PREPARATION OF LIPSTICK WITH RED DRAON FRUIT (*HOLYCEREUS POLYRHIZUS*) PIGMENT AND STORAGE STABILITY STUDY

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A project report submitted in partial fulfilment of the requirements for the award of Bachelor of Engineering (Honours) Chemical Engineering

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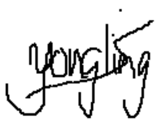
April 2020

DECLARATION

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

Signature : 

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APPROVAL FOR SUBMISSION

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ACKNOWLEDGEMENTS

I am extremely grateful for the overwhelming and continuous support received throughout this research. First and foremost, I would like to express my deepest gratitude to my research advisor, Dr. Woo Kwan Kit for giving me the opportunity to be a part of the research and also her invaluable advice, guidance and enormous patience throughout the development of the research. Without her unwavering encouragement and positivity, the unique idea that sparked into this creative and challenging research project would never have been possible. In addition, I would like to thank the lab officers for never-ending support and help in laboratory. Lastly, I am eternally grateful to my friends and family for always offering word of encouragement when I needed.

ABSTRACT

Synthetic dyes were mostly utilised in the production of commercial lipsticks. However, the usage of synthetic dye has been associated with many health problems. Hence, natural colorant might offer a promising solution to this problem. The betalains which are found in red dragon fruits (*Holycereus polyrhizus*) have drawn attention as a potential source of natural colorant to synthetic dye. However, the application of natural colorant in corresponding filed is limited due to its low stability towards light, temperature, oxygen, pH and colour deterioration across storage. The purpose of this study was to formulate lipsticks with encapsulated betalains and evaluate its feasibility as natural colorant in lipstick formulation and study its stability across one-month storage period. In this research, betalains was extracted from flesh of red dragon fruit using ethanol at ratio of 1: 3 and the concentrated betalains extract were microencapsulated with different proportion of maltodextrin (DE-10) and spray dried at 198 °C (concentrated betalains + 30 % maltodextrin) or freeze dried at -110 °C (concentrated betalains + 50 % of maltodextrin) into betalains pigment. The encapsulated betalains pigment were then used to formulate lipsticks with basic lipstick formula and the formulated lipsticks were store under light or dark condition for one-month storage period for stability study. The physico-chemical properties of the synthesised lipstick such as spreadability, skin irritation test, breaking point, surface anomalies, melting point, perfume stability, homogeneity and colour uniformity were determined and compared with commercial lipsticks. The functional group presence in betalains were identified by the Fourier Transform-infrared Spectroscopy (FT-IR). The stability of the synthesised lipstick found to be stable under dark condition while color loss was greater for lipstick in light condition. Although betalains in freeze dried lipstick stabilized better than spray dried lipsticks, spray dried lipstick achieved closer standard to commercial lipstick. However, due to the low pigmentation from both method, betalains is suggested incorporate in to lip balm application. This study has proven that betalains could replace synthetic dye in cosmetics industry for lip balm application.

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CHAPTER 1

INTRODUCTION

1.1 General Introduction

Nowadays, cosmetics products are essential in daily life and widely used by women to enhance their appearance. Lipstick is a cosmetics product that is applied on lips to give colour to the lips and protect it from drying or cracking. In the current cosmetics market, lipsticks are incredible in demand as it enhanced the aesthetic of make up by colouring the lips. There are hundreds of shade developed for lipstick just to meet the customer's desired shade. However, the most common colours of lipsticks are reddish, orange and pinkish in colour and it is the main factor affecting customer acceptance of a lipstick. Typically, the colour of a commercial lipstick is attributed by synthetic dye. However, the synthetic dye was found to be dangerous upon consumption as lipsticks often eaten away unintentionally by user. Some synthetic dyes were found to be toxic or even carcinogenic. Coal tars-the basic ingredient of synthetic dye was reported caused nausea, allergy, dermatitis, and drying of lips.

Due to the realisation of adverse effects of synthetic dye brings on human health, customers switching their preference towards natural products than synthetic products. However, the use of natural dye on cosmetics are limits from 5% to 15% (Setyawaty and Pratama, 2018). The present research was conceived by formulating a natural colorant lipstick with betalains (pigment extracted from red dragon fruit) that are safe to use and at the same time, fulfilled the satisfaction of customer in the aspect of colour and performance of a normal lipstick.

Red dragon fruit *(Holycereus polyrhizus)* are found to be betalains-rich and it is highly available in Malaysia as weather in Malaysia is suitable for the plantation of red dragon fruit. The most commercialised source of betalains in the current market are beetroots. However, red dragon fruit are claimed to exhibit higher pigment stability compared to beetroot and do not have unpleasant smell like beetroots (Moura, 2015). European Food Safety Authority (EFSA) claimed that betalains posed outstanding potential to replace synthetic dye in food, pharmaceuticals, nutraceutical and cosmetics industry as their antioxidant capacity and health promoting effects (Leong et al., 2018). The colour pigment in red dragon fruit is contributed by betalains which is a water-soluble pigment that can be classified into two groups: betacyanins (red-violet) and betaxanthins (yellow-orange). Interestingly, betalains offer a broad colour palette ranging from yellow to purple which can be achieved by mixing betaxanthins and betacyanins. The common lipstick colour such as orange, red, pink and purple lie coincidently in the colour palette covered by betalains. Thus, betalains is no doubt a potential natural pigment to replace synthetic dye in cosmetic industry.

In old time, many researchers had studied the stability of betalains at different parameters that affect its stability such as pH, temperature, light, additives, oxygen and storage condition of betalains. The significant contribution allowed us to understand the betalains biosynthesis, stability range, and also from chemical and physiological aspects. The key of application of betalains upon cosmetic industry is the uniformity of colour across batches. In this regard, microencapsulation technology by spray drying and freeze drying method offer great pigment stability and storage stability. In recent years, many researchers had studied the efficiency of solvents, extraction ratio and encapsulation ratio to obtain maximum betalains pigment. In current research, the optimum condition for betalains extraction and maximum encapsulation efficiency were utilized to produce betalains powder. This research aimed to evaluate the feasibility of betalains as natural colorant in lipstick formulation and studied its colour stability during one-month storage period. A basic lipstick formula was utilized to formulate lipstick with betalains powder and the lipstick produced were evaluated on spreadability, melting point, breaking point, surface anomalies and also skin irritation test. The colour stability of the betalains lipsticks during one-month storage period were monitored by tri-stimulus devices and analyse with CIE L*a*b* and CIE L*C*h*colour spaces coordinates.

1.2 Importance of the Study

The results of this present study may have significant impact on providing an alternative colorant to replace synthetic colorant in cosmetics application and understand the theory behind:

- The mechanism of structural changes in betalains at different condition.
- colour degradation of betalains.

1.3 Problem Statement

The synthetic dye used in lipstick formulation give perfect shade to the lips. However, in return, it brings adverse effects on human health as most of the synthetic dye are found to be toxic or carcinogenic. This issue raised the attention and awareness of public towards the impact of synthetic dye concerning health and safety. Red dragon fruit pigment-betalains has huge potential to become the substitute of synthetic colorant for colour range from yellow to violet colour in cosmetic production. Over the years, numerous researches have been done on the factors affecting the stability of red dragon fruit pigment (betalains). The primary factors affecting the stability of the betalains are light, temperature, oxygen and pH value.

One of the reason why natural pigment not widely commercialised in the industry was due to the discoloration of pigment due to exposure of light, pigment stability during storage, and also the storage environment condition. The stability of the betalains still undergo research to obtain optimum solution to protect the pigment from colour degradation and the most recent technology discovered was microencapsulation of sensitive pigment with coating through spray dryer or freeze dryer. More details research is required to find out the optimum condition and parameters to produce an effective natural colorant.

This research is emphasized on the preparation of encapsulated betalains powder with freeze drying and spray drying method. Besides, betalains pigment produced is used to formulate lipsticks as the model to study the colour stability during storage period. The priority of this research was to observe the suitability of betalains pigment to replace synthetic colorant.

1.4 Aim and Objectives

The aim of this study was to synthesis a lipstick with natural colour pigment-betalains which extracted from red dragon fruit and study its colour stability during storage period. The specific objectives of this research were to:

i. To prepare red dragon fruit pigment using spray drying and freeze drying method.

ii. To formulate lipstick using betalains.

iii. To study the colour stability of the betalains lipstick during storage period.

1.5 Scope and Limitation of the Study

1.5.1 Scope of study

The scope of this study was to study the suitability of betalains as substitute for synthetic colorant of lipstick application and to reveal the feasibility of natural pigment to be applied in cosmetic products in the future.

1.5.2 Limitation of Study

The stability of the betalains are affected by the factors such as temperature, pH, light and oxygen. The color tone of the lipsticks with different betalains extracted from different batches might vary. Therefore, tedious quality control during processing is important.

CHAPTER 2

LITERATURE REVIEW

2.1 Lipstick

Lipstick is a beauty enhanced cosmetic product that often used by women to apply on their lips to give nice colour and protection to the lips. The basic ingredients of a lipstick composed of oil, wax, and colorant.

The common wax used in lipstick are was beeswax, candelilla wax and carnauba wax. Waxes are added to in lipstick formulation to provide strength and structure to the lipstick. Various types of natural oils are used in lipstick formulation such as jojoba oil, sweet almond oil and castor oil. Oils act as moisturiser in lipstick and aid in blending the mixture to form a smooth and perfectly spreadable mixture. Butters such as shea butter act as moisturizer to form a stable lipstick mixture that is solid at room temperature yet will glides and melts when apply on the lips (Compound Interest, 2014).

Generally, lipsticks are manufactured using synthetic and lake dyes such as D&C Orange No. 17, Red No.21, Red 7, Red 34 and many more (Ahsan, 2017). The manufacturers of synthetic dye are able to provide synthetic dye with variety choices of colours for lipstick formulation. Synthetic dyes are favour by cosmetic industry at this moment as the colour of the shade remains stable with high pigmentation. The shades of lipstick are classified to few main colour like red, maroon, pink, nude, orange, and purple. Out of these choices, red colour is more favourable by the women in all aged.

The addition of fragrance oil and antioxidants into the lipstick formula is optional. Fragrance oil will enhance the aroma of the lipsticks and while antioxidant like vitamin E or lemon juice are added to prolong the shelf life by prevent oxidation. The fragrances are normally derived from petroleum or extract from flowers or fruits. In order to ensure the safety of consumer, the ingredients used to formulate lipstick must be acknowledged as safe by Food and Drug Administration before production.

2.1.1 Global Lipstick Demand

The rapid urbanization in developing countries increase the purchasing power of consumer. The inclination of internet usage further drives the demand for lipstick market globally. Apart from that, marketing strategies such as advertise lipsticks products in social networking site like Facebook, YouTube and Instagram are predicting to further pushes the demand for lipsticks over the coming year (Cision PR Newswire, 2019).

Global lipstick market stood at $7.15 billion in 2014 (Cosmetics Business, 2016) according to a report from Technavio and proliferate up to $9.2 billion in 2018. By the year of 2040, the revenue generate from lipstick market is expected to fall around $13.4 billion (Cision PR Newswire, 2019). The major factor contributes to the growth of market on account of the rising demand from young teenagers to working women across the globe.

The demand of lipstick will increase gradually in the upcoming future. With the raising of consumer awareness of side effect of synthetic dye on human health, the current research which aim to producing natural dye for lipstick provide the most promising solution for this issue.

2.1.2 Lipstick Formulation

The basic ingredients to formulate lipsticks consists of waxs, oils, colorant and fragrance. The proportion and weightage of each ingredient in lipstick formulation need to be controlled to produce a lipstick with appropriate softening and rupture point. The ingredient for lipstick formulation using *Daucus carota* extract were shown in Table 2.1 and the finished product were shown in Figure 2.1 (Swati et al., 2013).

Table 2.1: Lipstick Formulation Formulated from *Daucus carota* Extract (Swati et al., 2013).

Ingredients	Quantity (gm)
Paraffin wax	26
Olive oil	13
Bees wax	38
Daucus carota extract	15
fragrance	1
Lemon juice	0.5

Figure 2.1: Lipstick Formulated with *Daucus carota* Extract (Swati et al., 2013).

The herbal lipstick formulation was shown in Table 2.2 and the comparison parameters of lipstick between herbal lipstick and commercial lipstick were shown in the Table 2.3 (Kothari et al., 2018).

Table 2.2: Herbal Lipstick Formulation (Kothari et al., 2018).

Ingredients	Quantity
Coconut oil	1.5ml
Olive oil	1ml
Bees wax	1gm
Castor oil	1gm
Rose essence	3 drops
colourant	2gm
Vanilla essence	5-6 drops
Lemon juice	2ml

Table 2.3: Evaluation and Comparison between Formulated Herbal Lipstick and Commercial Lipstick (Kothari et al., 2018).

Parameters	Herbal Lipstick	Commercial Lipstick
Texture	smooth	smooth
Colour	red	red
Breaking Point	250gm	140gm
Melting Point	60°C-64°C	62°C-64°C
pH	6.3	6.6
Aging stability	smooth	smooth
Surface Anomalies	No defects	No defects
Perfume stability	+++	+++
Softening Point	60°C	62°C

Based on Table 2.3, each parameter of formulated herbal lipstick shown similar result as the commercial lipstick that sold in the market. Therefore, this formula demonstrated an excellent formula for lipstick formulation. It is believed that the formula can be used to produce a good lipstick with betalains extract.

Azwanida et al. (2015) reported a simple formula as shown in Table 2.4 that received well response from customer survey. The consumer acceptance survey was conducted on pH, colour, skin irritation and all the parameters shows high acceptance for the betalains lipstick.

Table 2.4: Lipstick Formulation (Azwanida et al., 2015).

Ingredients	Quantity(g)
glycerol	3
Betalains	2.4
Bees wax	21
Castor oil	33.6

2.1.3 Characterisation of an Ideal Lipstick

An ideal lipstick is a lipstick that apply smoothly and give colour to the lips with pleasant odour. Besides, an ideal lipstick must be toxic free, stable during storage period and do not melt at room temperature (Varghese, et al., 2017).

2.1.4 Defects of Lipstick

It is important to study the common defects found in commercial lipstick in order to tackle the issue of lipstick defects such as sweating, streaking and bleeding during processing. Sweating was claimed to be the most common defect found in lipstick formulation which the oil in the formulation was in excess. Bleeding defect denote the separation of colourless liquid form waxy base. Streaking of lipsticks was observed when a band of different colour, a substance or a thin line appears on the lipstick (Varghese, et al., 2017).

2.1.5 Evaluation of Lipstick.

In cosmetic industry, every batches of the produced lipstick will undergo quality check to verify its colour, odour and quality before being commercialized. The common quality control tests are spreadability test, melting point test, breaking point test, surface anomalies and skin irritation test.

2.2 Red Dragon Fruit

Red dragon fruit (*Holycereus polyrhizus*) is belongs to the cactus family of *Cataceae*. Red dragon fruit has unique appearance which is in bright pink skin with green scales. The flesh is red-violet in colour and embedded with abundance of tiny black seeds. Red dragon fruit is claimed to be betalains-rich and it is a great antioxidants sources, and contains high proportion of vitamin C, vitamin E and vitamin B3. Recently, betalains in red dragon fruit had drawn attention as potential source of natural colorant to synthetic dye as consumption of synthetic dye has been associated with many health problems.

2.2.1 Availability of Red Dragon Fruit in Malaysia

Red dragon fruit *(Hylocereus polyrhizus)* was introduced for commercial planting in Malaysia starting from 2000's. According to Then, (2017), the total cultivated area of red dragon fruit in Malaysia was increased from 502 hectares to 1641 hectare from year 2005 to year 2013 while the total production of red dragon fruit was recorded as 10961 tonnes with wholesale value of RM55.51 million. According to Matrade Government (2018), the selling price of red dragon fruit in the current market is RM6/kg.

The rapid development of cultivation of red dragon fruit in Malaysia is due to the availability of longer hour sunshine exposure (to induce flowering and fruiting) and also the suitable weather condition which is around 32 °C to support the growth of red dragon fruit during cultivation. Normally, red dragon fruit will be fruiting after a year.

2.3 Colorant

Food and Drug Administration define colour as a dye that can impart colour to food, cosmetics or to human body and must not chemically reacted with any other substances (Náthia-Neves et al., 2018).

2.3.1 Synthetic Colorant

Synthetic colorant is produced in complex matrix or by modification of precursor components. It is favourable in textile, paint, and food industry due to its greater stability and lower production costs compared to natural colorant. However, the usage of synthetic dye can be harmful for human when they are not fixed in the matrix. Toxic substances were formed when the structure of synthetic dye disrupted in the matrix and react chemically with other components to form unknown toxic that harmful to human body (Náthia-Neves et al., 2018).

As mentioned above, lipstick production with synthetic dye are restrained as some of the synthetic dyes are harmful. FD&C Red #40 is an azo dye reported to cause mutagenic and genotoxic that would destruct DNA structure and have shown to be carcinogenic in animals over a short period of time. Azo reductase, an enzyme found in azo dyes, was potential in reducing metabolically active amines in mammals which is cancer-causing (Moura, 2015). The main route of human exposure to dyes in cosmetics is through dermal contact where cosmetic product like eye shadow or lipstick applied. Besides, the formation of rough kin was identified with Acid Red 92 (Xanthenes dyes), a red shade dye which are very popular in cosmetics as it reacts with protein on human skin (Guerra et al., 2018). Some minor effects caused by synthetic dyes such as Yellow No.5 (Tartarzine) and Red No.2 are allergic reaction, skin irritation and drying on lips (Moura, 2015).

Due to the side effect of synthetic dyes mentioned above, it is believed that the natural colorant is demanding in the current market as it is safe and environmental friendly.

2.3.2 Natural Colorant

Natural colorants were derived from natural edible source such as flowers and fruits. The main criterion for application of natural dye is due to their stability during processing and also storage stability. The stability of the natural dyes are affected by many factors such as pH, light, oxygen, and temperature. There exist plenty of natural colour pigments in various colour such as carotenoids (orange-red), flavonoids (violet), iridoids (blue or yellow), chlorophylls (green) and betalains (yellow to violet) which illustrated in Figure 2.2.

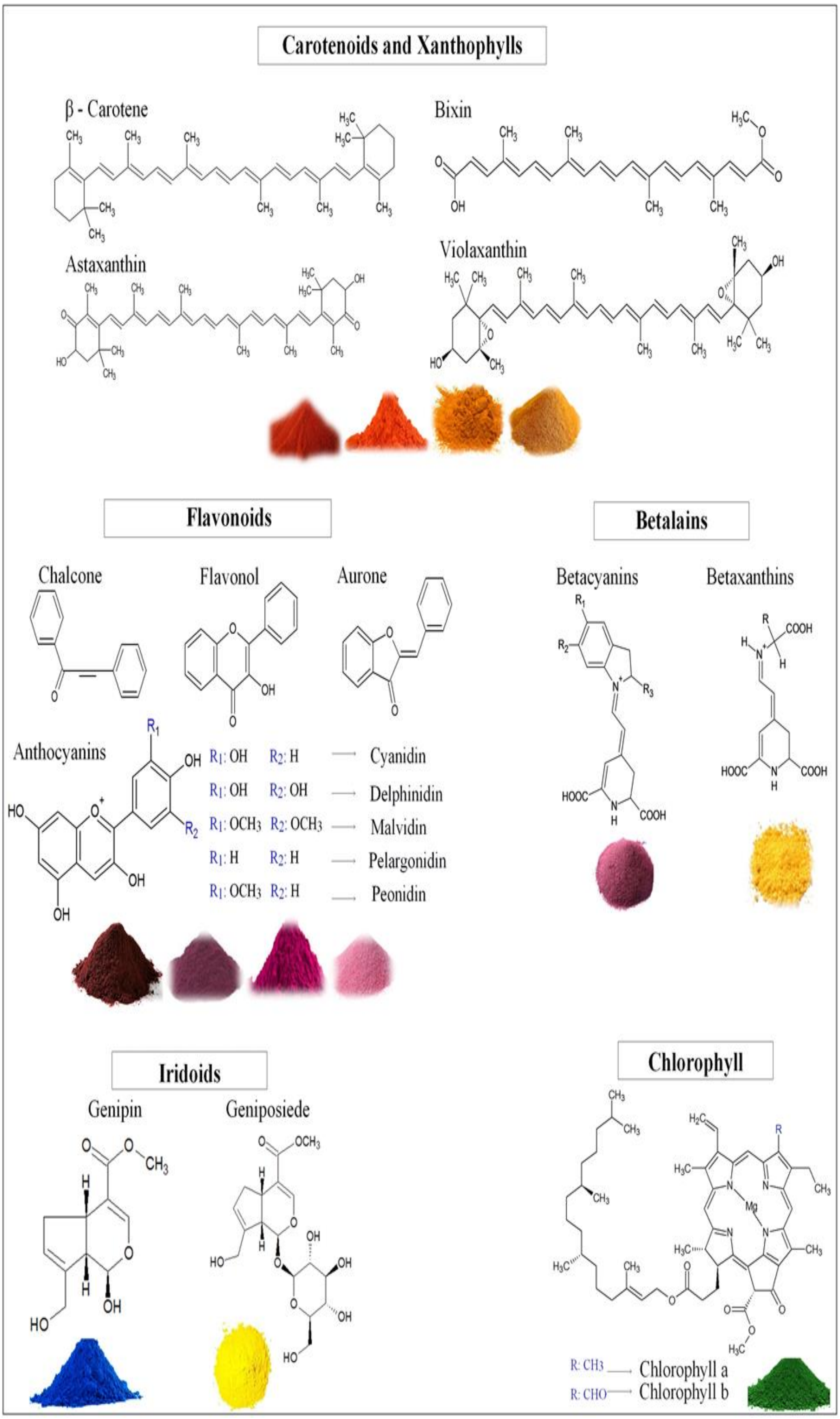

Figure 2.2: Source of natural pigment (Gandía-Herrero et al., 2010).

Carotenoids composed of few structural which are β-carotene, bixin, violaxanthin, and astaxanthin. The conjugated double bond of carotenoids structure influenced its properties and each of the polyene chains could be in cis or trans in the structure, resulting a number of possible isomers formed with its respective colour ranging from yellow, orange to red. The colour pigments of Carotenoids are soluble in oil, and can be found in carrot, papaya or pineapple (Gandía-Herrero et al., 2010).

Flavonoids which composed of chalcone, flavonol, aurone, and anthocyanins has natural colour ranging from soft pink to purple-blue. Anthocyanins is the dominants colorant in flavonoids group and can be found in blueberry and black berry. Anthocyanin was found to be stable at low temperature at pH ranging from pH 3.1 to pH 4.7 and prone to undergo oxidation which causes colour deterioration accompanied by undesired browning (Stintzing & Carle, 2014).

Another source of natural pigment is chlorophyll. Chlorophylls are soluble in oil and commonly extracted from grass or leaves of plants to obtain the greenish colour. The other sources of chlorophylls are avocado, spinach, and kiwi. However, chlorophylls degrade easily due to its poor stability. In acidic condition, chlorophyll lose its Magnesium Ion and turn into yellowish-brown in colour as the formation of phaeophytin (Delgaldo & Paredes, 2003). Besides, the destruction of chlorophyll indicates the ripening of fruit or flowering. As chlorophyll are unstable, thus it did not commonly apply in food industry.

The utilization of natural pigment in lipstick formulation were performed by Varghese, et al., (2017) using *Bixa orellana* (Cartenoids), *Beta vulgaris* (betalains), *Daucus carrota* (lycopene), *Holycereus polyrhizus* (betalains), and *Lycopersicon esculentum* (lycopene) to produce natural colorant lipstick. The research finding conclude the use of natural pigments have no side effect in lipstick formation.

2.4 Betalains as Alternative Dye for Lipstick Production

Betalains are water-soluble nitrogenous pigment which classified into two groups, betacyanins and betaxanthins. The red-violet colour is contributed by the variation in sugar and acyl group in betacyanins while the yellow-orange colour of betaxanthins is contributed by the conjugation of wide range of amine or amino acids. Their respective colour is attributed by the resonating double bond in the structure. The maximum absorption of yellow betaxanthins are from 457nm to 480nm while maximum absorption of red-violet betacyanins are from 532nm to 550nm (Azeredo, 2009).

Betalains (EEC No.162) were reported as red natural colorant by European Union and approved by Food and Drug Administration to be utilised as food colorant in food processing such as making yogurt, ice cream and candies (Delgado-Vargas et al., 2010). Betalains is a very potential natural colorant source in cosmetic industry compared to other sources like anthocyanin as its stability over a broad range of pH (Varghese, et al., 2017) and its ability to provide a broad range of colour ranging from yellow to purple as betalains were reported to comprise of more than 75 structures with variety of hue (Khan, 2016). The structure of betacyanins and betaxanthins were shown in Figure 2.3.

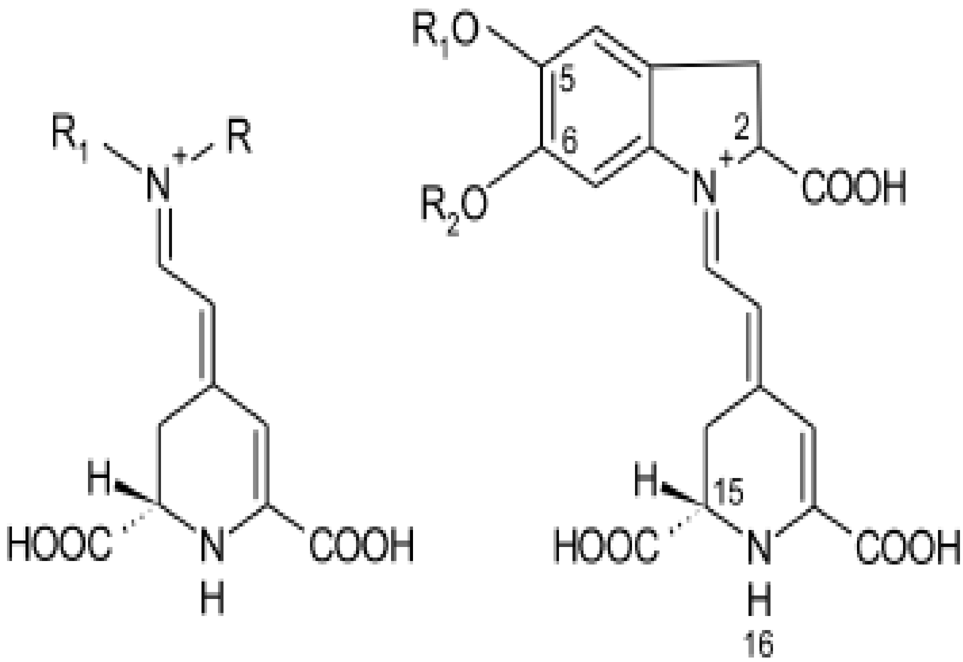

Figure 2.3: Structure of betacyanins and betaxanthins (Miguel, 2018).

The mechanism of degradation of betalains involves isomerisation, deglycosylation, decarboxylation, hydrolysis, hydrogenation and bond cleavage. A colour shifts from red-purple hue to yellow-orange tone was observed with red beet juice during thermal treatment (Celli and Brooks, 2017). Interestingly, betalains regeneration is possible even after thermal treatment. Therefore, the desired hue can be achieved by mixing betaxanthins with betacyanins (Stintzing et al., 2005). From the colour aspect, it is believed that betalains have great potential to replace synthetic dye in lipstick production as its ability to provide many hues for lipstick production. Figure 2.4 illustrated the chemical structure of betacyanins and the sites prone to its respective degradation.

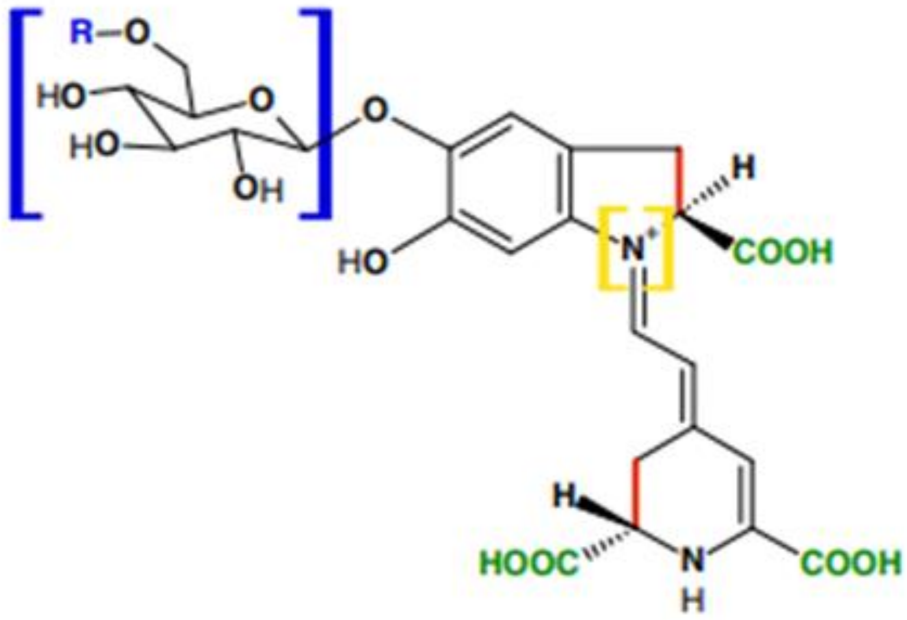

Figure 2.4: Chemical Structure of betalains and the site prone to its respective degradation (Khan, 2016).

The blue bracket in Figure 2.4 indicated the sugar or acyl group site which prone to undergo deglycosylation. The COOH labelled with green indicates the site prone to undergo decarboxylation while the red line indicated the site prone to undergo dehydrogenation. Lastly, the yellow bracket site is prone to nucleophilic attack. The degradation pathway of betalains with its respective hue were illustrated in the Figure 2.5 and the hue can be used to identify the structure of betalains for different hue of lipsticks.

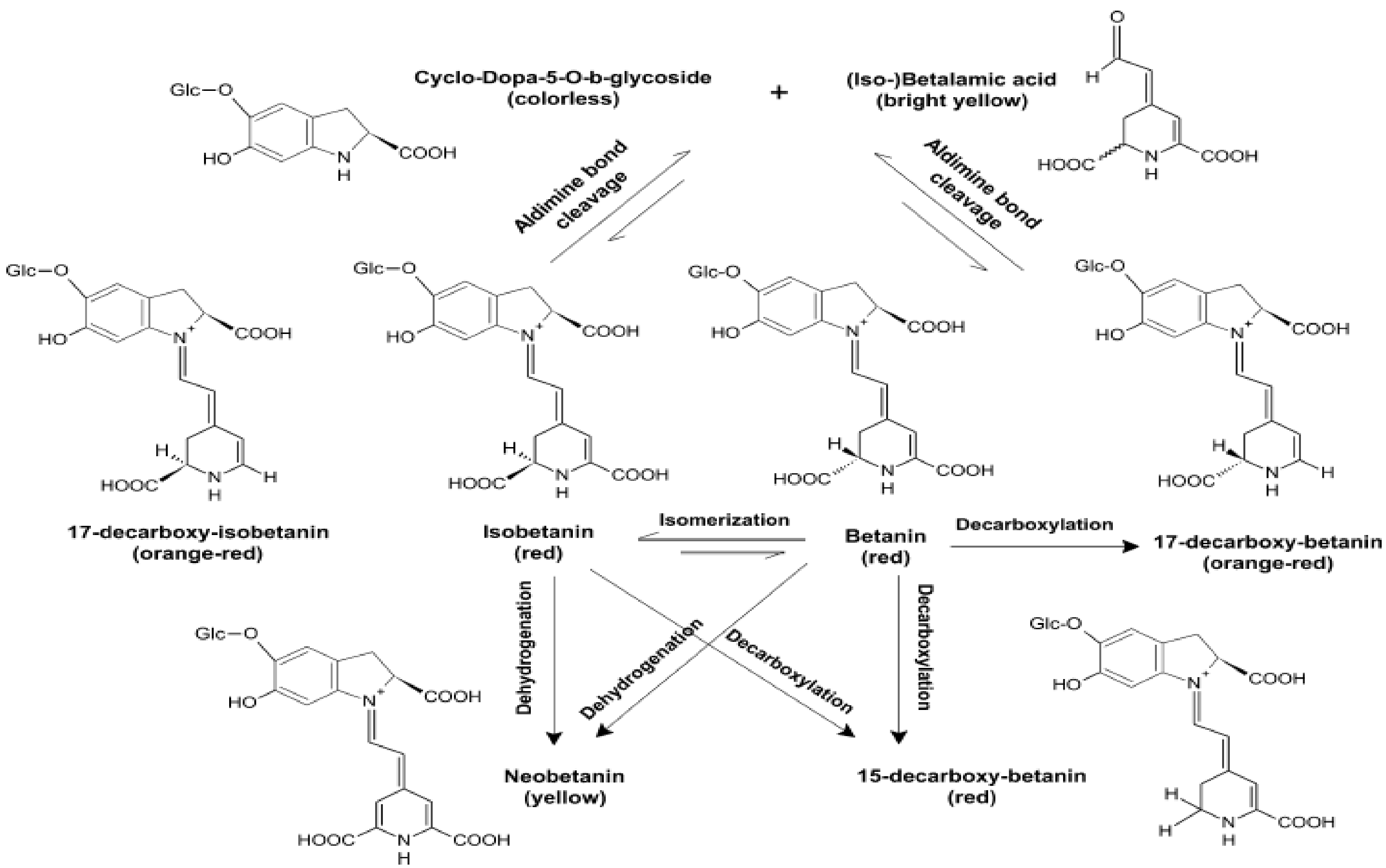

Figure 2.5: Degradation pathway of betalains with its respective colour (Azeredo, 2009).

2.4.1 Parameters Affect the Stability of Betalains

Parameters that affect the stability of betalains were studied. The degradation of betalains occur due to the different mechanism in the betalains structure (Herbach, et al., 2006). Therefore, several control need to be consider to ensure the stability of betalains and optimum colour retention in order to apply the betalains pigment into lipstick formulation. According to Herbach et al. (2016), the main factors affecting betalains stability are light, temperature, oxygen and pH.

2.4.1.1 pH

According to Woo, et., 2011, betalains are stable in a broad range of pH ranging from pH 3 to pH 7 and the maximum colour stability is at pH 5 (Azeredo, 2009) which confirmed their application at low acidity condition. Any pH condition out of this range will experienced the colour degradation. At strong acidic pH, isomerisation and dehydrogenation of betalains structure happened and while aldmine bond hydrolysis occur at strong alkaline condition in betalains structure (Herbach, et al., 2006).

2.4.1.2 Light

Light exposure was reported as the main factor for colour degradation in betalains (Woo, et al., 2011). Stability of betalains was found to has inverse relationship with light intensity. The presence of visible light or ultraviolet light promote the light absorption by exciting the pi electron in betalains structure to a more stable state in betalains chromophore. However, the effect of light exposure can be ignored under anaerobic condition as light degradation in betalains were found to be oxygen dependent. The yield of betalains storage in light and dark condition were identical under anaerobic conditions (Herbach, et al., 2006)

2.4.1.3 Oxygen

Oxygen contributed to colour degradation of betalains structure as betalains will react with oxygen in the atmosphere. Normally, the presence of oxygen caused darkening and colour loss. To stabilise betalains, oxygen removal or the addition of anti-oxidants agent to the betalains extract were recommended. According to Attoe & Joachim, (1984), betalains degradation with involvement of oxygen was pH dependent. The oxygen involvement in betalains structure increases when exceed betalains optimum pH. However, betalains degradation were improved significantly in nitrogen environment (Herbach, et al., 2006).

2.4.1.4 Temperature

Temperature was claimed to be the most vital factor affecting the betalains stability in food processing and storage stability. Colour retention of betalains drop significantly at elevated temperature in longer heating period. Besides, betalains degrade above 80 °C and at pH value that exceed the optimum pH value of 5 with the presence of oxygen. Betalains degrade by decarboxylation, isomerisation and cleavage by heat or acids resulting the loss of red tone to brownish colour while neobetanin (yellow) were formed through dehydrogenation of betanin (Azeredo, 2009).

Commercial lipsticks were stable at room temperature in atmosphere. Thus, the environment condition of the synthesised betalains lipstick must be similar to the commercial lipstick in order to determine the feasibility of betalains as the natural colorant in lipstick application.

2.5 Microencapsulation

Microencapsulation is a physical process of isolate the core pigment (solid particle or liquid droplets) with wall material to protect the thermal or light sensitive material from degradation and deterioration (Náthia-Neves et al., 2018). A thin film of wall material will form a physical barrier around the core material and protect the core material from other components thus prolong the shelf life of the sensitive pigment. Current microencapsulation technology is a done by either spray dry or freeze dry the mixture of core material with wall material to solid form.

A few researches were conducted to test the microencapsulation efficiency of different wall material on betalains stability. The common wall material used are maltodextrin, xanthan gum, gum Arabic and cladode mucilage. However, maltodextrin with grade of DE-10 was found to be the most effective coating agent in stabilising betalains (Handayani et al., 2018a).

Maltodextrin exists as white solid crystal and it offers high water solubility, low viscosity, and most importantly, tasteless and odourless in application. With the mentioned material properties, maltodextrin is capable to protect the sensitive pigment without disrupt the flavour and nutrition of the particle (Handayani et al., 2018). However, the percentage of the maltodextrin for maximum colour retention in betalains pigment were found to be at 30 % of maltodextrin to the betalains content in spray drying (Ruri, et al., 2015) while 50 % of maltodextrin to the betalains content in freeze drying (Handayani et al., 2018). The ratio of maltodextrin mentioned above were utilised in this research to produce betalains powder to formulate lipsticks.

2.5.1 Spray Drying

Spray drying is a process of dispersion of homogenized mixture of core material in entrapment material through nozzle, and the atomized droplets is evaporated by hot air in drying chamber. Tons of encapsulated powder can be produced in a very short while. However, a fraction of powder may remain entrained in the chamber resulting less powder are collected from the collection chamber (Andrews.N, 2017). Spray dryer are favour by the industry which manufacture thermal sensitive powder. The most common application of spray dryer is production of milk powder. The powder quality obtained is depends on the operating temperature, atomization speed and feed flow rate of spray dryer. Besides, spray dryer able to produce dry solid powder range from 0.2 μm–60 μm and the powder are consistent in particle size distribution.

Ravichandran et al., (2014) reported that microencapsulation with maltodextrin increased storage stability significantly and the spray dried powder stored at room temperature remain 98 % of its colour after one month. According to Lim et al. (2012), the mixture of sodium caseinate with maltodextrin DE-10 as wall material at core to wall ratio of 1:3 obtained the highest microencapsulation efficiency which was 98.06 % for spray drying at the condition of 150 °C as inlet temperature and 77 °C as outlet temperature. Other combination of potential wall material such as why protein, lactose, and gum Arabic were tested. However, the microencapsulation efficiency of those wall materials were found to be significantly lower without maltodextrin. It is believed that maltodextrin had contributed significantly on core material protection in improved betalains stability.

According to Ruri et al. (2015), spray dryer was capable to produce high colour strength betalains powder with low water activity. However, the yield of the spray dried powder affected by the powder adhere to the wall of drying chamber as the sample not dried completely. The main challenge for spray dryer utilization was due to the amorphous nature of food which prone to become sticky and rubbery. Thus, the spray dryer must be controlled carefully when perform the drying process. The results of the research indicated that the samples with lower DE value gave higher yield and the addition of 30 % of DE-10 Maltodextrin gave highest yield of 89.1 % with spray drying condition of 202 °C as inlet temperature. The longest storage stability and betalains retention was reported by Giridhar, (2018) which is 96.81 % of betalains retention after 2 years at 4 °C. Figure 2.6 illustrated a lab scale spray dryer which was utilised in current research to produce betalains powder for lipstick application.

Figure 2.6: Mini Spray Dryer manufactured by Buchi, Switzerland.

2.5.2 Freeze Drying

Freeze drying process are commonly known as lyophilisation. This method is in contrast with conventional drying method, which is supplying heat to evaporate the moisture. Freeze drying is a process of dehydration which carried out at low temperature to preserve perishable food or heat sensitive material such as protein, enzyme and blood plasma by extract out the moisture in the components thus prolong their shelf life. The low temperature minimal the damage to the material. Freeze drying are able to produce high quality product as the original shape of the product maintained. The nutrition and the flavour of the material are perfectly preserved and the quality of the material are maintained well after rehydrated. However, freeze drying are expensive compared to other drying method available in industry and involves long processing time (Ravichandran et al., 2014a).

Freeze drying involve three phases. The initial phase is the freezing phase. The materials are cooled at low pressure inside the freezer below its triple point to initiate sublimation. The second phase (primary drying) is sublimation phase. The pressure is lowered in the freeze dryer and heat is supplied to the material in order to ease the sublimation of water inside the material. At this particular stage, about 95 % of water are being eliminated from the material. The secondary drying phase (adsorption) is the final phase. In this stage, the ionically bounded water molecules were removed completely from the material by the elevated temperature causing the bonds between the material and water are broken. By using freeze dried method, 95 %-99 % of moisture can be removed from the material. However, the freeze dried materials always retain a porous structure (Ravichandran et al., 2014a).

According to Handayani et al. (2018), the maximum encapsulation efficiency of betalains with 50 % of maltodextrin using freeze dryer is 59.32 % with the betalains concentration of 75.95 mg/L. The microencapsulation efficiency of betalains using maltodextrin-gum Arabic with the ratio of 3:2 (w/w) by using freeze drying method was reported as 94.32 %. In Nemzer, et al. (2011), the beetroot extracts were loaded into commercial freeze-dryer at -23° for 24 h and the water content left in the sample were lower than 2.3 %. The report claimed that the stability of extracts after freeze dried improved significantly under light exposure. Thus, freeze drying of betalains demonstrate a promising solution to improve colour stability of betalains under light exposure. Figure 2.7 illustrated a bench top freeze dryer which utilised in the current research to produce betalains powder for lipstick application.

Figure 2.7: Bench Top Freeze Dryer, SP scientific (Pharmaceutical Online, 2011).

2.6 Colour Analysis

In cosmetics industry, colour analysis is very important to ensure same tonality of lipsticks are produced in every batches for lipstick with same colour code. Generally, in mass production lipstick industry, visual inspection and was performed to analysis colour tone between the produced lipstick with the standard lipstick as a reference model. Even tough human eyes are sensitive to perceiving colour, human eyes are less sensitive to minute changes in colour. Due to this limitation, colour control instruments-colorimeter were developed to interpret colour in colour space precisely in numerical term. The method developed such as CIE XYZ, CIE L*a*b*, and CIE L*C*h* allow users to identify colour attributes, evaluate colour inconsistencies accurately and expressed their findings in numerical terms.

2.6.1 Colour Space System

2.6.1.1 Tri-stimulus System (CIE-XYZ)

Tri-stimulus system is a colour measurement system that measure the intensity of red, green and blue light that reaching our eyes and the result were expressed as X, Y, and Z which known as tri-stimulus value. The values were evaluated on a standard chromatically diagram as shown in Figure 2.8. However, the x and y only interpret the chromaticity of the sample and power of illuminant is not fixed (Nassau.K, n.d.).

This colour control measurement is less accurately as variation of light source, viewing angle and field of view can alter the visual perception. The standard angle of 10° was claimed to be the most accurate degree of observation. The tri-stimulus value obtained were standardised to ensure precise colour matching and product compatibility in various industry. The tri-stimulus values evaluated can be transformed into other colour space properties such as CIE L*a*b* to evaluate the colour in colour space coordinates (Ramanathan, 2015).

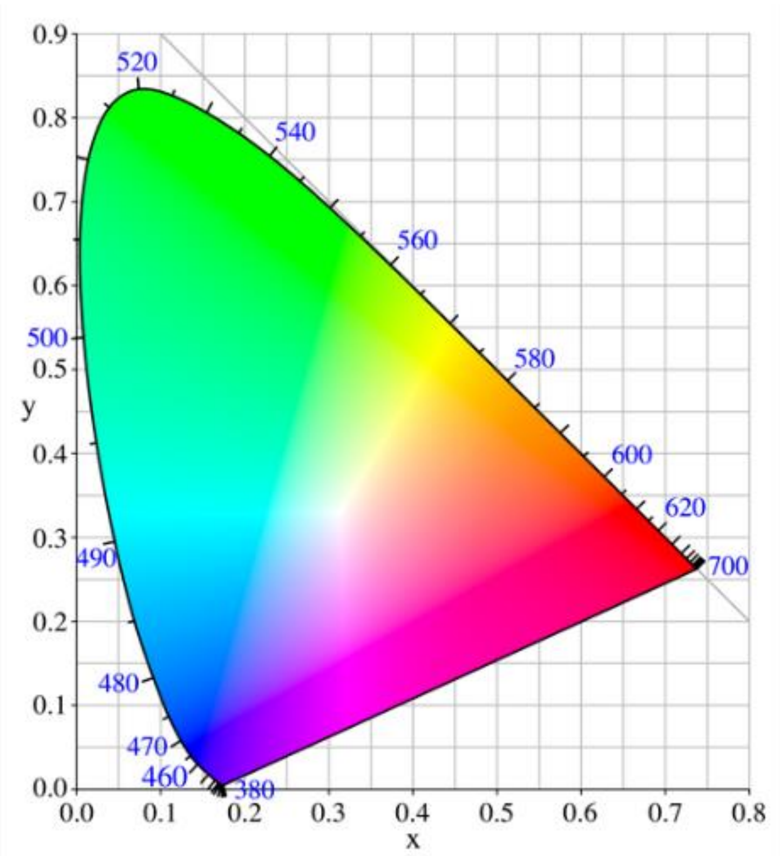

Figure 2.8: Standard Chromatically Diagram for Tri-stimulus colour system (Nassau.K, n.d.).

2.6.1.2 Hunter CIE L*a*b* and CIE L*C*h* System

CIE L*a*b* colour space was developed after a colour opponent theory stating that a colour cannot be red and green or blue and yellow at the same time. The value of L* indicates lightness, a* indicate value of red (+128) to green (-128), while b* represent value of yellow (+128) or blue (-128). The difference of the colour, ΔE can be expressed in numerical value.

$$\Delta E = \sqrt{(L_i^* - L_0^*)^2 + (a_i^* - a_0^*)^2 + (b_i^* - b_0^*)^2} \quad (2.1)$$

In CIE L*C*h* colour space, the L* represents lightness, C* represents Chroma, h* represent the hue angle. The L value of 0 indicate as black colour while L value of 100 indicate as white colour. The hue angle of 0°, 90°, 180°, 270° in colour wheel represent red, yellow, green and blue respectively. Chroma indicate the intensity and purity of the hue. Hue angle and Chroma value can be calculated after obtained the a* and b* value under illuminant of D_{65} (average daylight under visible light spectrum) and 10° observer angle.

The hue angle and Chroma can be calculated as below:

$$\text{Hue angle, } H^\circ = \tan^{-1} \frac{b^*}{a^*} \quad (2.2)$$

$$\text{Chroma, } C^* = \sqrt{a^{*2} + b^{*2}} \quad (2.3)$$

The colour retention over storage period can be calculated as follow:

$$\text{Colour retention} = \frac{Chroma\ at\ storage\ time_x}{Chroma\ at\ storage\ time_0} \times 100 \quad (2.4)$$

Figure 2.9 and Figure 2.10 shows the colour space coordinate for CIE L*a*b* and CIE L*C*h system respectively.

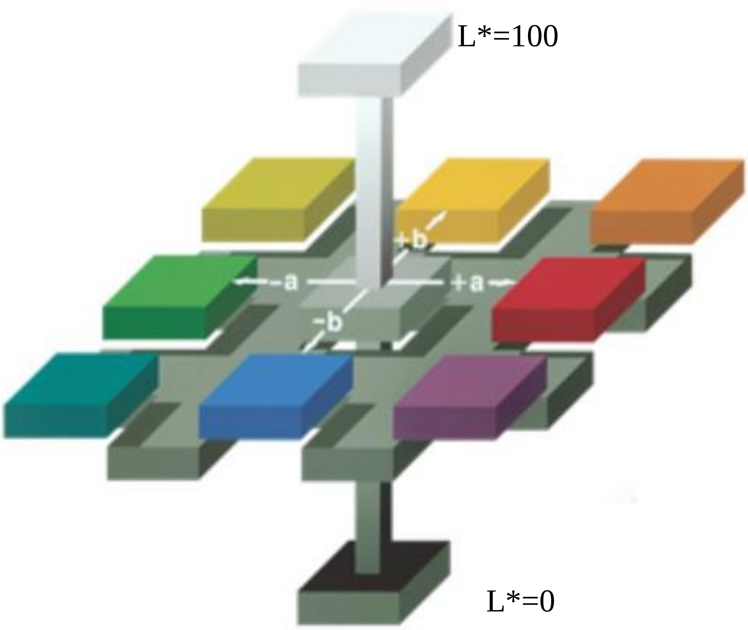

Figure 2.9: A. Hunter CIE L*a*b* rectangular colour space (Moura, 2015).

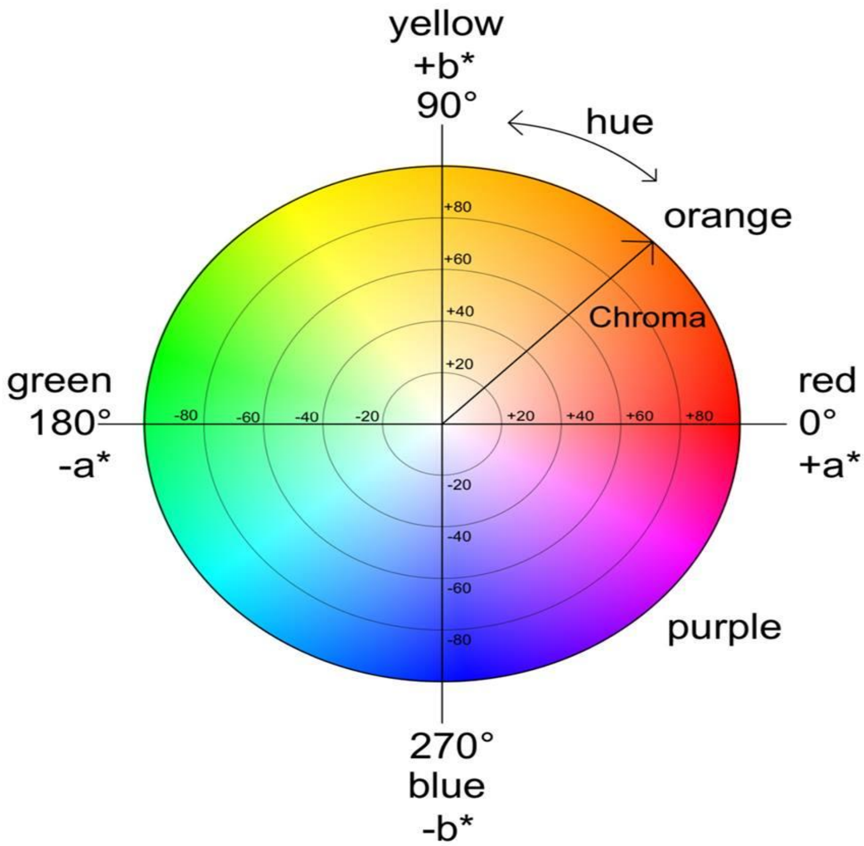

Figure 2.10: CIE L*C*h* colour space (Moura, 2015).

The variation of colour difference in cosmetics industry or other colour industry like paint, textile, automobile industry allows the manufacturer to perform quality checking on the colour whether the colour produced matched their standard and also customer acceptance.

The method mentioned above were utilised by Casterllar et al. (2006) to evaluate the commercial applicability of *Opuntia stricta* extract (a type of cactus). Its colour properties were determined by colorimeter and the data (as shown in Figure 2.11) were compared with other source of natural red colorant. The total colour difference, ΔE is a measurement of the extent to which the colour of the other colorants was similar to that of *Opuntia stricta* (ΔE=0, as standard reference) using the Equation 1.1 mentioned above while Figure 2.12 show the location of each colorant inside the colour space coordination of CIE L*C*h* system (Castellar et al., 2006).

Colourant	*L**	*a**	*b**	*C**	*h*°	Δ*E**
1. *Opuntia stricta*	69.8 ± 0.0	59.7 ± 0.1	−23.5 ± 0.0	64.2	338.5	0.0
2. Red beet	69.5 ± 0.1	57.9 ± 0.2	−1.0 ± 0.0	57.9	359.0	22.6
3. Cochineal	65.1 ± 0.1f	61.9 ± 0.0	−9.9 ± 0.1	62.7	350.9	14.6
4. Elderberry	60.2 ± 0.2	37.1 ± 0.2	11.5 ± 0.2	38.9	17.2	42.7
5. Red grape skin	57.7 ± 0.0	33.6 ± 0.1	5.9 ± 0.0	34.1	9.9	41.1
6. Red carrot	61.0 ± 0.1	33.5 ± 0.2	9.3 ± 0.2	34.8	15.5	42.9
7. Hibiscus	73.1 ± 0.1	49.5 ± 0.1	12.3 ± 0.1	51.0	13.9	37.4
8. Red cabbage	64.3 ± 0.2	52.5 ± 0.0	−18.7 ± 0.0	55.7	340.4	10.2

Figure 2.11: The Obtained Colour Properties of CIE System from Natural Red Food Colorant (Castellar et al., 2006).

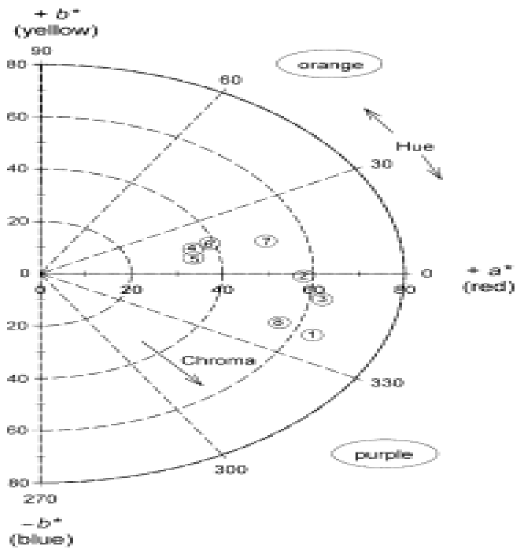

Figure 2.12: Chromatically diagram of CIE L*C*h* (Castellar et al., 2006).

2.6.2 Colour Measuring Tools

2.6.2.1 Colorimeter

Colorimeter is a device that used to measure the colour index in various colour spaces precisely. Colour space measured by colorimeter was CIE L*a*b* system, CIE L*C*h* system and CIE-XYZ (tri-stimulus system-RGB). Colorimeter were illustrated in Figure 2.13. The colour analysis method was simple by just putting the colorimeter on the finished product`s surface and press the analyse button and all the colour properties will be shown (China OEM, n.d.).

Figure 2.13: Colorimeter (Colorimeter 3nh Production, n.d.).

This device is useful to observe the maturation process of red dragon fruits. During maturation of red dragon fruit, the L* value was low which indicated that betalains were darker in colour due to the development of reddish colour whereas the C* value indicate the saturation and intensity of the vividness of red dragon fruit. The higher the chroma value, the greater the intensity of the vividness of red dragon fruit. The declination of hue reflects the development of the reddish colour (Phebe et al., 2009). The method of monitor the maturity of red dragon fruit could be applied for colour analysis of lipstick colour and monitor the colour degradation of lipsticks during storage period.

2.6.2.2 Color Name - Mobile Apps

Color Name is a mobile app developed by Vlad Polyanskiy to detect the light intensity of red (R), green (G), and blue (B) of a sample and convert it into numerical term. Colour analysis using by Color Name was pretty simple by just taking photo of the product and the value of R, G, B will be shown on screen. As UTAR did not facilitated with a colorimeter which cost around RM1500, a free mobile App-Colour Name which works the same as colorimeter is utilised in this research to detect the RGB value of the lipsticks.

CHAPTER 3

METHODOLOGY AND WORK PLAN

3.1 List of Material and Equipment

3.1.1 Raw Material and Chemical

Red dragon fruit were obtained from the local market around Bandar Sungai Long, Kajang and stored at 4 °C before used. The complete list of raw materials and chemicals that were utilized in this study are tabulated in Table 3.1.

Table 3.1: List of Raw Material and Chemical Used.

Chemicals/Material	Purity	Grade (Brand, Country)
Red dragon fruit	100 %	Grade A (Malaysia)
Ethanol	95 %	Technical Grade (Systerm, Malaysia)
Maltodextrin	100 %	(DE-10 Grain Processing, USA)
Beeswax	Refined	(Craftivity, Malaysia)
Carnauba Wax	-	(Craftivity, Malaysia)
Candelilia Wax	-	(Craftivity, Malaysia)
Castor Oil	100 %	(Craftivity, Malaysia)
Sweet Almond Oil	100 %	(Craftivity, Malaysia)
Jojoba Oil	100 %	(Craftivity, Malaysia)
Shea Butter	-	(Craftivity, Malaysia)
Vitamin E	-	(Craftivity, Malaysia)
Fragrance	-	(Craftivity, Malaysia)

3.1.2 Apparatus, Equipment and Instrument

The apparatus, equipment, and instruments that were utilized in this study are tabulated in Table 3.2.

Table 3.2: List of Apparatus, Equipment and Instruments.

Apparatus/ Instruments/ Equipment	**Specifications**
Rotary Evaporator	HEI-VAP, Heidolph, Germany.
Centrifuge Machine	Sigma 3-18K, Sigma, Germany
Mini Spray Dryer	B-290 advance, Buchi, Switzerland.
Freeze Dryer	Labconco FreeZone-7670530, Millrock Technology, New York.
Fourier Transform Infrared Spectrometer (FTIR)	Nicolet IS10 FTIR, Thermo Scientific, United State
Color Name	A smartphone app developed by Vlad Polyanskiy
Mini Photo Studio	Light source: White LED (12V) Measurement :25cmx20cmx25cm

3.2 Research Methodology

This research was separated into three parts: synthesis of betalains pigment, formulate betalains lipstick, and study the colour stability of betalains lipstick for one-month storage period. In the first part, the betalains was extracted from red dragon fruit using ethanol. The concentrated betalains extract was obtained after undergo centrifuge machine and rotary evaporator. Maltodextrin DE-10 was added to the concentrated betalains and the mixture was either spray dried or freeze dried to betalains pigment. The Fourier Transform-Infrared Spectroscopy spectrum were utilised to identify the functional groups on the synthesised betalains pigment. Second part of this research was the application of betalains pigment incorporated into basic lipstick formulation to formulate a lipstick. The synthesised betalains lipsticks were evaluated on melting point, breaking point, skin irritation test, surface anomalies, and spreadability test. Lastly, the colour stability of betalains lipstick during storage period were studied by tri-stimulus colour system and Commission Internationale de I`Eclairage (CIE L*a*b* & CIE L*C*h*) colour space coordinates.

3.3 Experiment Procedure

The detail procedures of the research will be discussed in the following section.

3.3.1 Extraction and Purification of Betalains from Red Dragon Fruit

Fresh red dragon fruits were purchased in the local market and washed with tap water to remove the dirt and dust on the surface of the red dragon fruit. Then, the red dragon fruit was cut and the flesh was blended for 30 s in a blender. After that, the blended flesh was homogenized at a ratio of 1:3 with ethanol (95 % v/v) (Thu & Mon, 2014). The solution was kept for 15 min for betalains to leach out from the red dragon fruit juice. This step was necessary to carry out to remove the pectic substances from the red dragon fruit. After that, the homogenized fruit juice was sieved to remove the little black seeds that found in red dragon fruit. Lastly, the betalains extract were send to centrifuge for 10 min at 10,000 rpm to get rid of the precipitates (Moura, 2015). The solution obtained were stored in schott bottle and placed inside the mini photo studio.

3.3.2 Monitoring Color Changes of Betalains Extracts

The colour of the solution was closely observed time by time with the mobile app-Color Name inside the mini studio under controlled environment. Three colours were selected to produce betalains lipstick which are orange, red and purple. Once the colour of the betalains extract reached the designed hue (RGB value) which detected by Color Name, the extract will be immediately covered with aluminium foil and undergo the following process. The Hue of the betalains extract were determined as shown in Table 3.3 and the results were illustrated in Figure 3.1.

Table 3.3: The Designed Hue for the Betalains Extract.

Hue Value	Lipsticks Colours		
	Orange	Red	Purple
R，G，B	191，81，0	109，0，0	142，0，75

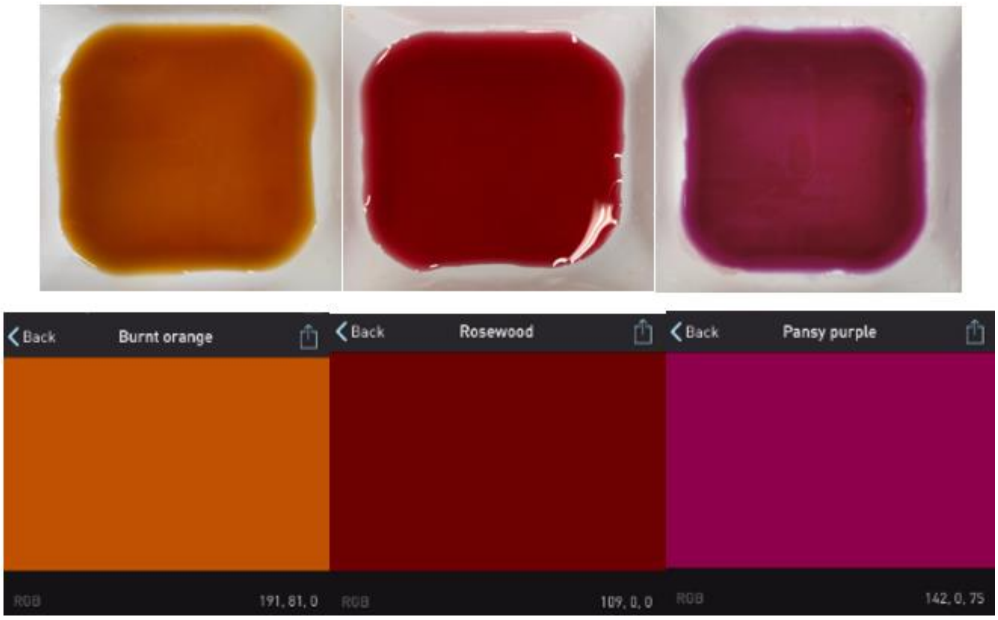

Figure 3.1: The Betalains Extract and its Hue Detected by Color Name.

3.3.3 Purification of Betalains

After obtained the desired hue for betalains extract, the betalains extract was concentrated by a rotary evaporator under vacuum at the operating condition of 40 °C for 2 hours (Thu & Mon, 2014) until it reached one fourth of its initial volume. Half of the concentrated betalains extract were mixed with 30 % of Maltodextrin (Thu & Mon, 2014) and stored in sample bottle covered with aluminium foil before feeding into spray dryer while half of the betalains extract were mixed with 50 % of Maltodextrin (Giridhar, 2017) were poured into centrifuge tubes with maximum 10 ml per centrifuge tube and freeze immediately in freezer for at least 24 hours before undergo freeze drying process.

3.3.4 Spray Dried Betalains Extract with Maltodextrin as Coating Agent

The mixture of homogenized betalains extracts with Maltodextrin was spray dried using Mini Spray Dryer B-290 produced by Buchi, Switzerland. The mixture was feed into the spray dryer at pump rate of 20 % - 40 %. The operating temperature was 198 °C as inlet temperature and 98 °C as outlet temperature (Ravichandran et al., 2014b). The sample bottles were covered with aluminium foil and the spray dried betalains was stored at room temperature for further work. Figure 3.2 illustrated the spray drying of betalains extract with spray dryer.

Figure 3.2: Spray Drying of Betalains Extract using Mini Spray Dryer.

3.3.5 Freeze Dried of Betalains Extract

The minimal amount of mixture (5 ml- 10 ml) of homogenized betalains extracts with maltodextrin were stored in centrifuge tube and frozen in ultra-low temperature freezer at –20 °C for overnight. Then, the betalains extracts were then freeze dried by freeze dryer (Labconco FreeZone-7670530, Millrock Technology, New York) at −110 °C for at least 24 hours to be freeze dried to dry porous solid. The dried betalains extracts were collected after 24 hours, and covered with aluminium foil for further work. Figure 3.3 illustrated freeze drying of betalains extract in freeze dryer.

Figure 3.3: Freeze Drying of Betalains Extract using Freeze Dryer.

3.3.6 Lipstick Formulation

The procedures mentioned above were repeated until three lipsticks colours pigment (orange, red and purple) was obtained from each drying method. The formulation of lipstick with betalains pigment was based on the formula recommended by Kothari et al. (2018) as shown in Table 3.4 with slight modification.

Table 3.4: Lipstick Formula (Kothari et al., 2018).

Ingredients	Composition per lipstick	Importance
Bees Wax	0.32g	Provide hardness and glossy
Carnauba Wax	0.15g	Provide hardness
Candelilla Wax	0.38g	Provide hardness
Shea Butter	1g	Used as emollient
Castor Oil	1.4g	Aid in blending
Jojoba Oil	0.5g	Used as moisturizer
Sweet Almond Oil	0.5g	Used as moisturizer
Fragrance	Few drops	Used to provide fragrance
Vitamin E	Few drops	Used as anti-oxidant
Betalains Powder	2g	Used as natural colourant

The dried powder obtained from spray drying and freeze drying were sieved or ground into fine powder to avoid forming clump in lipstick formulation. The dried solid betalains obtained for the three colours from spray drying and freeze drying were shown in Figure 3.4.

Figure 3.4: Betalains pigment obtained from Spray Drying and Freeze Drying.

The betalains powder were mixed with sweet almond oil and jojoba oil in a small beaker (beaker A) and stir continuously to ensure the pigment well dispersed in the mixture. All waxes, shea butter, and castor oil were measured in another small beaker (beaker B) and heated in water bath at 80°C with gentle stirring until beeswax melts completely. Both mixtures were then mixed together with spatula in water bath until uniform colour was achieved. Then, the mixture was remove from heat source. A few drops of vitamin E and fragrance oil were added to the mixture and the mixture was stirred again to ensure evenly blending of all ingredients.

A little amount of oil was applied on the surface of the lipstick mould to ease the process of removing lipstick from the mould as shown in Figure 3.5. After blending vitamin E and fragrance oil into the lipstick formula, the mixture was poured into lipstick mould. The mould was chilled immediately in the fridge for 15 minutes to solidified the lipstick mixture.

When demoulding, the empty lipstick stick was insert into the lipstick and the lipstick were lift upwards carefully. At this point, the lipstick was done. The betalains lipsticks were stored at room temperature with the storage concept shown in Figure 3.6 under light or dark condition.

Figure 3.5: Lipstick Mould (Walmart, 2019)

Figure 3.6: Demonstration of the lipstick at light or dark condition during storage period.

A short form denoted for each lipstick according to their drying method, storage condition and their colour for simplification. Table 3.5 shows the short form denoted for each lipstick in data tabulation. A total of 12 lipsticks were produced to study the colour stability of betalains.

Table 3.5: Short form of lipstick denoted for each lipstick.

Lipsticks	Spray Dried		Freeze Dried	
	Light	Dark	Light	Dark
Purple	SLP	SDP	FLP	FDP
Red	SLR	SDR	FLR	FDR
Orange	SLO	SDO	FLO	FDO

3.3.7 Colour Stability Test During Storage Period

After formulate betalains lipstick, the lipsticks were placed inside the mini photo studio as illustrated in Figure 3.7 and the colour of each lipstick (tri-stimulus RGB value) were evaluated with the mobile App-Colour Name. The steps of colour analysis using Color Name were illustrated in Figure 3.8. The value of RGB of the betalains lipsticks were constantly observed and recorded throughout the one-month storage period and the RGB data were converted into L*, a* and b* colour properties by ColorMine (Sinatra.F, n.d.) to evaluate colour stability in colour space coordinates with CIE L*a*b* and CIE L*C*h* system.

Figure 3.7: Mini Photo Studio with Betalains Lipstick.

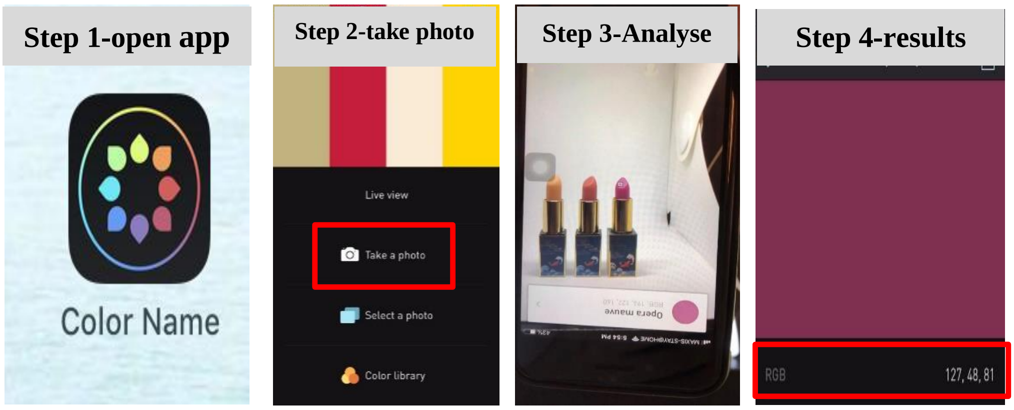

Figure 3.8: The steps of Colour Analysis using Colour Name.

3.3.8 Evaluation of Lipstick

The formulated lipsticks were tested with its spreadability, melting point, breaking point, surface anomalies and skin irritation test. The evaluation test of synthesised lipsticks was compared with the commercial lipsticks.

3.3.8.1 Spreadability Test

Spreadability test were conducted for all lipsticks at room temperature. The lipsticks were applied at least 3 cm onto a paper in order to visually observe the smoothness and uniformity of a protective layer formation from the lipsticks. The lipsticks were evaluated with the following standards listed in Table 3.6.

Table 3.6: Ranking and Standards for Spreadability Test on Lipsticks (Kothari, et al., 2017).

Ranking	Standards
Excellent (E)	No fragment, smooth and uniform surface application without deformation of lipsticks.
Intermediate (I)	Few fragments, uniform application with little deformation of lipsticks.
Unsatisfactory (U)	Many fragments, not uniform application with intense deformation of lipsticks.

3.3.8.2 Melting Point

The melting point of lipsticks were determined by heating lipsticks using double boiler method in water bath. A thermometer was immersed in the water bath to determine to melting point of the lipsticks. The melting point was determined by the thermometer at the moment when the lipsticks started to melt (Setywaty & Meldina, 2018).

3.3.8.3 Breaking Point

Breaking point of lipsticks were determined by the burden of suspended load of 10 gm on the lipsticks. The load was constantly added at 30 s interval. The breaking point of lipsticks were the weight load that causes lipsticks breaks (Varghese, et al., 2017).

3.3.8.4 Surface Anomalies

Surface anomalies analysis was used to determine the surface defect by visual observation. Surface defects such as crystal and fungi formation, wrinkles formation indicated that the lipsticks were contaminated (Shashi, et al., 2017).

3.3.8.5 Skin Irritation Test

The lipsticks were applied on skin and left for 5 minutes. The lipsticks were tested with few respondents with different skin conditions-dry skin, oily skin and sensitive skin (Varghese, et al., 2017).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Colour Stability Analysis upon Storage Period

Figure 4.1 shows the betalains lipsticks produced by spray dried betalains and freeze dried betalains at day zero.

Figure 4.1: A. Lipstick Produced by Spray Dried Betalains at day 0 and B. Lipstick Produced by Freeze Dried Betalains at day 0.

Colour Stability Analysis on betalains lipstick were performed at room temperature for one-month storage period. The colour properties such as Lightness, a*, b*, Chroma (C*), hue angle (h°), total colour difference (ΔE) and colour retention across storage period for each lipstick were tabulated in table A-1 in appendix A while the colour degradation pathway of betalains lipstick were illustrated in Figure 4.2.

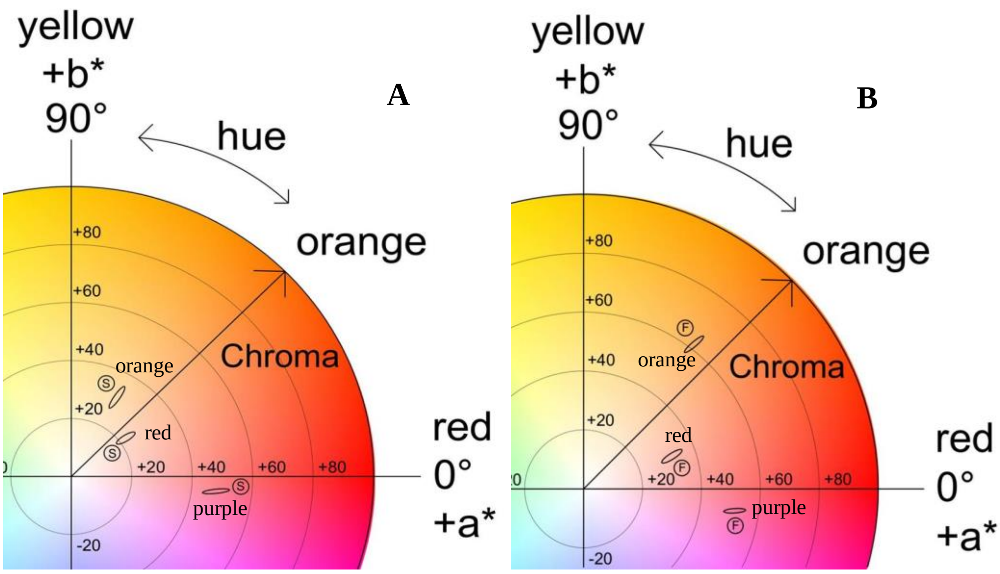

Figure 4.2: Colour Degradation Pathway of Betalains Lipstick in CIE L*a*b* and CIE L*C*h* Colour Space Coordinates in Light Condition A) Spray Dried Betalains Lipstick B) Freeze Dried Betalains Lipstick

The three oval shapes in Figure 4.2A and Figure 4.2B show the movement of colour degradation of orange lipstick (top oval), red lipstick (middle oval) and purple lipstick (bottom oval) in the direction **towards the centre** of colour wheel. The S or F indicate that the betalains pigments were spray dried or freeze dried. The colour degradation pathway of betalains lipsticks in dark condition were similar to the trend of light condition but with significantly shorter movement as colour difference of lipstick in dark condition is hardly noticeable by human eye.

While studying the colour properties of each lipstick throughout the storage period, some generalizations of colour degradation pathway can be observed. The lightness (L*) increased for all lipsticks across storage period. The changes in lightness was significant for lipsticks in light condition while slight changes in lightness was observed for lipsticks in dark condition. This results appear to confirm that light accelerate colour loss of thermos-sensitive natural extract (Azwanida et al., 2015). The higher increment of lightness in spray dried was observed compared to freeze dried method as colour of the spray dried betalains lipsticks is lighter than freeze dried betalains lipsticks.

The a* value (x-axis in Figure 4.2), which indicate the redness of a colour decreased across all lipsticks upon storage accompanied by the gradually increment of L* (Aztatzi-Rugerio et al., 2019). The shifting of a* towards the centre was observed in Figure 4.2 indicates that the colour of the lipstick become less red, lighter and paler. Similar to the changes in lightness, the changes in a* was significant for lipsticks in light condition while slight changes of a* was observed for lipsticks in dark condition. Besides, the reduction of a* was greater with spray dried method was noticed compared to freeze dried method. The may be due to the destruction of pigment during spray dried process of betalains as the process was carried out at very high temperature of 198 °C while freeze dried was performed at extra low temperature which is at -110 °C.

The changes in b* value (y-axis in Figure 4.2) were not statistically significant during storage. The shifting of b* towards the centre was observed with lipstick in orange and red colour indicates the lipstick become less yellow during storage. However, the b* in purple lipstick were fluctuate horizontally. This may be due to the position of the purple hue is located near to the y-axis of the colour space. As mentioned by Moura (2015), a colour cannot be green and red, or blue and yellow at the same time. Thus, the value of b* of the purple lipstick fluctuate around horizontal axis. The changes in b* for spray dried and freeze dried method were similar while the changes of b* of the lipsticks in dark condition was lesser compared to light condition. Even though the b* value fluctuate a little around y-axis, the overall trends were shifting downwards. Thus, the degradation pathway of b* is towards the blue. The shifting is could be related to the theory explained by Herbach et al. (2016) as the ability of betalains to regenerate even after heat treatment. The shifting towards blue may indicate the possibility of regeneration of a darker purple.

Chroma describes the purity of a colour. The higher the Chroma value, the closer the colour to its pure hue. Mixing of white, black or grey light with the pure hue will reduce the its Chroma value (Smith.K, 2019). This is proven in the colour analysis as lightness (addition of white light) increased in all lipsticks across the colour degradation, the Chroma value of all lipsticks decreased was observed in Figure 4.2. Freeze dried orange lipstick had the greatest initial Chroma value of 66.19 as it is nearest to the outer most of colour wheel while lipstick spray dried red lipstick had the smallest initial Chroma value of 25.55 as it is furthest away from the outer most of colour wheel.

The hue angle of the betalains lipsticks were compared with standard reference hue of commercial lipstick as hue of lipsticks did not varied much upon storage. Table 4.1 tabulated the hue angle comparison between commercial lipstick and synthesised lipsticks while Figure 4.3 shows the hue angle for commercial lipstick as standard reference.

Table 4.1: Hue angle comparison with the commercial lipstick (Moura, 2015).

Colour of Lipstick	Initial Hue Angle (°)		
	Commercial Lipstick	Betalains Lipstick (Spray Dried)	Betalains Lipstick (Freeze Dried)
Purple	352.03	353.24	348.03
Red	15.38	34.39	24.66
Orange	21.40	66.88	56.31

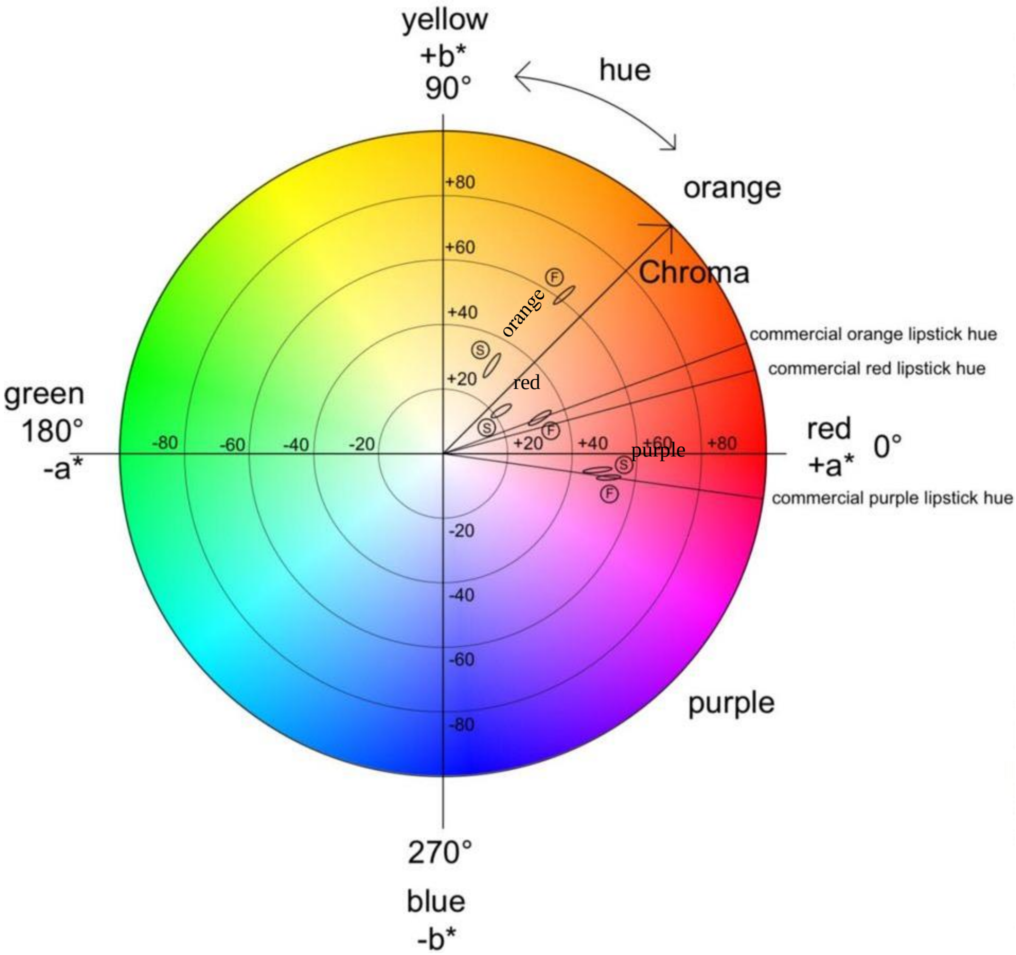

Figure 4.3: Comparison of Hue Angle between Commercial and Synthesised Lipstick.

The hue of common commercial lipstick for purple, red and orange in colour were used as a standard reference to compare with the initial hue obtained from the synthesised lipsticks. The results show that the purple lipsticks produced by spray dried and freeze dried lie closely with the commercial standard. The red lipstick produced by freeze dried lies slightly away from the standard while red lipstick produced by spray dried lies further away from the standard reference. However, a huge gap was observed between the standard reference with the orange lipsticks as the commercial lipstick were darken in colour and small in hue angle. Thus, the hue of purple lipstick and red lipstick produced from freeze dried method are more suitable as colour agent in lipstick production. The total colour difference (ΔE) and colour retention across storage period were shown in Figure 4.4 and Figure 4.5 respectively.

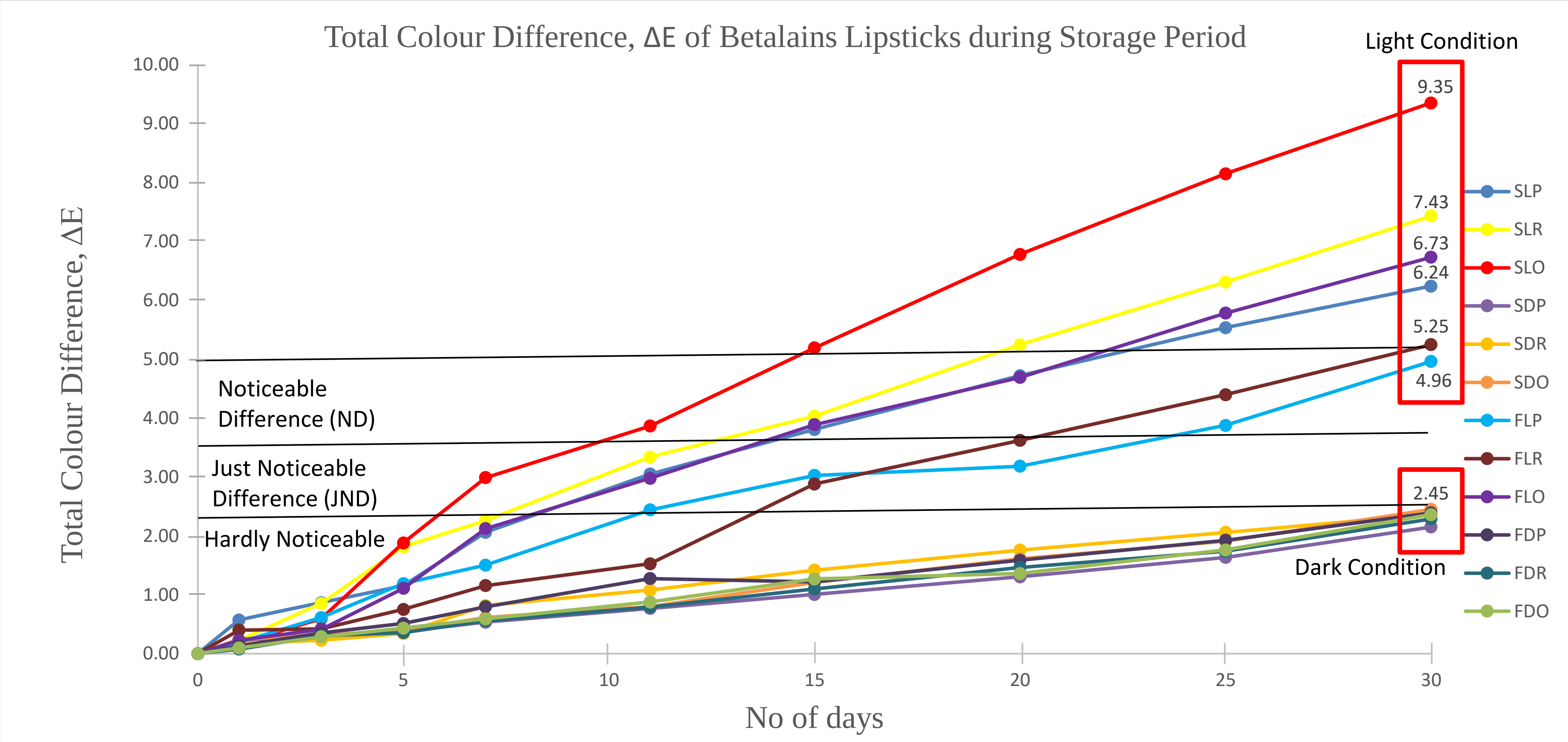

Figure 4.4:Total Colour Difference of Betalains Lipstick against Storage Period.

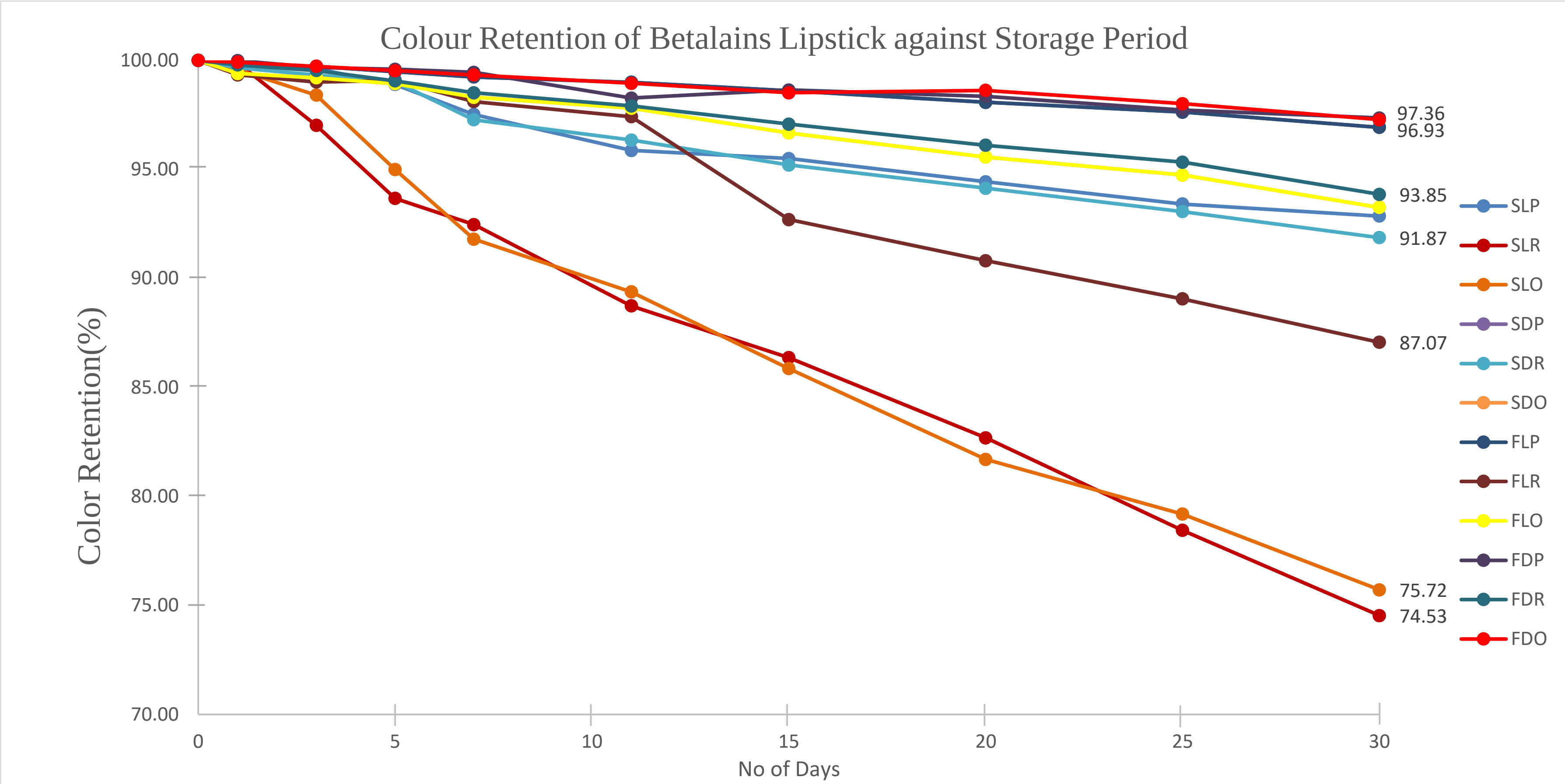

Figure 4.5: Colour Retention of Betalains Lipstick against Storage Period.

The total colour difference (ΔE) are used to quantify the colour changes in numerical term. A Just Noticeable Difference (JND) in a colour can be detected at ΔE about 2.36 while normal observer will notice differences in colour with ΔE at range of 3.5 to 5 (Moura, 2015). In Figure 4.4, it is observed that the ΔE of lipsticks at light condition have greater value than the lipstick at dark condition and all of the lipstick at light condition exceeded the Noticeable Difference (ND) of 5 except for FLP which has a value of 4.96 while SLO has the highest value of 9.35. Meanwhile, all the lipstick at dark condition poses ΔE value less than 2.45 which is close to the JND which indicates that the colour difference of lipstick in dark condition is hardly noticeable by human eye.

The colour retention (%) are similar to total colour difference in opposite way. Total colour difference measure how much colour had loss while colour retention measures how much colour remained in the lipsticks. From the observation of Figure 4.5, SLR and SLO have the lowest colour retention which remain 74.53 % and 75.72 % after one-month of storage time. Moderate retention of colour was found on FLR, SDR, SLP, SDO, FLO, and FDR which have colour retention of 87.07 % to 93.5 % while SDP, FLP, FDO and FDP have the highest colour retention of 96.93 % to 97.36 %. The purple colour in betalains remains the most in the lipstick while orange and red lipstick produced by spray dried reduced significantly.

4.2 Evaluation of Lipsticks Performance

It is very important to ensure the uniformity standard of the produced lipsticks. Hence, the lipsticks produced were evaluated on its spreadability, melting point, breaking point, surface anomalies and skin irritation test and the result were tabulated in Table 4.2.

Table 4.2:Evaluation of Formulated Betalains Lipsticks at room temperature (Azhary et al., 2017).

Evaluation Parameters	Standard Commercial Lipstick	Betalains Lipsticks											
		SLO	SLR	SLP	SDO	SDR	SDP	FLO	FLR	FLP	FDO	FDR	FDP
Spreadability	E	E	E	E	E	E	E	E	E	E	E	E	E
Melting Point (°C)	Above 50 °C	59	58	60	59	60	61	55	55	58	55	57	58
Breaking point (g)	95	105	107	110	105	110	110	110	110	107	105	106	107
Surface Anomalies	No	No	No	No	No	No	No	No	No	No	No	No	No
Skin irritation	No	No	No	No	No	No	No	No	No	No	No	No	No

*All parameters were evaluated in duplicate.

Spreadability: E-Excellent; I- Intermediate; U-Unsatisfactory

The spreadability test all lipsticks were excellent as no fragment upon spreading with no deformation of lipstick was observed. The melting point of a lipsticks indicates the safe limit of storage. The standard melting point of a commercial lipstick is above 50 °C in order to remain its rigid structure and do not melt in room temperature. Since the melting point of each lipstick determined are more than 50 °C, the synthesized lipsticks were consider achieved the requirement for safe limit storage. The breaking point of the synthesized lipsticks were fall on 105 g to 110 g which were slightly higher than the breaking point of a commercial lipstick of 95 g. The ideal softening and rupture point in a lipstick is achieved by holding a good balancing between all ingredients in lipstick formulation. The most tedious part of formulating lipstick is finding appropriate ratio of wax in relation to oil in the lipstick formula. As wax in relation to oil increase, the melting point and the hardness of the lipstick will increase but at the same time, facilitating stick breakage. The results of melting point and breaking point of the synthesized lipsticks show that the formulation of lipsticks were with appropriate wax to oil ratio. For surface anomalies test, no fungi and crystallization were detected in the surface of lipsticks. Lastly, the synthesized lipsticks were applied on skin at back arms and skin behind the ear for 5 minutes to test for skin irritation as the skin area at those locations are the most appropriate location to test for skin irritation. The results showing no skin irritation was observed as all ingredients used in the formulation are natural ingredients.

Besides the evaluation mentioned above, the lipsticks were evaluated on color uniformity by visual inspection. The color of the synthesized lipsticks are uniform and stable under room temperature. However, the lipstick color produced by freeze dried betalains was darker than spray dried betalains and closer to the color of commercial lipstick. The homogeneity of spray dried betalains lipsticks surface were smooth with no coarse grains while coarse grains were found in freeze dried lipsticks. This is due to the fact that freeze dried method produced porous and sticky flakes with high sugar content rather than fine solid powder. The flakes produced by freeze dried is hard to ground into fine solid by dry mill when formulating lipsticks. Lastly, the odor of all lipsticks are pleasant throughout the storage period.

4.3 Feasibility of Spray Dried Betalains and Freeze Dried Betalains as Natural Colorant in Cosmetic Industry

The mechanism of degradation of betalains had been studied. Heat was found not significantly change the colour of the betalains as the degradation of betalains were mainly due to the exposure of light (Herbach et al., 2006). When the betalains solution were left at second day, the hue of the solution was changed from red-purple tone to dark red tone. The loss of the purple tone is the strong evidence to prove that betalains is more sensitive towards light than heat. Azwanida et al. (2015) reported that deterioration of betalains at 80°C were stable. Hence, betalains is suitable as the lipstick mixture were heated up to 80°C when formulating lipstick. Table 4.3 shows the comparison of spray dried betalains and freeze dried betalains from different aspects.

Table 4.3: Comparison of Spray Dried Betalains and Freeze dried Betalains with different aspects.

Aspect	Spray Dried Betalains	Freeze Dried Betalains
Colour	Lighter	Darker
Solid form	Fine powder	Porous flakes
Pigmentation	low	low
Cost	low	high
Time	fast	slow
Yield	low	high
Mode	Continuous	Batch

By comparing from the color aspect, freeze dried betalains are more preferable as the color produced are darker and similar to the color of the commercial lipsticks as low temperature at vacuum condition works better in preserving the colour of the betalains while betalains produced by spray dried are lighten and paler in colour. While from the view of solid product formed from both method, fine solid powder produced by spray dried method are preferable in formulating lipstick to achieve smoother, no-grainy and homogeneity in lipsticks. The freeze dried betalains are produced in the form of flakes and it is hard to ground into fine powder for homogenous dispersion of colorant across the lipsticks thus, formed coarse grains (tiny flakes) in lipsticks. As spray drying dehydrate the moisture in betalains by hot air at relatively high temperature, causing the breakdown of sugar component in betalains, hence, spray drying method is more suitable as it is capable of producing non-sticky and fine betalains powder for lipstick production. In the aspect of pigmentation to lips, both betalains were suggested incorporate into lip balm formula rather than a pigmented lipstick formula as low pigmentation on lip were observed but a shining protective layer are formed uniformly on lips.

According to Ravinchandran et al. (20194a), the cost of freeze drying process was reported 30 to 50 times more expensive than spray drying process thus, spray drying process is more cost-effective. Moreover, spray drying process operates in continuous mode and the product can be obtained within a very short period of time (minutes) while freeze dried process required more than 24 hours to fully dehydrate the content in batch mode. However, the deteriorating and microbiological activity in betalains are arrested at low temperature in freeze drying thus produced higher quality product and retain the nutrition value of betalains. The yield of betalains from spray dried are lower than freeze dried method as some of the betalains stick on the wall of cyclone and drying chamber, leads to reduction in yield. During freeze drying, the betalains extract were frozen in the centrifuge tube and the end product was collected within the same bottle hence the product loss is insignificant. Although the betalains were found better stabilized using freeze dried method with higher yield, the simplicity of spray dryer is suggested as it offers low production cost, good encapsulation efficiency, provide wider choice of wall material, and provide great stability for mass production in continuous mode.

4.4 Fourier Transfrom-Infrared Spectroscopy (FTIR)

Fourier Transform-Infrared Spectroscopy (FTIR) of Spray Dried Betalains powder and Freeze Dried Betalains Powder were illustrated as shown in Figure 4.6 and Figure 4.7.

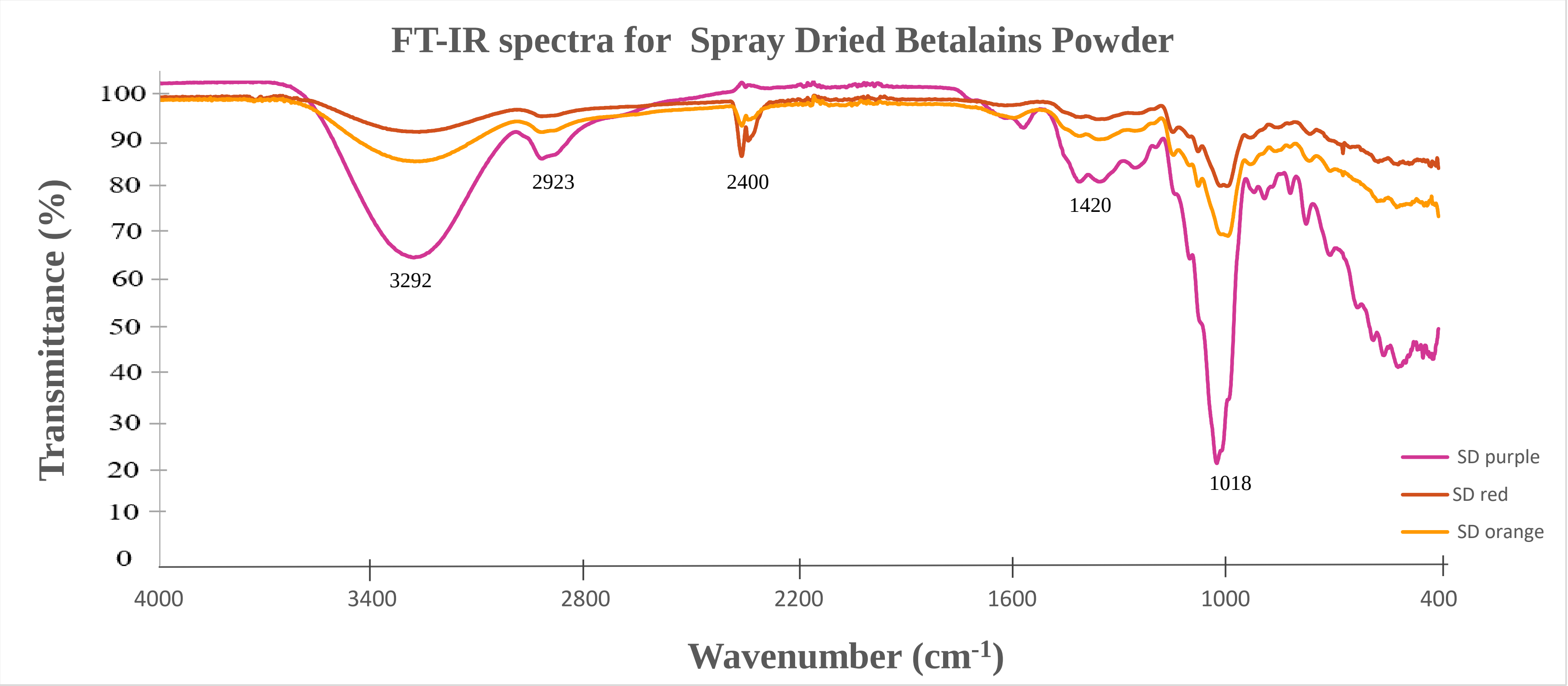

Figure 4.6: FT-IR spectra for Spray Dried Betalains Powder

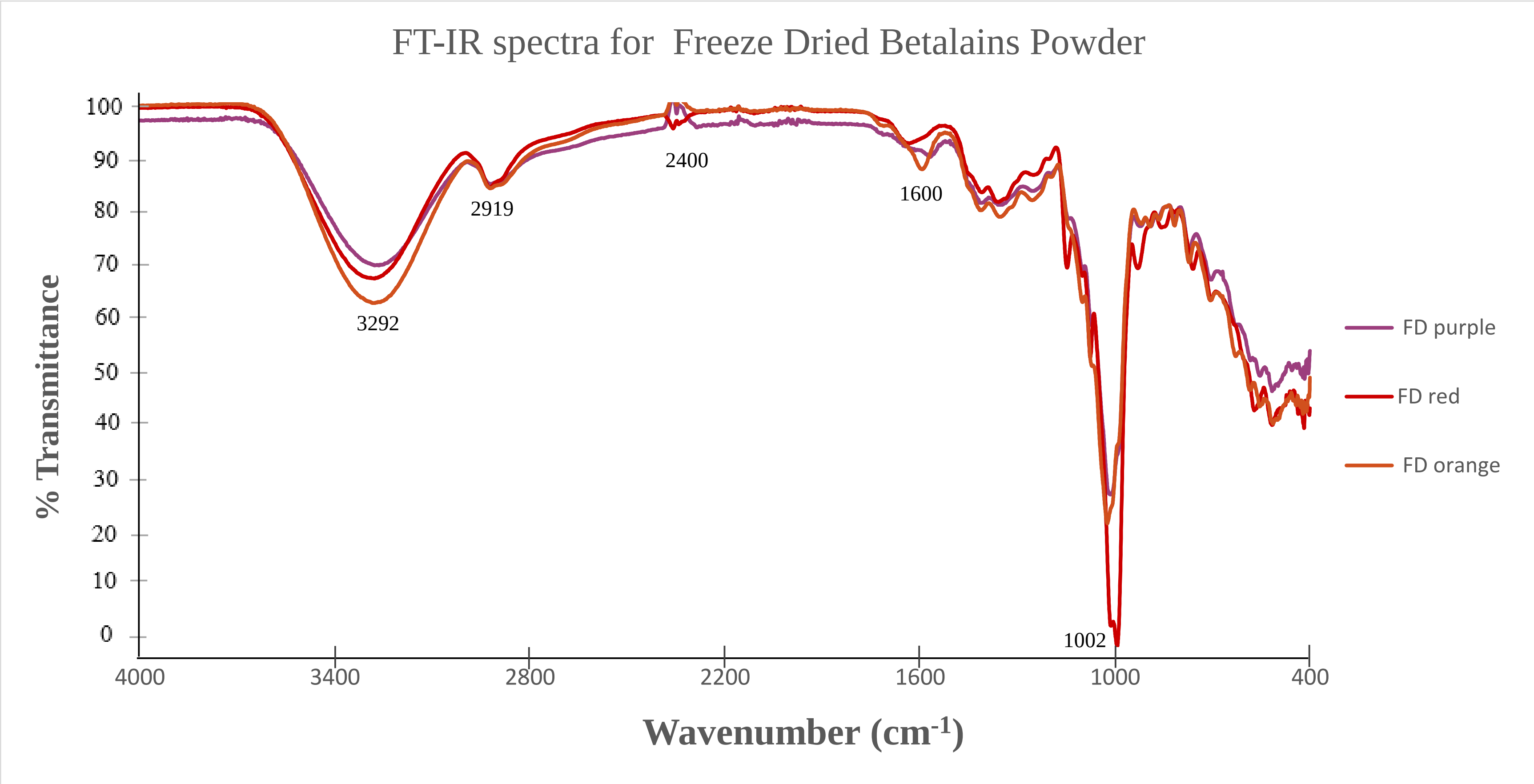

Figure 4.7: FT-IR spectra for Freeze Dried Betalains Powder.

The concentrated betalains extract were mixed with maltodextrin and spray dried or freeze dried to solid betalains. FT-IR analysis was performed on the solid betalains with the mixture of maltodextrin from wavelength of 400cm^{-1} to 4000cm^{-1} as shown in Figure 4.6 and Figure 4.7. The functional groups presence in the sample was identified from this analysis. The aim of this study is to identify the functional groups of betalains that are presence in the sample.

By comparing the overall trend of the graphs, the trend and location of peaks for both graphs were similar. The peak at 1002 cm^{-1} to 1023 cm^{-1} represents the C-O stretching vibrations while the peak 1410 cm^{-1} to 1415 cm^{-1} correspond to the aromatic group containing C-C stretch stretch (Santiago-Adame et al., 2015). The peak at 1564 cm^{-1} to 1589 cm^{-1} corresponds to C=C double bond, C=O and C=N stretching vibrations in structure of betalains. The peaks of 2913 cm^{-1} to 2919cm^{-1} indicates C-H stretch vibration while the absorption band at 3270 cm^{-1} to 3292 cm^{-1} indicates the characteristic of O-H stretch and N-H stretch.

The most active components in betalains such as C=O and O-H usually found in carboxylic acid. The carboxyl group promotes strong hydrogen bonding thus promote a large shift towards lower frequencies. The COOH stretching confirms the presence of carboxyl group in Betalains (Syafinar et al., 2015).

The major difference between freeze dried sample and spray dried sample are found to be at wavenumber of 2347 cm^{-1} to 2358 cm^{-1} corresponds to the $–CH_2$ and $–CH_3$ in aromatic phenolic compounds found in maltodextrin (Acordi Menezes et al., 2018) as the proportion of Maltodextrin used in freeze dried method were more than spray dried method. The lowering band were observed in freeze dried as more interaction between the maltodextrin and betalains. The bands between 900 cm^{-1} to 1100 cm^{-1} were interpreted as C-O vibrations and C-O-C stretching vibrations of glucose found in Maltodextrin. Thus the presence of functional group of betalains and maltodextrin was confirmed in the test sample.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The aim of this study was to evaluate the feasibility of betalains to be used as natural colorant in lipstick application and study its stability during storage period. The spray dried and freeze dried betalains incorporated into lipstick formula provide attractive colour on lipsticks ranging from purple to orange. The betalains lipsticks stored under dark condition poses higher stability than the betalains lipstick stored under light condition as light accelerate colour loss in betalains. The L* and Chroma of lipstick increases upon storage while the a* and b* decreases upon storage. The synthesised lipsticks, FDP, FDR and SDP have the hue similar to commercial lipstick. The betalains lipsticks at light condition show significant total colour difference while the total colour difference of betalains lipsticks at dark condition is hardly notice by human eye. The synthesised lipstick, SLR has the lowest colour retention of 74.53 % after one-month storage period while FDP has the highest colour retention of 97.36 %.

The formulated lipsticks show positive results on spreadability, melting point, breaking point, surface anomalies, and skin irritation test. This indicate that the lipstick formula was close to the standard of a commercial lipsticks. Spray dried betalains lipsticks achieved colour uniformity, homogeneity and perfume stability. However, freeze dried lipstick are poor in homogeneity (form coarse grain in lipstick) as freeze dried betalains is flakes rather than fine solid powder.

All lipsticks showed remarkable stability even in accelerate environment (light condition). Although freeze dried method found better in stabilizing the betalains, spray dried method is preferable as it is low cost, less time consuming and available for large scale production. Due to the low pigmentation of betalains lipstick, the betalains is suggested to incorporate into lip balm formula instead of lipstick formula. Overall, the results show that betalains is potential to be used as alternative to synthetic dye in cosmetic industry.

5.2 Recommendations for future work

Due to the time constraints and the limitations of the facilities, the project was not being studied intensively. Apart from that, there were some abnormalities during the study were being ignored. Most of the abnormalities were due to the human error and the limitations of the equipment. Hence, in order to further improve the research outcome, the following recommendation were suggested.

1. The red dragon fruit used in the research should be obtained from the same batches. This is on account of the fact that the maturity and of the red dragon fruit and the species of red dragon fruit may vary as it is obtained from different seller (Ding et al., 2009).
2. To improve extraction of betalains, Ultrasonic Assisted Extraction (UAE) method is recommended as ultrasonic will cause a cavitation effect and break down the cell wall to release betalains, thus, maximising the extraction yield (Asra et al., 2019).
3. The combination of 20 g of maltodextrin with 0.5 g of xanthan gum to 100 ml of betalains extract is suggested as the recovery of betalains was higher than maltodextrin alone (Ravichandran et al., 2014b).
4. Three roll mill were suggested to grind the betalains pigment for homogenous dispersion of pigment (Torrey Hill Technologies, 2013).
5. A colorimeter is recommended to test the colour properties of the lipsticks for more reliable results (Aztatzi-Rugerio et al., 2019).
6. Use carotenoids from carrot or papaya (oil-soluble natural pigment) to make orange lipstick (Swati et al., 2013).
7. Add surfactant such as acacia and ripe fruit powder of shikaki to overcome the precipitation of colour in lipsticks (V et al., 2014).
8. If time constraint, the degradation of betalains from purple-red tone to orange-red tone can be achieved by heating at 75 °C for 120 min (hue° changed from 359° to 62°) (Aztatzi-Rugerio et al., 2019).

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APPENDICES

APPENDIX A: Colour Properties of Each Betalains Lipsticks across One-Month Storage Period.

Table A-1: Colour Properties of Each Betalains Lipstick across One-Month Storage Period.

Days	**0**	**1**	**3**	**5**	**7**	**11**	**15**	**20**	**25**	**30**
Spray Dried Purple Lipstick, Light (SLP)										
Lightness	51.6	51.3	51.6	51.92	52.39	52.71	53.45	54.12	54.68	55.3
a*	51.61	51.87	51.31	50.92	50.15	49.23	48.96	48.35	47.77	47.43
b*	-6.12	-6.53	-6.92	-6.99	-7.34	-7.67	-8.13	-8.42	-8.65	-8.91
Chroma	51.97	52.28	51.77	51.40	50.68	49.82	49.63	49.08	48.55	48.26
Hue angle	353.24	352.82	352.32	352.18	351.67	351.14	350.57	350.12	349.74	349.36
ΔE	0.00	0.57	0.87	1.16	2.06	3.05	3.81	4.72	5.53	6.24
Color Retention (%)	100.00	100.59	99.62	98.90	97.52	95.87	95.50	94.43	93.41	92.86
Spray Dried Red Lipstick, Light (SLR)										
Lightness	59.9	59.7	60.23	60.56	60.98	61.5	61.77	62.61	62.87	63.44
a*	21.08	21	20.56	20.01	19.75	18.99	18.61	17.82	16.93	16
b*	14.43	14.5	13.84	13.12	12.96	12.38	11.85	11.34	10.72	10.32

Days	0	1	3	5	7	11	15	20	25	30
Chroma	25.55	25.52	24.78	23.93	23.62	22.67	22.06	21.12	20.04	19.04
Hue angle	34.39	34.62	33.95	33.25	33.27	33.10	32.49	32.47	32.34	32.82
ΔE	0.00	0.23	0.85	1.82	2.26	3.34	4.03	5.25	6.31	7.43
Color Retention (%)	100.00	99.90	97.02	93.67	92.47	88.74	86.36	82.68	78.44	74.53
Spray Dried Orange Lipstick ,Light (SLO)										
Lightness	61.54	61.48	61.57	61.06	61.58	61.59	62.35	62.89	64.58	64.65
a*	14.24	14.2	13.89	13.5	13.34	13	12.33	11.45	10.98	10.42
b*	33.36	33.21	32.88	31.7	30.51	29.7	28.6	27.33	26.54	25.41
Chroma	36.27	36.12	35.69	34.45	33.30	32.42	31.14	29.63	28.72	27.46
Hue angle	66.88	66.85	67.10	66.93	66.38	66.36	66.68	67.27	67.52	67.70
ΔE	0.00	0.17	0.59	1.88	2.99	3.86	5.19	6.78	8.15	9.35
Color Retention (%)	100.00	99.58	98.40	94.99	91.80	89.38	85.86	81.69	79.18	75.72
Spray Dried Purple Lipstick, Dark (SDP)										
Lightness	51.6	51.82	51.47	51.57	51.43	51.36	51.22	51.18	51.07	52.36
a*	51.61	51.52	51.45	51.31	51.18	51.03	50.82	50.53	50.27	49.87
b*	-6.12	-6.14	-6.27	-6.34	-6.39	-6.56	-6.61	-6.72	-6.89	-7.13

Days	0	1	3	5	7	11	15	20	25	30
Chroma	51.97	51.88	51.83	51.70	51.58	51.45	51.25	50.97	50.74	50.38
Hue angle	353.24	353.20	353.05	352.96	352.88	352.67	352.59	352.42	352.20	351.86
ΔE	0.00	0.24	0.25	0.37	0.54	0.77	1.00	1.30	1.63	2.15
Color Retention (%)	100.00	99.83	99.73	99.48	99.24	99.00	98.61	98.08	97.63	96.93
Spray Dried Red Lipstick, Dark (SDR)										
Lightness	59.9	59.77	59.98	60.03	60.08	60.24	60.46	60.61	60.74	60.99
a*	21.08	21.05	21.01	20.98	20.72	20.54	20.31	20.16	19.95	19.54
b*	14.43	14.32	14.23	14.13	13.72	13.56	13.38	13.11	12.93	13
Chroma	25.55	25.46	25.38	25.29	24.85	24.61	24.32	24.05	23.77	23.47
Hue angle	34.39	34.23	34.11	33.96	33.51	33.43	33.38	33.04	32.95	33.64
ΔE	0.00	0.17	0.23	0.34	0.82	1.08	1.42	1.76	2.06	2.37
Color Retention (%)	100.00	99.66	99.33	99.02	97.28	96.35	95.21	94.14	93.06	91.87
Spray Dried Orange Lipstick, Dark (SDO)										
Lightness	61.54	61.54	61.56	61.56	61.58	61.59	61.61	61.61	61.62	61.64
a*	14.24	14.25	14.25	14.12	13.97	13.88	13.72	13.52	13.46	13.21
b*	33.36	33.12	33.04	32.99	32.81	32.65	32.27	31.92	31.62	31.14

Days	0	1	3	5	7	11	15	20	25	30
Chroma	36.27	36.06	35.98	35.88	35.66	35.48	35.07	34.67	34.37	33.83
Hue angle	66.88	66.72	66.67	66.83	66.94	66.97	66.97	67.04	66.94	67.01
ΔE	0.00	0.24	0.32	0.39	0.61	0.80	1.21	1.61	1.91	2.45
Color Retention (%)	100.00	99.40	99.20	98.93	98.31	97.81	96.67	95.57	94.74	93.26
Freeze Dried Purple Lipstick, Light (FLP)										
Lightness	43.01	42.95	43.02	43.66	43.86	44.85	45.21	45.21	45.67	46.03
a*	52.21	52.13	51.87	51.23	50.98	50.63	50.22	49.99	49.47	48.34
b*	-11.07	-10.91	-10.56	-10.89	-11.21	-11.34	-11.67	-11.67	-11.73	-11.79
Chroma	53.37	53.26	52.93	52.37	52.20	51.88	51.56	51.33	50.84	49.76
Hue angle	348.03	348.18	348.49	348.00	347.60	347.38	346.92	346.86	346.66	346.29
ΔE	0.00	0.19	0.61	1.19	1.50	2.44	3.03	3.18	3.88	4.96
Color Retention (%)	100.00	99.79	99.18	98.13	97.80	97.22	96.60	96.18	95.26	93.23
Freeze Dried Red Lipstick, Light (FLR)										
Lightness	54.29	54.32	54.5	54.72	54.91	55	55.12	55.42	55.8	56.09
a*	32.08	32	31.8	32	31.76	31.65	30.12	29.57	29.12	28.61
b*	14.73	14.34	14.5	14.12	13.81	13.45	12.79	12.38	11.85	11.23

Days	0	1	3	5	7	11	15	20	25	30
Chroma	35.30	35.07	34.95	34.98	34.63	34.39	32.72	32.06	31.44	30.74
Hue angle	24.66	24.14	24.51	23.81	23.50	23.02	23.01	22.72	22.14	21.43
ΔE	0.00	0.40	0.42	0.75	1.15	1.53	2.88	3.62	4.40	5.25
Color Retention (%)	100.00	99.34	99.01	99.08	98.11	97.42	92.70	90.81	89.06	87.07
Freeze Dried Orange Lipstick, Light (FLO)										
Lightness	45.19	45.3	45.55	45.89	46.12	46.49	46.92	47.36	48.12	48.88
a*	36.72	36.59	36.91	36.21	35.76	35.19	34.56	33.94	33.2	32.86
b*	55.07	54.92	55.01	54.38	53.42	52.87	52.34	51.98	51.54	50.97
Chroma	66.19	65.99	66.25	65.33	64.28	63.51	62.72	62.08	61.31	60.64
Hue angle	56.31	56.33	56.14	56.34	56.20	56.35	56.56	56.86	57.21	57.19
ΔE	0.00	0.23	0.41	1.11	2.12	2.98	3.89	4.69	5.78	6.73
Color Retention (%)	100.00	99.70	100.08	98.71	97.12	95.95	94.76	93.79	92.62	91.62
Freeze Dried Purple Lipstick, Dark (FDP)										
Lightness	43.01	43.14	43.27	43.38	43.51	43.78	43.91	44.24	44.42	44.85
a*	52.21	52.2	52.01	51.94	51.81	51.22	51.42	51.25	50.92	50.71
b*	-11.07	-11.09	-11.19	-11.3	-11.54	-11.29	-11.3	-11.35	-11.29	-11.34

Days	**0**	**1**	**3**	**5**	**7**	**11**	**15**	**20**	**25**	**30**
Chroma	53.37	53.37	53.20	53.15	53.08	52.45	52.65	52.49	52.16	51.96
Hue angle	348.03	348.01	347.86	347.73	347.44	347.57	347.61	347.51	347.50	347.39
ΔE	0.00	0.13	0.35	0.51	0.79	1.27	1.22	1.59	1.92	2.39
Color Retention (%)	100.00	99.99	99.68	99.60	99.45	98.27	98.64	98.35	97.73	97.36
Freeze Dried Red Lipstick, Dark (FDR)										
Lightness	54.29	54.31	54.37	54.41	54.46	54.53	54.62	54.72	54.79	54.92
a*	32.08	32.01	31.83	31.76	31.64	31.47	31.21	30.94	30.68	30.25
b*	14.73	14.71	14.88	14.63	14.43	14.29	14.15	13.92	13.83	13.51
Chroma	35.30	35.23	35.14	34.97	34.78	34.56	34.27	33.93	33.65	33.13
Hue angle	24.66	24.68	25.06	24.73	24.52	24.42	24.39	24.22	24.27	24.07
ΔE	0.00	0.08	0.30	0.36	0.56	0.79	1.10	1.46	1.74	2.29
Color Retention (%)	100.00	99.80	99.54	99.06	98.51	97.91	97.08	96.11	95.33	93.85
Freeze Dried Orange Lipstick, Dark (FDO)										
Lightness	45.19	45.27	45.41	45.49	45.58	45.72	45.99	46.15	46.36	46.72
a*	36.72	36.7	36.64	36.52	36.46	36.28	36.15	36.47	35.97	35.76
b*	55.07	55.02	54.91	54.83	54.71	54.53	54.27	54.14	53.99	53.55

Days	0	1	3	5	7	11	15	20	25	30
Chroma	66.19	66.14	66.01	65.88	65.75	65.50	65.21	65.28	64.87	64.39
Hue angle	56.31	56.30	56.29	56.33	56.32	56.36	56.33	56.03	56.33	56.27
ΔE	0.00	0.10	0.28	0.43	0.59	0.88	1.27	1.36	1.76	2.36
Color Retention (%)	100.00	99.92	99.73	99.53	99.33	98.95	98.52	98.62	98.01	97.28