membrane contains lecithin, that helps to prevent enzymatic oxidation, lipolysis and keep the fat globule suspended in water phase (Potter and Hotchikiss, 1998). Body of milk fat as illustrated as below, consist of 98.3 % (w/w) triacylglycerol (TGA) and the remaining 1.7 % involves phospholipid diacylglycerol, sterol, free fatty acid and monoacylglycerols in descending order (Deosakar, Khedkar and Kalyankar, 2016).



(Prezi, 2017)

Figure 2.1 Illustration Diagram of Milk Fat Globule.

2.1.4 Butter Making Theory

Butter is made from milk fat, which can be found in cream or whole milk. (Mahony, 1988). Cream is a raw material that is isolated from milk and contains about 30-35% fats (Potter and Hotchikiss, 1998). It is an oil in water emulsion, in which tiny globules of fats are suspended into water (Oklahoma, 2006). Butter manufacturer will first pasteurize the cream before churning at slightly higher temperature than milk pasteurization to kill off bacteria as high fat content of cream tend to have protective effect on bacteria (Potter and Hotchikiss, 1998).

Churning process is a mechanical disturbance that reverses the natural emulsion in cream. During churning, temperature control is critically important. The ideal churning temperature is at 10-12℃ in hot climate and at 14-17 C in cold climate (Potter and Hotchikiss, 1998). If the cream is too cool, there will be an interruption in butter formation and small gains produced is difficult to handle. On the other way, if cream's temperature is too high. Large proportion of fat will remain in buttermilk, and butter produced will be in mushy and porous in texture (Potter and Hotchikiss, 1998).

During churning, nucleation happened when large amount of air is incorporated into the cream and then encourage the formation of crystal (Herrera and Hartel, 2000). After forming the crystals, agitation process breaks the formed crystals into fragments and then spread them across the food system (Kloek, Vliet and Walstra, 2005). Then, the crystal fragments served as nucleation sites to carry out secondary nucleation. As result, larger amount of crystals is formed (Herrera and Hartel, 2000). At the same time, agitation process also breaks the surface membrane of milk fat globules, causing fat globules to collide with each other. As the process continues, the cream becomes rougher due to clumping of fat globules and crystals and form tiny butter granules (Mahony, 1988).

Eventually, the rough milk fats form semi-solid butter granules, which rapidly clump together and grow in size. The by-product left is a milk-like liquid with tiny butter grains floating in it which is "buttermilk" (Oklahoma, 2006). Then, buttermilk is discarded and butter is washed few times with iced water before salting. The state of emulsion now is inversed. Butterfat is the major and continuous phase and about 15 % of buttermilk is the discontinuous phase which suspended as fine droplet in the butter (Ronholt, et al., 2012).

2.2 Rheological Properties of Butter

Body and texture characteristic of a butter are very important for consumer acceptability (Buldo, 2012). A good butter shall be smooth and firm. It should have optimum resistance against cutting and can be spread smoothly. When eaten, it should melt evenly in the mouth cavity without leaving an oily aftertaste (Deosakar, Khedkar and Kalyankar, 2016). Texture of butter is closely related to water content, fatty acid and chemical composition, distribution of milk fat crystal network structure, polymorphism and processing steps (Buldo, 2012), milk fat structure and composition (Deosakar, Khedkar and Kalyankar, 2016).

Milk fat compositions and the arrangement of fatty acid in triacylglycerol (TGA) molecule of the cream can effect the crystallization behaviour and then affect the microstructure, macrostructure and even texture of the butter (Deosakar, Khedkar and Kalyankar, 2016). Hence, mastering the chemical and rheological properties of food by different processing conditions is paramount importance in developing a new product.

Knowledge of butter emulsion's rheological properties enables a better understanding of the structural organization of butter, processing, storage, transportation, stability and mouth feel of the products (Emadzadeh, et al., 2013). To make a clearer picture, fat crystallization; crystal network and polymorphism are further discussed.

2.2.1 Fat Crystallization

Careful control fat crystallization during butter production can produce butter with the pleasant structural properties. During crystallization, organized molecules randomly come together and form 3-dimensional molecular structure, known as crystal (Davey and Garside, 2000). The crystallization process is made up of two steps: nucleation and crystal grow (Marrero, 2017).

In nucleation, a crystal nucleus is formed when energy is absorbed from the system (Timms, 2007). Before the first crystal is formed, a specific amount of energy is required to enter the cluster. As cluster grows, entropy of the system will decrease (Wesdorp and Marangoni, 2013). According to Boskish (1998), nucleus produced from nucleation is an intermediate stage of converting itself from an unorganized nucleus to an organized crystallized structure. Yet, the nucleuses were not stable and can be degraded anytime. Therefore, they must reach the size between 0.1 and 1.0 µm in order to grow into crystal. Nucleus that fail to grow into crystal will result in degradation.

Secondary nucleation may happen if part of the first crystal breaks. Then, crystallization is favored as the crystals fragments started to grow. The rate of crystal growth is proportionate to the degree of undercooling (Smith, 2008). As crystallizes grow, they start to collide. As result, the growth rate of crystal is slowed down (Buldo, 2012).

2.2.2 Polymorphism

Triacylglycerols in fat globules tend to crystallize in 3 different modifications which are: α (alpha), β’ (beta prime) and β (beta) (Guarley and Marangoni, 2005). α modification present in hexagonal form with the short spacing of 4.15 Å. It is characterized by low melting point, low density, and low stability. The β’ form has short spacing of 3.8 Å, 4.2 Å and it is structured in an orthorhombic subshell with high melting point, high density and high stability (Marangoni, 2016). However, the arrangement of β’form crystal lattice structure is less organized and stable than β modification (Juriaanse and Heertje, 1988). Lastly, the β polymorph is in triclinic structure and it has the highest melting point, density, and stability among all the polymorphs (Marangoni, 2016). Although β modification is the most stable crystals, it causes poor crystal network and it is often perceived as sandiness (Juriaanse and Heertje, 1988). Among the 3 polymorphisms, β’ form is most desirable in butter due to its optimal crystal morphology and crystal network that give the desired texture (Buldo, 2012).

Cooling rate is one of the processing conditions that can affect the crystallization behaviour of milk fat by influencing the polymorphology and further alter the microstructure and texture of butter (Buldo, 2012).

The cooling rate of milk fat can cause a difference in size and amount of crystal formed. Slow cooling rate allows TAG to organize themselves in a more stable form of crystal: β' (Wiking et al., 2009). As a rule, slow cooling rates enhances large and stable crystals to form, whereas fast cooling rates produce plenty of small and unstable crystals (Herrera and Hartel, 2000).

2.2.3 Crystal Network

Under the vision of confocal laser scanning microscope. Intact and partially disrupted milk fat globules, fats crystals, aqueous and air droplets are embedded in a 3-dimensional fat crystal network (Marrero, 2017). The crystals are connected to each other by two types of bonds: primary and secondary bond to form a complex crystal network (Juriaanse and Heertje, 1988). Primary bonds are responsible for crystals grow; whereas secondary bonds are weak Van der Walls force that hold crystals together (Deosakar, Khedkar and Kalyankar, 2016). Therefore, properties of fat crystals and amount of water in the butter are important for the strength of crystal network (Ronholt, et al., 2012).

Small crystals contribute more contact points for bonding and lead to a finer crystal network. As result, a firmer butter is produced. Contrary, large crystals provide lesser contact points for bonding and crystal network formed is more vulnerable. As consequence, softer butter is produced. On the other hand, unbroken fat globule will disrupt the crystal network, resulting in a weaker structure and good spreadability (Deosakar, Khedkar and Kalyankar, 2016). This explains the need of long churning time in order to disrupt intact milk fats in the cream to produce a firmer butter.

Moreover, water droplets are dispersed in the butter, forming a network with the other fat globules (Pedersen, et al., 2012). It was found that high moisture content will greatly interfere the crystal network and resulted in soft and watery butter (Ronholt, et al., 2012).

2.3 Matcha Green Tea

2.3.1 Background of Matcha Green Tea

Powdered tea is a processed tea whereby the leaves are broken into tiny pieces and whisked swiftly together with hot water to produce tea. It was first discovered during the Song Dynasty in China. This method of preparing tea was introduced to few Japanese Zen monks before they bring it back to Japan and implement the culture of consumption powdered tea by their own. Then Eisai, a Japanese Zen priest successfully discovered matcha tea in the 11 century by himself. Soon, the Matcha was popularized by the Samurais and elite groups of the society (Snyder, et al., 2015). Since then, matcha became one of the must in tea ceremonies and eventually became one of the Japanese culture.

Matcha was recorded to have oxygen radical absorbing capacity (ORAC) of 1573 per gram. It is the highest rated food rank ever recorded in the ORAC scale (Snyder, et al., 2015). Matcha is derived from the green tea plant *Camellia sinensis* (Auxter, 2015). Few weeks before harvesting, the tea plants are deliberately covered in shade to avoid direct exposure of sun light. This retard the growth of plant, and energy is converted into leaves by increasing the amino acid and chlorophyll level (Smith, 2016). Increase of amino acid in the leaves can bring up a sense of sweetness (Towler, 2010).

During harvesting, fresh and tender leaves are handpicked before they are cleaned, steamed and air dried (Snyder, et al., 2015). Tencha is the name of tea after air drying. Since the leaves can only be harvested 2 times a year. Most tencha are kept refrigerated within 48 hours after harvesting for constant production of matcha throughout the year (Smith, 2016). The remaining tencha that are ready to be process are dehydrated to further reduce moisture content of leaves by 3 %. In traditional method, tencha leaves are grind by stone in very gentle and slow manner. Speeding up the grinding process could introduce unnecessary heat and friction that cause the final product to be bulky (Smith, 2016).

Usually 30 g of matcha is produced by 1 hour of grinding (Towler, 2010). In modern factory nowadays, more tencha can be grinded in large mechanical grinder but the processing time is still long in order to retain the bright green and unique flavour of tea (Smith, 2016).

2.3.2 Health Benefits of Matcha Green Tea

2.3.2.1 Cancer Prevention

Exposure to ultraviolet (UV) radiation by sunlight is one of the unavoidable phenomena faced by most people especially in tropical regions. UV radiation that managed to penetrate through atmosphere is made up of 5 % UVB and 95% UVA. Both UVA and UVB are able to enter into dermis and epidermis of the skin. Extensive exposure of UVA causes production of hydroxyl free radicals and singlet oxygen in skin. They can injure the cellular macromolecules and tringle skin sagging by photo oxidation. On the other hand, UVB is carcinogenic and often cause skin diseases (Katiyar , 2011).

Matcha tea leaf is extremely high in antioxidant, polyphenols and epigallocetechin gallate (EGCG). EGCG is a very powerful and nature occurring antioxidant that can be also found in cocoa and blackberries. EGCG in matcha can prevent DNA damage due to radicals and reactive oxygen species. , Beside, EGCG can activate detox emzymes like quinone that is capable to prohibit development of tumor cell (Snyder, et al., 2015). In addition, polyphenols in tea can inhibit damage done on skin by UV radiation (Cheadle and Kilby, 2016) and can trigger apoptosis (Snyder, et al., 2015).

2.3.2.2 Promote Healthy Heart

Coronary heart diseases can be caused by many risk factors. While, diet high in saturated fats can greatly lift the risk of heart disease and has causes 31 % of total coronary heart disease cases worldwide (World Heart Federation, 2017). Diet is always closely related to heart disease as it can alter all the other cardiovascular risk factors. The intake of saturated and trans fats can potentially increase cholesterol level and block the arteries. Heart disease can happen if no corrective action is taken (World Heart Federation, 2017).

The EGCGs present in matcha can drive cardioprotective effect by supressing oxidation, vascular inflammation and thrombogenesis. Besides, EGCGs was found capable in lowering the absorption of total cholesterol by forming insoluble cholesterol precipitates to disturb the biliary micelle system in the lumen of the intestine. As result, more cholesterol is excreted as faeces and blood lipid profiles is improved (Zheng, et al., 2011).

Studies also show that, people who drink matcha green tea are unlikey to develop heart disease by 11 % than people who don’t (Snyder, et al., 2015). While, people who drink 5 cups or more matcha green tea everyday are 26 % less likely to engage with cardiovascular disease compare with those who take one cup a day (Cheadle and Kilby, 2016).

2.3.2.3 Help in Weight Loss

Matcha green tea is recognized for being outstandingly rich in flavonoids and catechins. Catechin can inhibit Cytosolic Catechol-O-Methyltransferase (COMT), an enzyme that can breakdown norepinephrine. Norepinephrine is an enzyme that work together with sympathetic nervous system to regulate thermogenesis and fats metabolism. Due to the absent of COMT, norepinephrine can works longer and inhibit prolonged thermogenesis and fats oxidation (Dulloo, et al., 1999). As result, intake of matcha diary can increase thermogenesis from 8 to 10%. Consuming matcha green tea before physical activity can further increase fat burn by 25% (Snyder, et al., 2015).

2.4 Respond Surface Methodology (RSM)

Respond Surface Methodology **(**RSM) is a collection of statistical and mathematical methods (Manohar, et al., 2013). It is commonly applied to achieve the desired conditions whereby the desired outcomes are influenced by several factors of interest (Bezerra, et al., 2008). RSM is often coupled with Box–Behnken designs to acquire high accuracy of optimization by running lesser experiment sets compared to traditional method (Qui, et al., 2014)**.**

Box Behnken design was developed by Box and Behnken. In this design, samples are reworked on parameters of different sets if they are aligned to the 2 level factorial design. The parameters which do not involve in the factorial design are keep at the mean value (Cavazzuti, 2013). Box Behnken design model is commonly used in industrial research, due to its convenience and cost saving properties. In each design, only 3 levels on every factor namely -1, 0, +1 are required (Khuri, and Mukhopadhyay, 2010).

Prior to the experiment, the levels of factors need to be identified and the internal space between each level must be constant. Number of experiment is determined by this formula: $N = 2k(k - 1) + cp$, where cp is the number of the central points and k represent number of factors (Bezerra, et al., 2008). Since, number of factors is 3. By applying formula $N = 2n(n - 1) + cp$, number of experiment is 15. After finding total number of experiments, combination of level of factors should be defined.

Referring to Table 2.1. First, level in factor "C" is locked at centre level "0". Then, the other levels (-1, 1) are permutated in factor "A" and "B" until no other combination can be made. After that, the levels of the factor "B" follow the previous steps until all possible combinations are produced. It is then followed by factor "A". Lastly, the levels of factor in the remaining experiments are all set to centre level "0" (Mustafa, et al., 2012).

Table 2.1. Box-Behnken Experimental Design of 15 Runs.

Rank	Box Behnken Experimental Design		
	A	**B**	**C**
1	-1	-1	0
2	1	-1	0
3	-1	1	0
4	1	1	0
5	-1	0	-1
6	1	0	-1
7	-1	0	1
8	1	0	1
9	0	-1	-1
10	0	1	-1
11	0	-1	1
12	0	1	1
13	0	0	0
14	0	0	0
15	0	0	0

(As abstracted from Mustafa, et al., 2012)

CHAPTER 3

MATERIAL AND METHODOLOGY

3.1 Box Behnken Design Setup

Salt, sugar and matcha powder were believed to effectively affect the sensory characteristic of matcha butter. Therefore, 3 level factors Box-Behnken design of Response Surface Methodology was used. Table 3.1 showed the levels and amount of each variables added to 200 g of freshly churned butter.

Table 3.1 Variables in Box–Behnken Design to Produce Matcha Butters.

Independent Variables	**Levels**		
	-1	**0**	**+1**
Matcha Powder	4.0 g	7.0 g	10.0 g
Salt	2.0 g	2.8 g	3.6 g
Sugar	7 g	10 g	13 g

Table 3.2 Randomized Box-Behnken Design.

No.	Independent Variable		
	Matcha Powder (g)	**Salt (g)**	**Sugar (g)**
1	7.0	3.6	7.0
2	10.0	3.6	10.0
3	7.0	2.8	10.0
4	7.0	2.8	10.0
5	4.0	2.0	10.0
6	10.0	2.8	7.0
7	4.0	2.8	7.0
8	4.0	2.8	13.0
9	7.0	2.8	10.0
10	10.0	2.8	13.0
11	4.0	3.6	10.0
12	7.0	2.0	7.0
13	7.0	2.0	13.0
14	10.0	2.0	10.0
15	7.0	3.6	13.0

(Refer to 200g fresh butter)

Table 3.2 showed the randomized Box-Behnken design, and matcha butters were produced based on randomized Box Behnken experiment design. The two dependent variables were sensory scores and different in lightness (ΔL^*) from standard matcha green tea. JMP software was used to analyse the significant of each factor and produce optimum matcha butter by maximizing sensory scores and minimizing difference in lightness (ΔL^*) from standard matcha's lightness profile.

3.2 Experimental Design

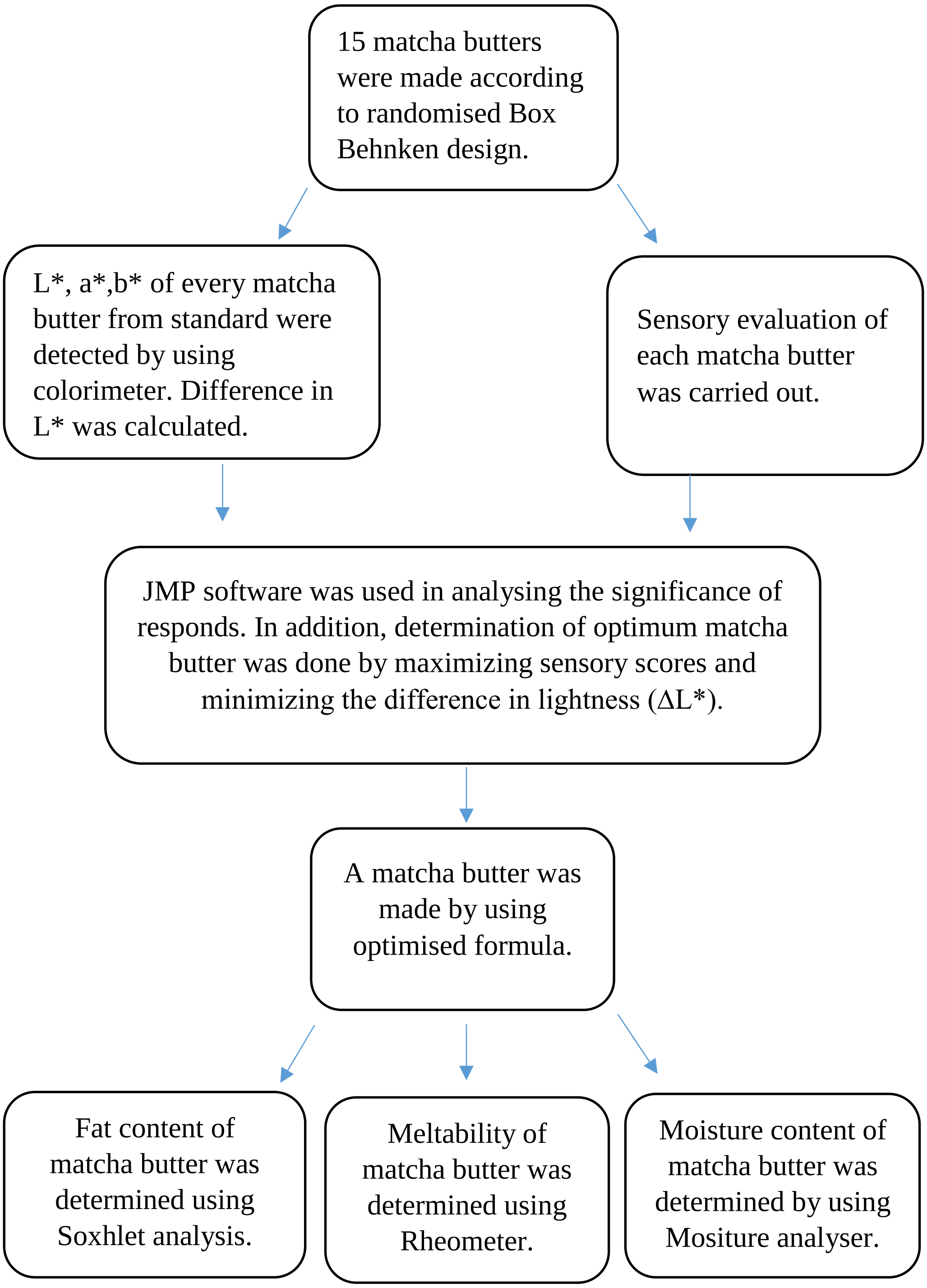


Figure 3.1: The Experimental Design throughout this Project.

3.3 Preparation of Matcha Butter

Ingredients needed to produce matcha butter were whipping cream, fine sugar, fine salt and matcha powder. Matcha powder used was from Tehmag food cooperation, Taiwan. Whipping cream used was manufactured by Emborg, Denmark. Fine sugar used was produced from MSM Pria Berhad, Pulau Pinang, Malaysia. Fine salt was packed by Seng Hin Brother Enterprises SDN BHD, Selangor. Whipping cream, sugar and salt were purchased from Tesco supermarket in Kampar. While, matcha powder was purchased from Siti bakery shop, Kampar. Apparatus used for butter preparation were Hobart mixer, food grade thermometer, cheesecloth and spatula.

In the first stage, whipping cream was introduced into the mixing bowl. The temperature of whipping cream was adjusted to 15.6 ℃. Temperature of whipping cream was checked from time to time by using thermometer. When temperature of whipping cream reached 15.6 ±0.6 ℃, the cream was agitated at low speed (speed 1) for 8 minutes using Hobart mixer. Then, the agitation speed was adjusted to high for another 12 minutes (speed 2) for phase separation. The butter was allowed to sit for 5 minutes for better separation of buttermilk. Then, butter milk was discarded. Butter was washed several times until wash water turned clear before straining using cheesecloth (Helweg, 2010). The strained butter was put back to mixing bowl together with sugar, salt and matcha powder and mixed for 15 minutes. The amount of sugar, salt and matcha powder were added by referring to Table 3.2. After mixing, matcha butter was wrapped using cling wrap in rectangular container and stored in refrigerator.

3.4 Analysis of Colour on Matcha Butter

Analysis of colour was carried out with colorimeter namely CM-600d by Konica Minolta, Japan. Hunter Lab colour space was used as the parameter to measure colour. L* value indicated lightness and it ranged from 0 (black) to 100 (white). a* value represented greeness * (-a) and redness (+a). b* indicated blueness (-b) to yellowness (+b) (Sahin and Sumnu, 2006).

First, cell culture dishes were half filled with freshly made matcha butter. Matcha butter was gently pressed with a spatula until the bottom was fully filled. Cell culture dishes were capped and set aside. Then, colorimeter was turned on and calibrated. Head of colorimeter was place vertically above the bottom surface of cell culture dish to take measurement. All of the samples were run in triplicate. L*, a*, b* readings were recorded.

The Hunter L*a*b* colour profile of premium matcha powder that marks the desired and highest quality were: 60.39, –6.43, 18.71 respective to L*, a*, b* (Ayhan, et al., 2014). The $L_{standard}$ is 60.39. Only the differences of lightness (ΔL^*) of 15 matcha butters were obtained and used for optimization of matcha butter.

3.5 Sensory Evaluation on Matcha Butter

Sensory evaluations were carried out at sensory lab, Faculty of Science, University Tunku Abdul Rahman, Kampar, Malaysia. 9 point hedonic scale was used in this sensory evaluation, detailed showed in Appendix. Sensory attributes to be evaluated were: colour, appearance, taste, mouthfeel and overall acceptability of matcha butter. The butter was spread on Massimo white sandwich loafs as flavour carrier due to its affordable pricing and mild flavour of loaf that will not take over the flavour of matcha butter. 25 untrained panellists were recruited for every testing and only 3 types of matcha butters will be provided to avoid confusion. In addition, samples presented were coded with random 3 digit numbers to prevent bias (Lawless and Heymann, 1999).

3.6 Temperature Sweep of Matcha Butter

Rheometer model TA Discovery HR1 by TA INSTRUMENT coupling with 60mm cone plate were used in this test. Surface of peltier plate was cleaned with few drops of alcohol. Zero calibration was carried out before spreading a thin layer of matcha butter on peltier plate. Storage modulus and Loss modulus were determined in this test by increasing temperature from 5 to 40 ℃ and decrease back to 5℃. The test was performed with 5 ˚C interval and 1 % strain (Vithanage, Grimson, and Smith, 2009). The measurements were taken in triplicate.

3.7 Determination of Moisture Content on Matcha Butter

Moisture analysis was carried out by using Mettler Toledo moisture analyzer HB43-S Greifensee, Switzerland. Moisture analyser was turned on and temperature was set to 40℃. A piece of fibre glass sheet was placed on the pan. Then 1 g of matcha butter was weighted and spread evenly on the glass fiber sheet. All of the samples were run in triplicate. The percentage of the moisture content was recorded.

3.8 Determination of Fat Content on Matcha Butter

The lipid analysis carried out was Soxhlet method. The machinery involved in the test was Gerhardt, SOXTHERM automatic extraction system produced from Königswinter, Germany.

Prior to extraction, six clean and dry extraction beakers were prepared. 3 boiling stones were added into every extraction beaker allowed to dry in 105℃ for 1 hour. After that, the extraction beakers were cooled down in a desiccator for 1 hour. Dried and cooled extraction beaker was carefully removed from desiccator one by one using a thong and weighted on a 4 decimal place digital weighing balance. The weight of every extraction beaker was recorded as *Mi*.

Then, 3 g of matcha butter was weight and recorded as *Mo*. The sample was wrapped in filter paper. This step was repeated for another 5 times to produce 6 samples. Samples together with filter papers were put into the respective thimble. Then, cotton wools were added to cover the mouth of thimbles. After that, thimbles were inserted into the bottom of extraction beaker and approximately 90ml of petroleum ether was added into each extraction beaker in the fume hood. At the same time, water supply and pump of Soxtherm were turned on. The 6 extraction beakers were inserted to the Soxtherm apparatus using thong and extraction process was run for around 2 hours.

After extraction, extraction beakers were removed from Soxtherm apparatus with a thong. Thimbles were removed from extraction beakers and dried in oven at 105℃ for 1hour. Dried extraction beakers were then cooled down in the desiccator for 1 hour. Cooled and dried extraction beakers were weighted by 4 decimal place digital weighing balance. The weight of every extraction beaker was recorded as *M2*. All of the samples were run in triplicate. The percentage of the fat content was calculated using the formula below:

Equation 3.1 Percentage of Fat

$$Percentage\ of\ Fat = \frac{M2 - M1}{M0} \times 100\%$$

CHAPTER 4

RESULT

4.1 Formulation Optimization

Box–Behnken design of three variables consists of 15 experiments. Readings of L* lightness, a* redness, b* yellowness and ΔL* difference in lightness were recorded in Table 4.1. Besides, score of each attribute (colour, appearance, taste, mouthfeel and overall acceptability) and their means were tabulated in Table 4.2.

Based on Table 4.1, Hunter Lab colour scale ranged from 55.33 to 66.83, -14.05 to -11.23 and 31.06 to 38.25, respective to mean values of L* lightness, a* redness, and b* yellowness. However, only ΔL* difference in lightness was selected as the response variable for colour analysis and it was calculated by dividing the $L_{standard*}$ 60.39. The range of difference in lightness was greatest at 8.44 and lowest at 0.14.

The general mean for the taste recorded the highest (72.30 %) followed by overall acceptability (70.99 %), colour (70.46 %), appearance (70.25 %), while the mouthfeel showed the lowest at 69.90 %.

Table 4.1 Results of Lightness (L*), Redness (a*), Yellowness (b*), and Differences in L* (ΔL*) in 15 Matcha Butter Samples.

No.	**Matcha Powder (g)**	**Salt (g)**	**Sugar (g)**	**L***	**a***	**b***	**Difference in L* (ΔL*)**
1	7.0	3.6	7.0	62.00±0.48	-13.82±0.10	37.39±0.33	1.61
2	10.0	3.6	10.0	56.15±1.56	-14.00±0.39	38.11±0.67	4.24
3	7.0	2.8	10.0	63.70±0.21	-14.05±0.07	37.82±0.51	3.31
4	7.0	2.8	10.0	60.25±1.36	-13.31±0.30	36.80±1.01	0.14
5	4.0	2.0	10.0	66.10±0.63	-12.02±0.13	34.00±0.38	5.71
6	10.0	2.8	7.0	56.81±0.65	-13.56±0.17	38.08±0.17	3.58
7	4.0	2.8	7.0	66.65±0.46	-11.60±0.26	32.88±0.21	6.26
8	4.0	2.8	13.0	68.64±0.42	-11.34±0.10	31.79±0.04	8.25
9	7.0	2.8	10.0	62.58±0.47	-12.55±0.11	35.10±0.21	2.19
10	10.0	2.8	13.0	57.29±0.71	-14.02±0.27	38.25±0.89	3.10
11	4.0	3.6	10.0	68.83±0.29	-11.23±0.08	31.06±0.54	8.44
12	7.0	2.0	7.0	63.90±0.10	-12.67±0.12	34.45±0.29	3.51
13	7.0	2.0	13.0	63.44±0.42	-13.03±0.11	35.01±0.53	3.05
14	10.0	2.0	10.0	55.33±1.46	-13.13±0.28	35.33±0.82	5.09
15	7.0	3.6	13.0	64.96±0.39	-12.53±0.12	34.26±0.29	4.57

Remark: The difference in lightness (ΔL*) was set against L* 60.39

Table 4.2 Results of Response Variables in 15 Matcha Butter Samples.

No	Matcha Powder (g)	Salt (g)	Sugar (g)	Sensory evaluations				
				Colour (%)	Appearance (%)	Taste (%)	Mouthfeel (%)	Overall acceptability (%)
1	7.0	3.6	7.0	74.67	71.56	79.56	73.78	75.56
2	10.0	3.6	10.0	70.22	68.89	75.11	68.89	69.78
3	7.0	2.8	10.0	76.00	73.33	72.44	73.33	72.89
4	7.0	2.8	10.0	70.67	69.78	73.33	69.78	70.67
5	4.0	2.0	10.0	65.33	67.11	67.56	72.89	72.44
6	10.0	2.8	7.0	69.78	67.11	68.89	69.33	67.11
7	4.0	2.8	7.0	72.00	70.67	70.22	72.00	75.56
8	4.0	2.8	13.0	71.56	71.11	68.44	74.67	77.33
9	7.0	2.8	10.0	71.11	70.67	74.67	64.89	66.67

Table 4.2 Continued Results of Response Variables in 15 Matcha Butter Samples.

No	Matcha Powder (g)	Salt (g)	Sugar (g)		Sensory evaluations				
					Colour (%)	Appearance (%)	Taste (%)	Mouthfeel (%)	Overall acceptability (%)
10	10.0	2.8	13.0		75.56	75.56	72.89	71.11	72.00
11	4.0	3.6	10.0		61.33	67.11	70.67	68.89	69.78
12	7.0	2.0	7.0		72.44	73.33	72.89	70.22	71.56
13	7.0	2.0	13.0		74.22	73.33	74.22	67.11	72.00
14	10.0	2.0	10.0		71.56	74.67	68.00	66.67	65.78
15	7.0	3.6	13.0		60.44	59.56	75.56	64.89	65.78
				General Mean	70.46	70.25	72.30	69.90	70.99

Results tabulated from Table 4.2 and Table 4.1 were analysed using ANOVA, analysis of variance in order to select the relevant responses by comparing their significances and tabulated in Table 4.3.

In Table 4.3, R^2 can be denoted by the coefficient of determination and it represented the properties of fit to the polynomial model equation. In this model, the R^2 was highest in "taste" by scoring 0.97. "Colour" with R^2 of 0.91 was ranked second and then followed by "differences in L*" with R^2 of 0.81, "appearance" with of 0.76, "overall acceptability" with R^2 of 0.57 and lastly "mouthfeel" with R^2 of 0.51.

Besides, "differences in L*", "colour" and "taste" were significantly different ($p<0.05$) thus indicating significant and good fitness to the model. On contrast, "appearance", "mouthfeel" and "overall acceptability" were found not having significantly different at 95 % ($p<0.05$). Moreover, the F-value in Lack of fit was highest in differences in ∆L* (0.74), followed by colour (0.60), overall acceptability (0.59), taste (0.50), mouthfeel (0.50) and lowest in appearance (0.17).

Based on regression coefficients shown in Table 4.3, differences in L* from colour analysis and colour (%), taste (%) from the sensory evaluation were chosen as influencing factors to establish the full quadratic models due to P value < 0.05, lack of fit > 0.5 and R^2 > 0.80.

Table 4.3. Regression Output from JMP Software of the Response Variable.

Regression Coefficients	Differences in L* (ΔL*)	Colour	Appearance	Taste	Mouthfeel	Overall acceptability
β_0	1.79	72.59*	71.26*	73.48*	69.33*	70.08*
β_a	-5.01*	4.26*	3.48*	1.54*	-0.19	-0.43
β_b	0.40	-2.1	-2.67	2.28*	-0.06	-0.11
β_c	1.49	-2.63*	-1.86	-0.35	-1.64	-1.10
β_{ab}	-0.47	0.67	-1.45	1.00	1.56	1.67
β_{ac}	-1.53	-2.73	-2.40	0.37	-3.0	-3.48
β_{bc}	0.86	-4.00*	-3.00	-1.33	-1.45	-2.56
β_{aa}	-1.77	-0.10	1.39	-4.00*	2.08	1.33
β_{bb}	1.19	-5.38*	-3.21	0.86	-2.08	-1.97
β_{cc}	0.21	3.23	1.39	1.12	1.75	3.11
R^2	0.81	0.91	0.76	0.97	0.51	0.57
Lack of fit	0.74*	0.60*	0.17*	0.50*	0.50*	0.59*

Remark: a,b,c represent matcha powder, salt and sugar respectively. * Represent there was statistically significant ($p<0.05$).

4.2 Optimization of Matcha Butter

Equation 4.1 Polynomial Regression Model Generated from the Design for Equation of Differences in L* (ΔL*)

$$Y = 1.79 - 5.01a + 0.40b + 1.49c - 0.47ab - 1.53ac + 0.86bc - 1.77aa + 1.19bb + 0.21cc$$

Equation 4.2 Polynomial Regression Model for Colour

$$Y = 72.59 + 4.26a - 2.10b - 2.63c - 0.67ab - 2.73ac - 4.00bc - 0.10aa - 5.38bb + 3.23cc$$

Equation 4.3 Polynomial Regression Model for Taste

$$Y = 73.48 + 1.54a + 2.28b - 0.35c + 1.00ab + 0.37ac - 1.33bc - 4.00aa + 0.86bb + 1.12cc$$

The optimized results for maximizing sensory scores and minimizing lightness difference were 10, 3.6 and 7 g respective to matcha powder, salt and sugar. The interactive effects between the simultaneous variables were shown in next page.

4.3 3D Response Surface Graph

4.3.1 3d Response Surface Graph on Colour's Sensory Score

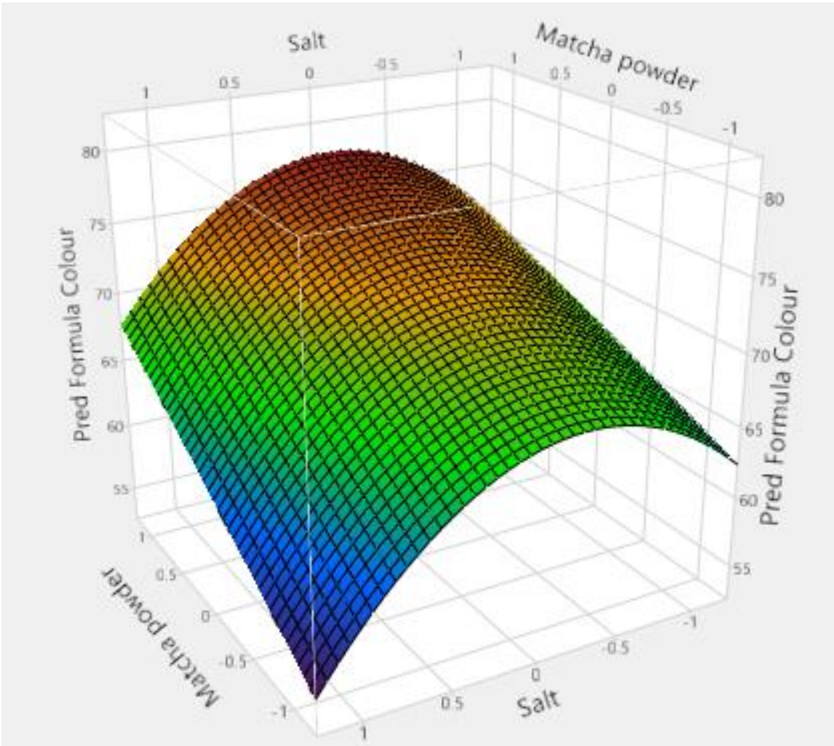


Figure 4.1 3D Response Surface Graph of the Effect of Matcha Powder and Salt on Colour Preference.

Based on Figure 4.1, orange region represented desirability. Sensory score of colour increased by increasing amount of matcha powder. Addition of salt at level 0 further increased the sensory score.

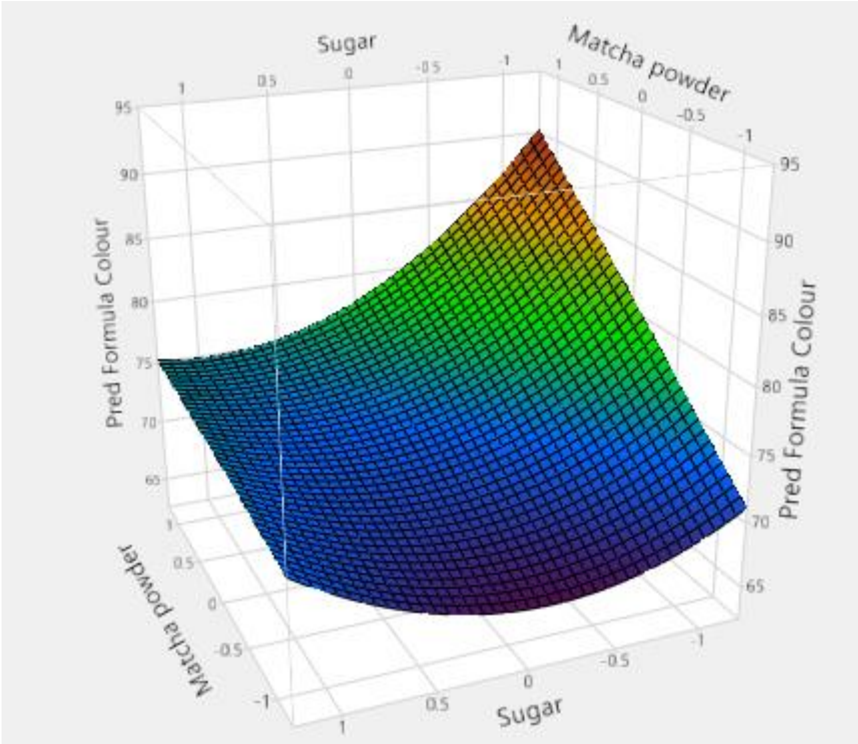


Figure 4.2 3D Response Surface Graph of the Effect of Matcha Powder and Sugar on Colour.

Based on Figure 4.2, orange region represented desirability. Sensory score of colour increased by increasing amount of matcha powder. Decreasing sugar level further increased the sensory score.

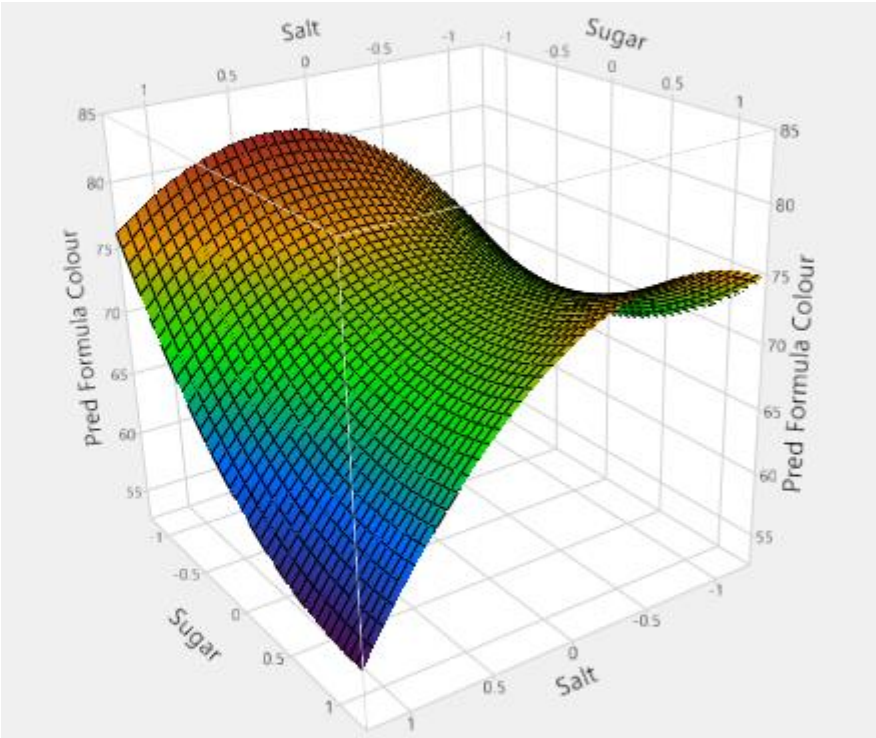


Figure 4.3 3D Response Surface Graph of the Effect of Sugar and Salt on Colour.

Based on Figure 4.3, orange region represented desirability. Sensory score of colour increased by reducing sugar level. Increasing salt level also boosted the sensory score. However, the sensory score dropped once the salt level exceeded a point.

4.3.2 3D Response Surface Graph On Taste's Sensory Score

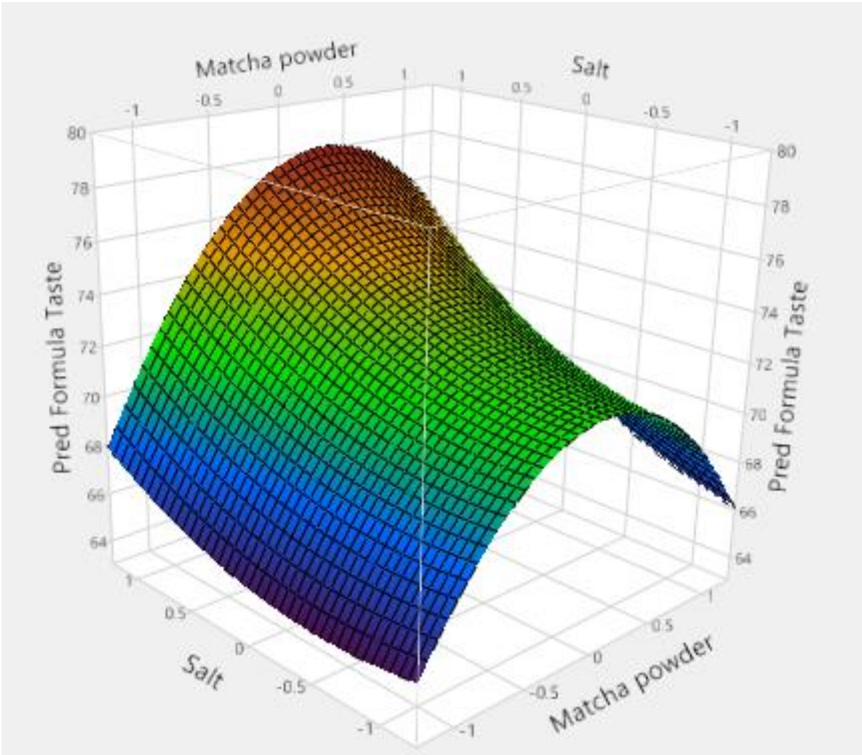


Figure 4.4 3D Response Surface Graph of the Effect of Matcha Powder and Salt on Taste.

Based on Figure 4.4, orange region represented desirability. Sensory score of taste increased by increasing salt level. Increasing matcha powder also raised the taste preference drastically. Yet, the taste preference dropped once matcha powder exceed a limit.

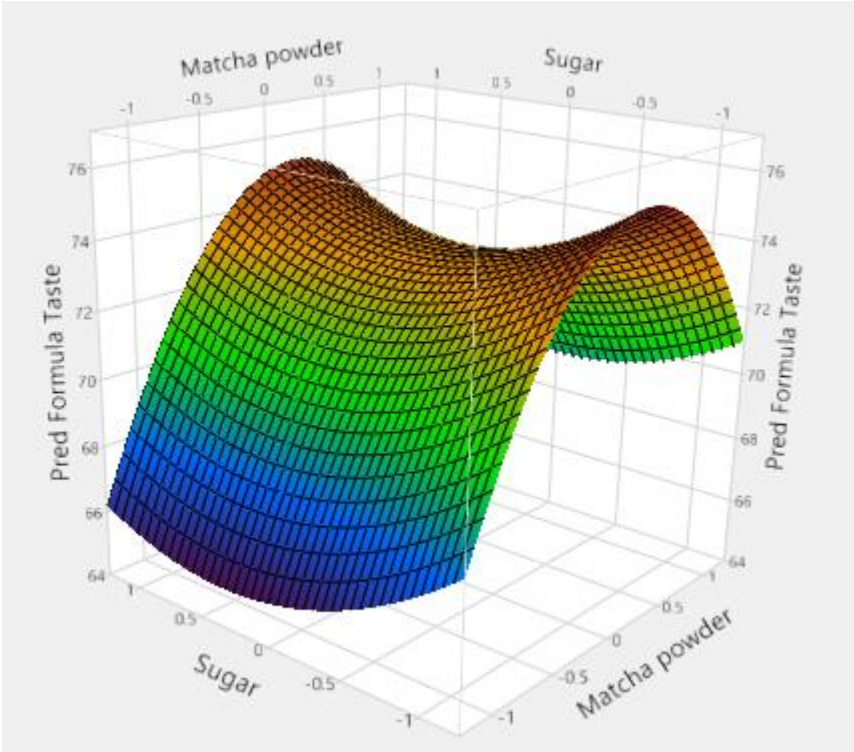


Figure 4.5 3D Response Surface Graph of the Effect of Matcha Powder and Sugar on Taste.

Based on Figure 4.5, orange region represented desirability. No matter how much sugar was added, the taste score was found highest when the amount of matcha powder added was in between level 0 to 0.5.

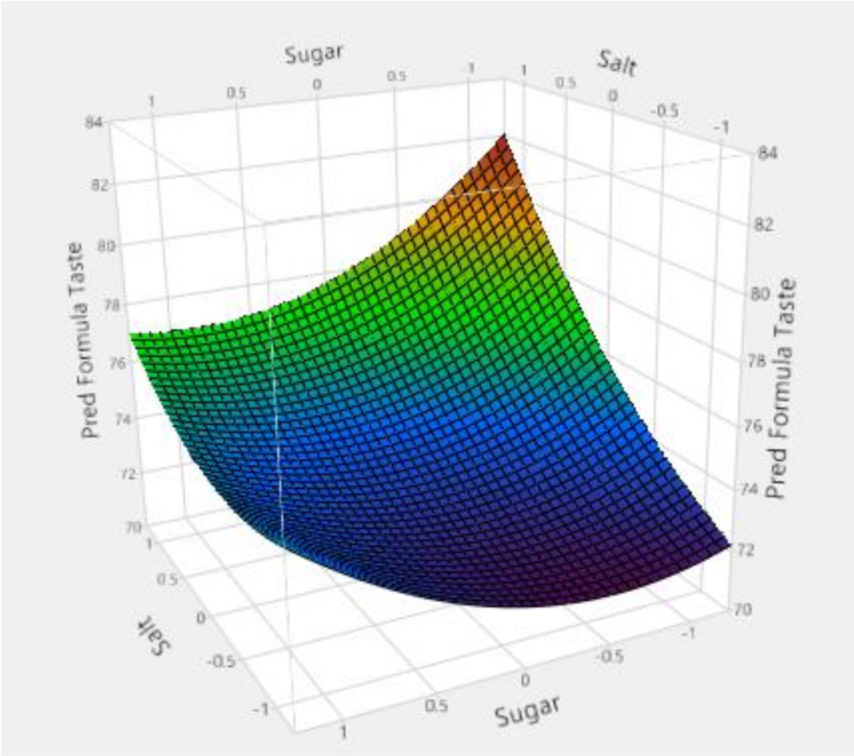


Figure 4.6 3D Response Surface Graph of the Effect of Salt and Sugar on Taste.

Based on Figure 4.6, orange region represented desirability. Sensory score of taste increased by increasing salt level. Reducing sugar level also increased the sensory score of taste.

4.3.3 3D Response Surface Graph on Different in Lightness ∆L

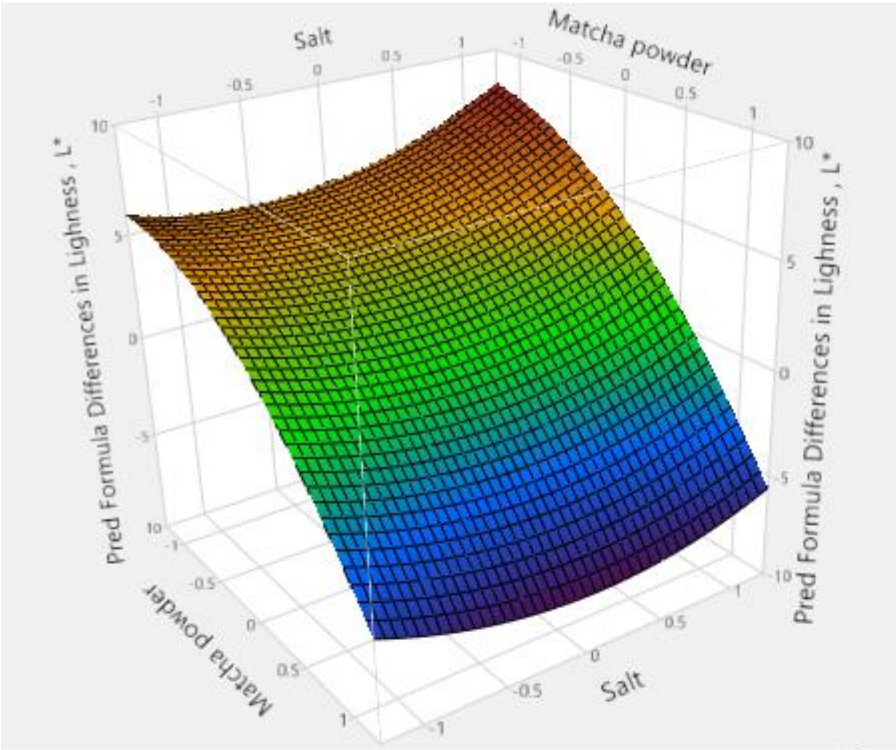


Figure 4.7 3D Response Surface Graph of the Effect of Matcha Powder and Salt on Difference in Lightness, ∆L*.

Based on Figure 4.7, orange region represented desirability. The difference in lightness, ∆L* decreased by reducing matcha powder. Addition of salt did not cause much changes to the lightness of matcha butter.

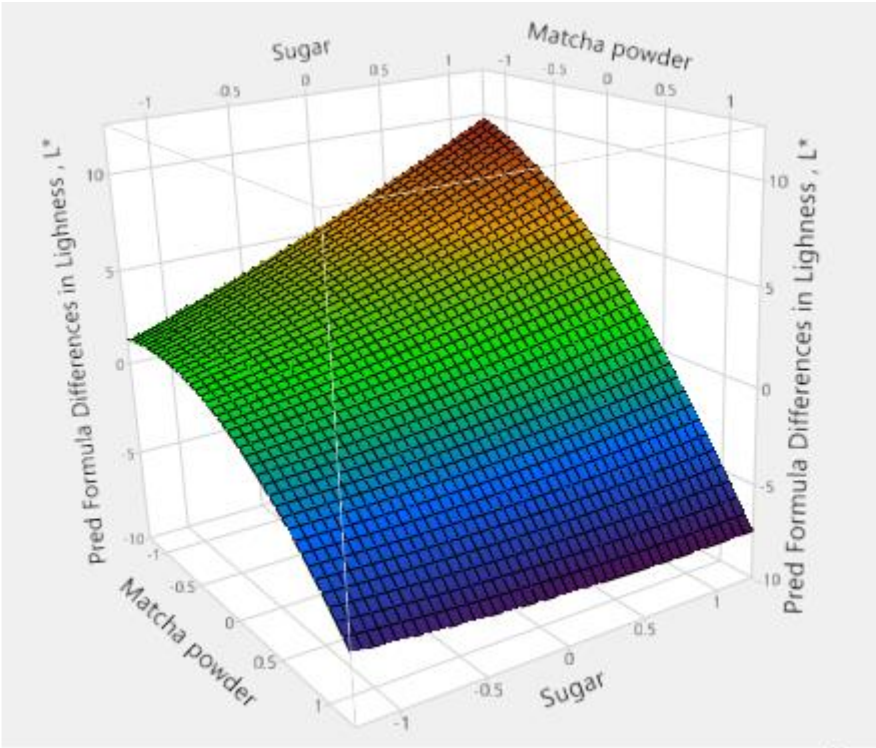


Figure 4.8 3D Response Surface Graph of the Effect of Matcha Powder and Sugar on Difference in Lightness, ∆ L*.

Based on the Figure, orange region represented desirability. The difference in lightness, ∆L* decreased by reducing matcha powder. Increasing sugar level also helps in reducing the difference in lightness, L*.

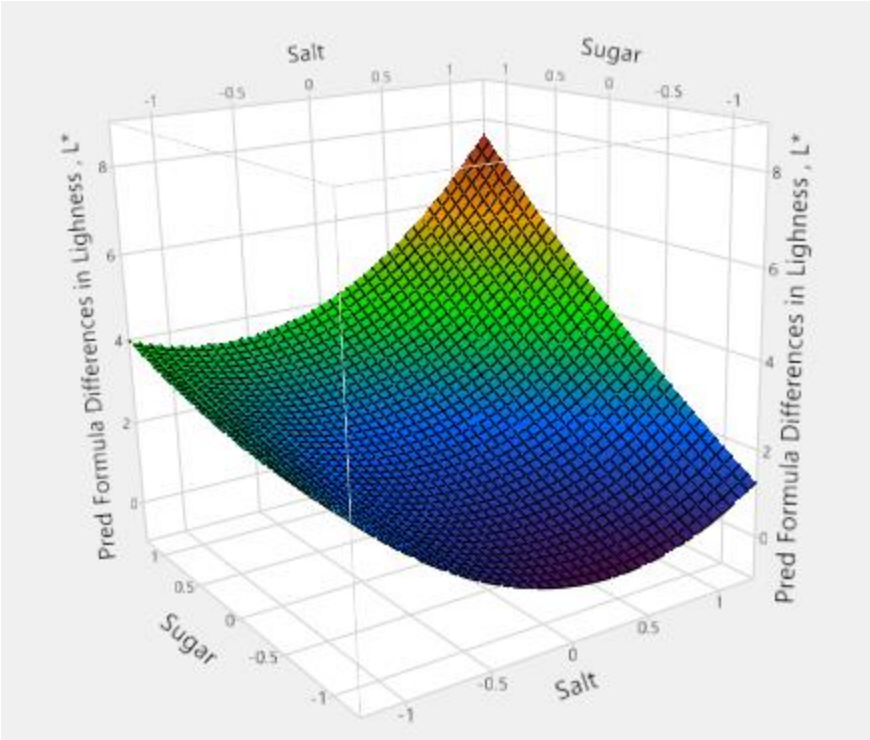


Figure 4.9 3D Response Surface Graphs of the Effect of Salt and Sugar on Difference in Lightness, ΔL*.

Based on the figure, orange region represented desirability. The difference in lightness, ΔL* decreased by increasing both sugar and salt levels.

4.4 Determination of Moisture Content and Fat Content on Optimized Matcha Butter

Based on Table 4.4, the moisture content of optimized matcha butter was 12.42 ± 0.035 %. Besides, the fat content of optimized matcha butter was 78.12 ± 0.75 %.

Table 4.4 Mositure Content and Fat Content of the Optimized Matcha Butter**.**

Moisture content (%)	12.42 ± 0.04
Fat content (%)	78.12 ± 0.75

4.5 Temperature Sweep on Optimized Matcha Butter

Table 4.5 Means of Storage Modulus Logarithms (G'), Loss Modulus Logarithms (G'') of Optimized Matcha Butter at Different Temperatures.

Temperature (℃)	Log G' (Pa)	Log G'' (Pa)
4.98	5.76	5.45
9.99	6.28	5.85
14.99	4.93	4.73
20.00	3.21	3.10
25.00	3.12	2.96
30.00	2.85	2.64
35.01	1.45	1.32
40.01	0.50	0.37
40.00	0.45	0.27
34.97	0.62	0.38
29.98	0.74	0.46
24.98	0.89	0.56
19.96	1.14	0.77
14.99	3.92	3.38
9.99	5.59	5.01
4.98	5.64	5.02

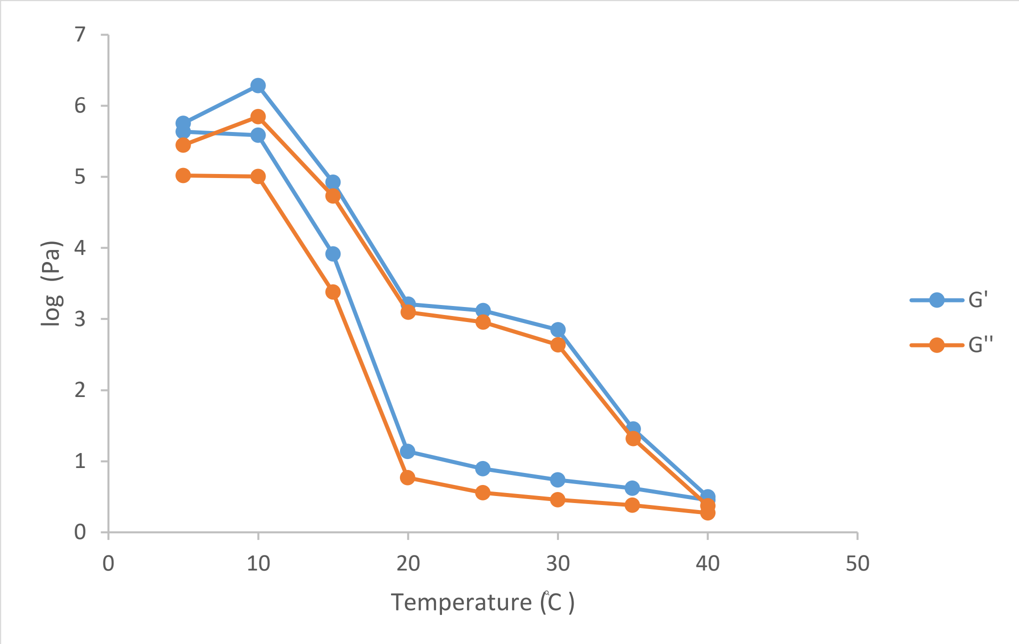


Figure 4.10 Graph of Temperature Sweep of the Optimized Matcha Butter

Based on Figure 4.10, log G’(storage modulus) and log G’’(loss modulus) were plotted as temperatures changed from 5 to 40 ̊C then back to 5 ̊C. Throughout the graph, the values of log G’ were always higher log G’’. Besides, both the G’ and G’’ were highest at 10 ̊C and lowest at 40 ̊C.

CHAPTER 5

DISCUSSION

5.1 Formula Optimization

Often, R^2 with value more than 0.8 signified a good fit model (Lu, et al, 2017). In this model, the R^2 was highest in “taste” by scoring 0.97. This showed that the model could not explain 3% of the total variation. Yet, it represents a good fitness to the model. “Colour” with the R^2 of 0.91, indicating 9 % of total variation was not able to be explained by the model. Then it was followed by “differences in L*” with R^2 of 0.81 showing 19 % of total variation were not explainable. R^2 below 0.8 indicated unfit and will not be taken into consideration.

Besides, “differences in L*”, “colour” and “taste” were found significantly different at 95 % ($p<0.05$) thus indicating significant and good fitness to the model. On contrast, “appearance”, “mouthfeel” and “overall acceptability” were found not having significantly different at 95 % ($p<0.05$).

Moreover, the F-value in lack of fit was highest in differences in L* (0.74), followed by colour (0.60), overall acceptability (0.59), taste (0.50), mouthfeel (0.50) and lowest in appearance (0.17). High F-value in lack of fit represented insignificant to pure error. When the F value of lack of fit was 0.74, there was no significant relation to pure error, as there was only 0.26 chance that the F-value can be resulted due to noises (Lu, et al, 2017).

Based on Regression Coefficients shown in Table 4.3, differences in L* from colour analysis and colour (%), taste (%) from the sensory evaluation were chosen as influencing factors to establish the full quadratic models due to P value < 0.05, lack of fit > 0.05 and $R^2 > 0.80$.

5.2 Single Factor Evaluation

5.2.1 Influence of the Added Amount of Matcha Powder

The influences of matcha powder added into matcha butter were shown in Figure 4.1 to Figure 4.9. From sensory evaluation, sensory scores of colour were high as the amount of matcha powder added increased. Next, the sensory scores of taste increased as the amount of matcha powder added increased, until an extend. Sensory score of taste were found to be highest when the matcha powder added at level 0 to 0.5. Excess amount of matcha powder added resulted in lower sensory score of taste. In addition, difference in lightness, ΔL^* was optimized as the amount of matcha powder added decreased. In conclusion, the optimum amount for matcha powder added was 10 g (Level + 1) to freshly churned butter.

5.2.2 Influence of the Added Amount of Salt

The influences of salt added into matcha butter were shown in Figure 4.1 to Figure 4.9. Sensory scores for colour was found highest when the salt was added at level 0. According to Hoecker (1941) when salt was added, butter milk remaining in milk fat globule attached itself to surface and resulted in lighter colour. As result, ligher colour of matcha butter increased the sensory scores of colour. Next, the sensory scores of taste increased by increasing amount of salt added. In addition, difference in lightness, ΔL^* was optimized as amount of salt added increased, due to the explanation above. As result, the optimum amount for salt added was 3.6 g (Level + 1) freshly churned butter.

5.2.3 Influence of the Added Amount of Sugar

The influences of sugar added into matcha butter were shown in Figure 4.1 to Figure 4.9. Sensory scores of colour were high as the amount of sugar added decreased. When sugar was added to matcha butter and mixed, the resulting matcha butter posed lighter colour. This was a common practice by mixing butter with sugar in many bakeries. However, there was no supporting evident to explain this trend. Next, sensory scores of taste increased by increasing amount of sugar added. In addition, difference in lightness, ΔL^* was optimized when large amount of sugar was added. Conclusively, the optimum amount for sugar added was 7 g (Level - 1) to freshly churned butter.

5.3 Determination of Moisture Content on Optimized Matcha Butter

Moisture content can be closely associated with the quality of butter (Ronholt, et al., 2012). Hence, test for moisture content is crucial during butter production (Ranken, Kill and Baker, 1997). According to the Codex Alimentarius (2011), butter shall contain no more than 16 % water. As result, most butter manufacturers tend to increase the moisture content of butter near to 16 % in order to maximize yield (Ranken, Kill and Baker, 1997).

The moisture content of butter must be well controlled. Since butter is a crystal network of fat globules, water, and fat crystals. Increasing water content in the butter changed the ratio of solid and liquid toward more liquid (Ronholt, et al., 2012). Consequently, the number of contact points between fat crystals decreased, fat crystal network is weakened (Pedersen et al, 2012). Hence, water droplet stability and product firmness were greatly reduced (Ronholt, et al., 2012).

By referring to Table 4.4, the moisture content of optimized matcha butter was 12.42 ± 0.035 %. The moisture content obtained was complied with General Standard for the Use of Dairy Terms and the General Standard for the Labelling of Pre-packaged Foods (Codex Alimentarius, 2011).

5.4 Determination of Fat Content on Optimized Matcha Butter

Based on Table 4.4, fat content of optimized matcha butter obtained was 78.12 ± 0.75%. According to Codex Alimentarius (2011), a butter shall contain minimum 80 % of milkfat. Since the fat content was lower than 80 %, it can be categorized as light butter (Frede, 2002).

In commercial butter production, cream was pasteurized, aged and churned at fixed temperature (Ranken, 1993). The processing temperatures cannot be well defined as they were varied from every author. However, it was found that inflation of cream's temperature during churning resulted in lower fat content of butter (Ranken, 1993). This evident explained the low fat content matcha butter obtained. Referring Chapter 3.2, the cream was churned for total 20 minutes to obtain butter. It was believed that, heat from surrounding and friction of the agitator raised the temperature of cream during churning. As result, fat content of butters produced were not up to 80 %.

In addition, commercial butter producers tend to insert proforated line into the churning machine to assist churning process (Ranken, 1993). Lacking of the machinery also contributed to the lower fat content in matcha butter.

5.5 Temperature Sweep on Optimized Matcha Butter

Temperature sweep is a common technique that used to study the melting ability of butter and other fats spread (Kanna, 2003). Often, storage modulus (G') and loss modulus (G'') were used to reflect the stress responds of an object. G' can be defined as the measurement of the capability of an object to store energy. On contrast, G'' is the measurement on the capability of a material to deplete mechanical energy in the form of heat using molecular motion. (Mark and Borch, 2002). The reading of G' and G'' shown were present in the form of log to emulate the format of another journal (Vithanage, Grimson and Smith, 2009).

By referring to Figure 4.10, all the G' were greater than G'', which signified a gel behavior (Emadzadeh, et al., 2013). When the temperature was increased from 5 ℃ to 10 ℃, the storage and loss modulus increased slightly, signaling a solid like behavior of matcha butter at that temperature (Vithanage, Grimson and Smith, 2009). This phenomenon was also reported by Mortensen, Knudsen, and Ronholt in 2013 where the hardness of butter increased when the temperature was raised from 8 ℃ to 15 ℃.

However, as temperature increased further, log G' and log G'' dropped sharply. According to Ronholt and other (2012), rising of temperature can greatly reduce the hardness of butter. Melting process started from the smaller fraction of fat crystals then followed by larger fat crystals (Mortensen, Knudsen, and Ronholt, 2013). During melting, fats globules were degraded and water molecules coalescence happened (Ronholt, et al., 2012). Thus, the microstructure was changed and resulted in low G’ and G’’.

Thousand species of triacylglycerol can be easily found in milk fat. Every type of triacylglycerol was characterized with different melting temperature. Therefore, the melting temperature of milk fat was ranged between -40 to 40℃ (Wringht, et al., 2001). Based on the graph of temperature sweep, the optimized matcha butter showed melting behavior in the whole system at 18℃.

Following that was a diatonic decrease of the log G’ and log G’’ values as temperature raised to 40 ℃. At 40 ℃, matcha butter turned and behaved like liquid form as the value of log G’ and log G’’ were near to 0 (Vithanage, Grimson and Smith, 2009).

During cooling, the values of log G’ and log G’’ increased in monotonic pattern until it reached 24℃. As cooling continued, log G’ and log G’’ dropped slightly and followed by sharp raising when the sample was cooled to 10℃. When the temperature dropped, crystallization happened as the remaining crystals on plate absorbed the surrounding liquid fats. As the process continued, larger crystals grew and resulted in interlink of the fat crystals. Consequently, storage and loss modulus were restored due to the formation of primary bonds (Mortensen, Knudsen, and Ronholt, 2013).

It was found that the reading of log G’ and log G’’ were slightly higher in the heating process if compared to the cooling process. This data can be explained by the different forms of microstructure subjected by the cooling rate (Mortensen, Knudsen, and Ronholt, 2013). Fast cooling caused a fusion of different triacylglycerols within the crystal structure and encouraged the formation of α crystal (Campos, Narine and Marangoni, 2002). As result, soft and unstable butter was produced, contributing to lower storage and loss modulus (Pedersen, et al., 2012).

5.6 Future Recommendation

In this study, optimised matcha butter was made and tested for the moisture content, fat content and meltability. In order to understand more about the microstructure of matcha butter, freeze fracture microscopy imaging is recommended to capture the fat crystals and the water in oil suspension network. Besides, frequency oscillation test can be carried out to determine the spreadability of butter. In addition, volatile compounds in the matcha butter can be identified by using gas chromatography mass spectrometry. Next, shelf life testing can be carried out to evaluate the shelf life of matcha butter.

CHAPTER 6

CONCLUSION

In order to make a matcha butter with maximum sensory scores, and minimum lightness difference. All the data (sensory scores for each attributes and different in lightness) were keyed in into the JMP software to examine their regression coefficients. Different in lightness, taste and colour's sensory scores were found to have strong interaction by having statistical significant at ($p<0.05$), lack of fit > 0.5 and $R^2 > 0.80$. Thus they were chosen to establish the full quadratic models. As result, the optimized formula was obtained by adding 10 g matcha powder, 3.6 g salt and 7 g sugar to 200 g of freshly churned butter with 0.87 desirability. The influences of matcha powder, salt and sugar to taste sensory scores, colour sensory scores and different in lightness were studied using 3 dimensional respond surface graphs.

For profiling purpose, moisture content and fat content test were on the optimized matcha butter. As result, moisture content was 12.42 % ± 0.04 and fat content was 78.12% ± 0.75. Moisture content level was found complying with Malaysia Food Regulations 1985 by not exceeding 16 % moisture. However, fat content was found not complying with Malaysia Food Regulations 1985 as the fat content was lower than the standard 80 %. Yet, it still can be categorized as light butter.

When subjected to temperature sweep test, butter was heated from 5℃ to 40℃ and cooled down to 5℃. Storage and loss modulus showed elastic behaviour and indicated overall melting behaviour of optimized matcha butter at 18℃.

REFERENCE

Auxter, J., 2015. *Concious Afternoon Tea*. Bloomington: Balbao Press

Ayhan, T., Cüneyt, D., Mehmet, T., İsmail, T., ŞAHİN, N., Ayhan, H., Feramuz, O., 2014. Turkish Journal of Agriculture and *Forestry. Physicochemical properties of Turkish green tea powder: effects of shooting period, shading, and clone*, 38(2), pp 233-241.

Bezerra, M.A., Santelli, R.E., Oliveira, E.P., Villar, L.S., Escaleira, L.S., 2008. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Tala nta*, *76*(5), pp. 965-977.

Boskisch, M., 1998. *Fats and oil handbook*. Hamburg: AOCS Press.

Buldo, P., 2012. *Crystallization of fat in and outside milk fat globules -Effect of processing and storage conditions.* PhD. Aarhus University.

Campos, R., Narine, S.S., and Marangoni, A.G., 2002. Effect of cooling rate on the structure and mechanical properties of milk fat and lard. *Food research international*, 35(10), pp. 971-981.

Cavazzuti, M., 2013. *Optimization Method: From Theory to Design*. Modena: Springer.

Cheadle, L. N., 2016. *The Book of Matcha*. London: Jacqui Small LLP.

Codex Alimentarius, 2011. *Milk and Milk Products*. [online] Available at :< http://www.fao.org/docrep/015/i2085e/i2085e00.pdf> [Accessed 6th August 2017].

Davey, R.J. and Garside, J., 2000. *From molecules to crystallizers: an introduction to crystallization*. 1st edition, Oxford: Univ. Press.

Deosakar, S.S., Khedkar, C.D., Kalyankar, S.S., 2016. *The Encyclopedia of Food and Health*. [e-book] Oxford: Elsevier. Available through: Google Books: <https://books.google.com.my/books?id=Ot9BAAAQBAJ&printsec=frontcover&dq=encyclopedia+of+food+and+health+2016&hl=en&sa=X&redir_esc=y#v=onepage&q=encyclopedia%20of%20food%20and%20health%202016&f=false> [Accessed 13th June 2017].

Dulloo, A.G., Duret, C., Rohere,D., Girardier, L., Mensi, N., Fathi, M., Chantre, P., Vandermander, J., 1999. Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans1,2,3. *American Journal of Nutrition,* 70(6), pp.1040-1045.

Emadzadeh, B., Razavi, S.M.A., Schleining, G., 2013. Dynamic Rheological and Textural Characteristic Of Low-Calorie Pistachio Butter. *International Journal of Food Properties,* 16(3), pp. 512-526.

Frede, R., 2002. Butter and Other Milk Fat Products | Properties and Analysis Encyclopedia of Dairy Sciences (Second Edition), 1, pp. 227-236.

Global Food Forum, 2017. *2017 Food trend*. [online] Available at: <https://www.globalfoodforums.com/food-news-bites/2017-food-trends/> [Accessed 13th June 2017].

Guarley, M. and Marangoni., 2005. *Physical Properties of Lipid*. New York: Marcel Dekker.

Helweg, R., 2010. *The Complete Guide To Making Cheese, Butter and Yugurt At Home: Everything You Need to Know Explained Simply.* [e-book] Florida: Atlantic Publishing. Available through: Google Book < https://books.google.com.my/books?id=FoeoctrDYC&printsec=frontcover&dq=butter+make&hl=en&sa=X&redir_esc=y#v=onepage&q=butter%20make&f=false > [Accessed 6th June 2017].

Herrera, M.L. and Hartel, R.W., 2000. Effect of processing conditions on physical properties of a milk fat model system. *Journal of the American Oil Chemists' Society*, 77(11), pp. 1177–1188.

Hoecker. W.H., 1941. *Distribution of salt in butter and its effect on bacterial action*. PhD. Iowa State University.

Juriaanse, A.C. and Heertje, I., 1988. Microstructure of Shortening, Margarine and Butter- A Review. *Food Microstructure Journal*, 7(2), pp.181-188.

Kanna, B.M.M., 2003. *Texture in Food: Semi-Solid Food.* [e-book] Cambridge: Woodhead. Available through: Google Book :<https://books.google.com.my/books?id=4xOkAgAAQBAJ&pg=PA54&dq=temperature+sweep+rheology+butter&hl=en&sa=X&redir_esc=y#v=onepage&q=temperature%20sweep%20rheology%20butter&f=false>[Accessed 6th August 2017].

Katiyar, S.K., 2011. Green tea prevent non melanoma skin cancer by DNA repair. *PubMed,* [e-journal] 508(2), pp. 152-158. Available through google website< https://www.ncbi.nlm.nih.gov/pubmed/21094124 > [Accessed 22nd July 2017].

Kementerian Kesihatan Malaysia, 2014. *LAWS OF MALAYSIA P.U.(A) 437 OF 1985 FOOD ACT 1983 FOOD REGULATIONS 1985.* [online] Available at: < http://fsq.moh.gov.my/v5/ms/103-butter/ > [Accessed 21st January 2017].

Khuri, A.I., Mukhopadhyay, S., 2010. Response surface methodology. *WIREs Comp Stat*, 2(2), pp. 128-149.

Kloek, W., Vliet, T. and Walstra, P., 2005. Mechanical properties of fat dispersions prepared in a mechanical crystallizer. *Journal of Texture Studies*, 36(5), pp. 544–568.

Konica Minolta, 2017. *Identifying Color Differences Using L*a*b* or L*C*H* Coordinates.* [Online] Available at: < http://sensing.konicami nolta.us/2014/04/ identifying-color-dif ferences-using-l-a-b-or-l-c-h-coordinates/> [Accessed 23th July 2017]

Lawless, H.T., Heymann, H., 1999. *Sensory evaluation of food.* New York: Springer.

Lu, H.S., Lv, C.L., Zhang, M.H., Liu, S.Y., Liu, J.T., Lian, F., 2017. Optimization of hydrothermal pre-treatment for co-utilization C-5 and C-6 sugars of cassava alcohol residue. *Energy Conversion and Management*. 132, pp. 251-260.

Mahony, F.O., 1988. *Rural Diary Technology*. Ethiopia: Addis Ababa.

Manohar, M., Joseph, J., Selvaraj, T., Sivakumar, D., 2013. Application of Box Behnken design to optimize the parameters for turning Inconel 718 using coated carbide tools. *International Journal of Scientific & Engineering Research*, 4(4), pp. 620- 639.

Marangoni, A., 2016. *Structure and Mechanical Properties of Fat Crystal Networks.* [online] Available at: <http://www.oleosegorduras.org.br/site/assets/arquivo/0c3b7f4b8b4416828b12676e9a8537b7.pdf> [Accessed 21st January 2017].

Mark, R.E., Borch, J., 2002. *Handbook of Physical Testing of Paper, Volume 1.* [e-book] New York: Marcel Dekker. Available through: Google Book :< https://books.google.com.my/books?id=Wh_KqCXPn3gC&pg=PA86&dq=storage+modulus+and+loss+modulus&hl=en&sa=X&redir_esc=y#v=onepage&qstorage%20modulus%20and%20loss%20modulus&f=false>[Accessed 6th August 2017].

Market research future, 2016. *Global Matcha Tea Market Information- by type (caffeinated, decaffeinated), by form (powder, tea bags, loose leaf, and others), by formulation (flavored and unflavored), by distribution channel (supermarket, specialty stores, e-commerce, retail stores and others) and by Region Matcha tea Forecast to 2022*. [online] Available at<https:/www.marketresearchfuture.com/reports/matcha-tea-market-2382> [Accessed 13th July 2017].

Marrero, R.M., 2017. *Butter: Microstructure, Raw Material and Manufacturing*. [online]. Available at :< https://prezi.com/wjs6toixea0-/butter-microstructure-raw-materials-and-manufacturing/> [Accessed 23th June 2017].

Mortensen, K., Knudsen, J.C., Ronholt, S., 2013. The Effective Factors on the Structure of Butter and Other Milk Fat-Based Products. *Comprehensive Reviews in Food Science and Food Safety*. [e-journal] 12(5), pp. 468–482. Available through Google Website < http://onlinelibrary.wiley.com/d oi/10.1111/15414 337.12022/full>[Accessed 6th August 2017].

Mustafa, A.T., Hülya, B., Berrin, O., Yasemin, G., 2012. Box- Behnken Experimental Design In Factorial Experiments: The Importance Of Bread For Nutrition And Health. *Turkish Journal of Field Crops*, 17(2), pp. 115-123.

OECD Agricultural Outlook, 2017. *OECD-FAO Agricultural Outlook 2016-2025.* [online] Available at: <http://stats.oecd.org/Index.aspx?datasetcode=HIGH _AGLINK_2016# > [Accessed 21st January 2017].

Oklahoma. A., 2006. *The Chemistry of Butter.* [online]. Available at:< http://www.clover.okstate.edu/fourh/aitc/lessons/butter.pdf> [[Accessed 21st June 2017.

Pedersen, T.B., Ronholt, S., Kirkensgaard, J.J.K., Mortensen, K., Knudsen,J.C. 2012. Polymorphism, microstructure and rheology of butter. Effect of cream heat treatment. *Food Chemistry*, 135(3), pp. 1730-1739.

Potter, N.N. and Hotchkiss, J.H., 1998. *Food Science. 5th Edition*. New York: Springer.

Qiu, P.P., Cui, M.C., Kang, K.L., Park, B., Son, Y.Y., Khim, E.Y., Min, J., Khim, J.H., 2014. Application of Box–Behnken design with response surface methodology for modeling and optimizing ultrasonic oxidation of arsenite with H2O2. Central European Journal of Chemistry, 12(2), pp. 164-172.

Ranken, M.D., 1993. *Food Industry Manual*. [e-book] New York: Springer. Available through: Google Book :< https://books.google.com.my/books?id=Ca_SBwAAQBAJ&pg=PA81&dq=butter+with+low+fat+content&hl=en&sa=X&redir_esc=y#v=onepage&q=butter%20with%20low%20fat%20content&f=false>[Accessed 6th August 2017].

Ranken, M.D., Kill, R.C., Baker, C.G.J., 1997. *Food Industries Manual*. [e-book] Wantage: Blakie Academy. Available through: Google Book :< https://books.google.com.my/books?id=VM3gBwAAQBAJ&pg=PA128&dq=butter+moisture+content&hl=en&sa=X&redir_esc=y#v=onepage&q=butter%20moisture%20content&f=false> [Accessed 6th August 2017].

Ronholt, S., Kirkensgaard, J.J.K., Mortensen, K., Knudsen, J.C., 2012. Effect of cream cooling rate and water content on butter microstructure during four weeks of storage. *Food Hydrocolloids,* 34 pp. 169-176.

Sahin, S., Sumnu, S.G., 2006. *Physical properties of food*. United State: Springer.

Smith, K., 2016. World Atlas of Tea. London: Octopus Publishing.

Snyder, M., Clum.L., Zulaica, A.V, 2015. *The Matcha MIRACLE Boost Energy, Focus and Health with Green Tea Powder.* [e-book] Berkeley: Ulysses Press. Available through: Google Book < https://books.google.com.my/books?id=EOZpCgAAQBAJ&printsec=frontcover&dq=matcha+benefit&hl=en&sa=X&redir_esc=y#v=onepage&q=matcha%20benefit&f=false > [Accessed 21st January 2017].

Tetra, 2007. *Crystallization Temperature.* [online]. Available at < http://solutionsguide.tetratec.com/index.asp?Page_ID=734&AQ_Magazine_Date=Current&AQ_Magazine_ID=2238> [Accessed 25th June 2017].

Timms,R.M., 2007. *Fat Crystallisation: mechanism and methods for studying*. [online]. Available at < file:///C:/Users/User/Downloads/RalphTimms %20(5). Pdf> [Accessed 25th June 2017].

Towler, S., 2010. Cha Dao: The Way of Tea, Tea as a Way of Life. London: Singing Dragon.

Vithanage, C.R., Grimson, M., Smith, B., 2009. The effect of temperature on the rheology of butter, a spreadable blend and spreads. *Journal of Texture Studies*, [e-journal] 40(3), pp. 346-369. Available through Google Website <https://researchspace.auckland.ac.nz/handle/2292/18376>[Accessed 6thAugust 2017].

Wesdorp, L.H., Marangoni, A.G., 2013. *Structure and properties of fat crystal network.* Second edition. Boca Raton, FL: CRC Press/Taylor & Francis.

Wiking, L., Graef, D.V., Rasmussen, M. and Dewettinck, K., 2009. Relations between crystallisation mechanisms and microstructure of milk fat. *International Dairy Journal.* 19(8), pp. 424-430.

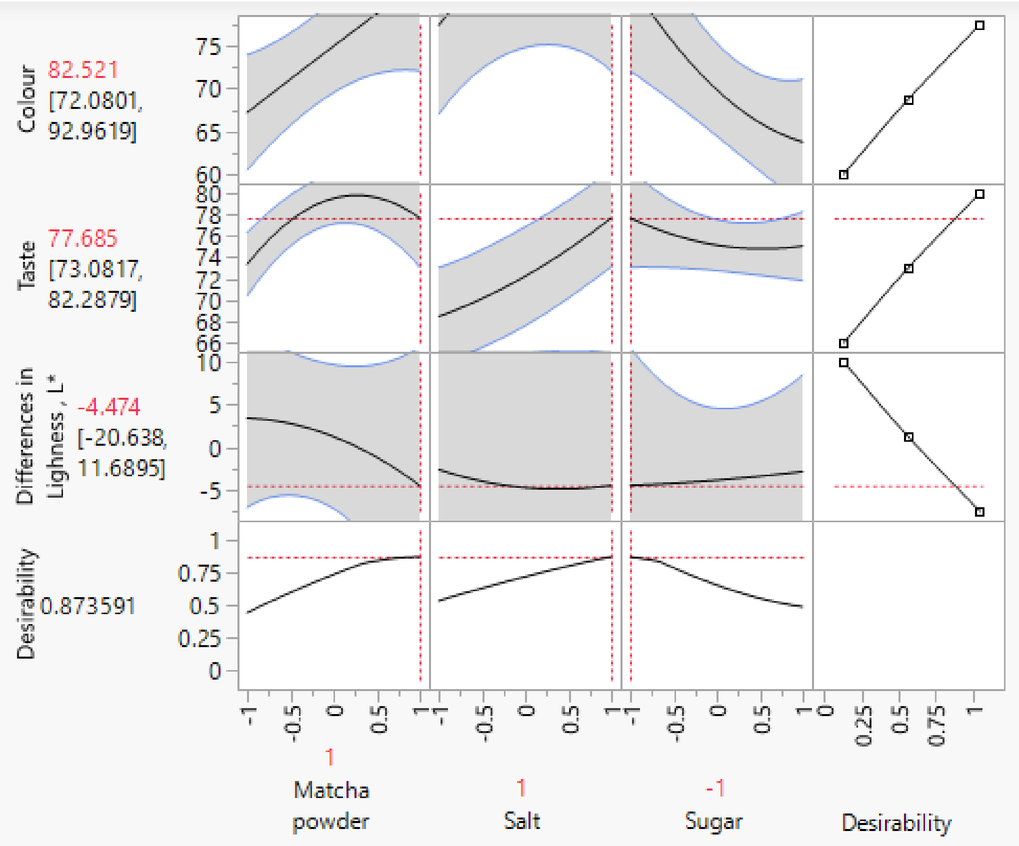
World Heart Federation, 2017. *Risk Factors*. [online] Available at:< https://www.world-heart-federation.org/resources/risk-factors/>[Accessed 12nd July 2017].

Wringht, A.J., Marangoni, A.G., Hartel, R.W., Scanion, M.G., 2001. Rheological Properties of Milkfat and Butter. *Journal of Food Science.* [e-journal] 66(8), pp. 1056–1071. Available through Google Website < http://onlinelibrary.wiley.com/doi/10.1111/j.13652621.2001.tb16082.x/abstract >[Accessed 6th August 2017].

Zheng, X.X, Xu, T.L, Shao, H.L., Xu, X,L., Rutai, H., Xiao, H.H., 2011. Green tea intake lower fasting serum total LDL cholesterol in adults: a meta-analysis of 14 randomized controlled trials. *American Journal of Nutrition.* 94(2), pp. 601-610.

APPENDIX A

OUTPUT OF RESPDONS SURFACE METHODOLOGY



APPENDIX B

QUESTIONNAIRE FOR HEDONIC SCALING TEST

Panel No.	:	Date	:
Product	: **Matcha Butter**	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:

Thank you.