Study of Extraction Method and The Stability of Red Pigment From Various Natural Sources for Food Colouring

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ABSTRACT: Nowadays, colorants, additives, or supplements used in food industry, cosmetics and pharmaceuticals are came from various kinds of pigments. However, synthetic pigments colorant has adverse effect that cause toxicity and carcinogenity in the human body. This will affect the usage of synthetic pigments in the industry. Therefore the natural pigments become the best replacement for synthetic pigments. The objectives of this research is aimed to study the extraction method of red pigment from natural sources dragon fruit, roselle, and red grapes. To study the effect of different storage parameters (time, temperature, taste, and colour) on the stability of the red pigment incorporated in food product and to study the effect of microencapsulation of red pigment extracted. In extraction of powder, Roselle with distilled water has 22.1 g of powder whereas roselle with ethanol has 6.1 g only. For Grape skins, the quantity of the powder of roselle with distilled water in greater than in roselle with ethanol which is 21 g and 0.73 g respectively. Next, for dragon fruits skin, the quantity of the powder is also comparable where with distilled water is 14.5 g and solvent is 1.77 g. A muffin is cooked and the extracted food dye is fused into the muffin. The resulted muffin will be tested through several tests such as light presence test, storage time test, temperature test, and taste and colour test. The stability test of coloured muffin temperature test is assumed that the muffin placed at 4^{o}C surrounding stand a better chance of surviving longer than the muffin placed at 60^{o}C. As for the presence of light test, the expected result is the muffin placed in dark place lasts longer. Last but not least, for the taste and colour test for coloured muffin, the covered muffin at a dark space will be longer lasting compared to the muffin exposed to an open space.

KEYWORDS: dragon fruit, roselle, red grapes, anthocyanin, natural food colouring, stability tests

1. INTRODUCTION

For long time ago years, both synthetic pigment colorant and natural pigment colorant have been broadly used in many fields such as food colouring, textile industries, agricultural practices and researches, water science and technology (Tibor, 2007). Nevertheless, the synthetic pigment colorant has adverse effect to human health in food industry such as toxicity and carcinogenity. The example of synthetic pigment is tartrazine (E102) and sudan red. (K. K. Wo et al., 2011). As a replacement for synthetic pigment colorant, natural pigment colorants from natural sources came into considerations especially from plants, food, and fungi. Natural colorants and pigments from plants are widely use in colouring food, beverages, confectionery, and bakery products. Colours of plant source are environmentally friendly and non-toxic and therefore it was preferred rather than synthetic colours. Natural colours that provided by plant pigment to food can be grouped into four primary classes which are chlorophylls, carotenoids, flavonoids/anthocynins and betalains. (A. Shamina et al., 2007). In this study, only anthocynins and betalains involved. Betalain is water-soluble pigment and can be classified into two main groups which is betacynin and betaxanthin. Betacynin is will give out red-violet color whereas betaxanthin is will give the yellow-orange color. The potential sources of betacynanin are red beet, cactus pear and pitaya. Pitaya is also known as Dragon fruits. But in this experiment, pitaya where choose because it is easily to get in the Local Supermarket. In addition, dragon fruits alson rich in betalains which similar of colour pigment founds in beetroot. Beetrot has been most important betalain source for natural red colouring. However, high nitrate concentration in the beetroot will give a carcinogen effect to human. Thus, this is the main reason why beetroot is unselected rather than dragon fruits. (R. Zuliana et al., 2008). Betalains have anti-oxidant properties which can prevent oxidation. This anti-oxidant properties is important during the extraction process and the storage of pigment. (Sri Priatni et al., 2015)

Besides, Anthocyanin is also same as betacyanins. It is a pigment that is found in many plants, fruits and flowers. This pigment is responsible for the colours that we can see in all plants, fruits and flowers. These intensely coloured water-soluble pigments are responsible for all the pink, magneta, red, violet, and blue colours in the petals, leaves, and fruits of higher plants (Mazza and Miniati, 1993). Anthocyanins are part of a very large and widespread group of plant components known in a group as flavonoids, but they are differ from other flavonoids by strongly absorbing visible light. Stability of color of anthocyanins depends on a combination of many factors including structure of anthocyanins, pH, temperature, oxygen, and light and water activity. (Selim, K. A et al., n.d). Anthocyanin is also a respectable source of natural anti-oxidant, but they are reasonably unsteady during processing and storage (Tsai et al., 2005). This pigment is unsteady and can be destroyed easily by a number of factors such as temperature, light and PH value. (Brownmiller et al., 2008; Buckow et al., 2010). In the development of science and technology, there are many synthetic food colourants are widely used. These synthetic food colourants are incorporated to recover colour loss and to enhance the appearance of food products. But sadly, they are gradually eliminated due to discovery of harmful effect to human health by using synthetic food colourant. (Wissgott and Bortlik, 1996).

Therefore, there are some limitations to use natural food colorants as a replacements of synthetic food colorants such as there are low stability in the food processing procedures, formulation and storage conditions and also give an undesirable odour or flavour to the food. (A. Shamina et al., 2007). The objectives of this research is aimed to study the extraction method of red pigment from natural sources dragon fruit, roselle, and red grapes. To study the effect of different storage parameters (time, temperature, taste, and colour) on the stability of the purple pigment incorporated in food product and to study the effect of microencapsulation of purple pigment extracted.

2. LITERATURE REVIEW

FOOD COLORING

The acceptable of food is influences by its attractive colours. Based on the colour several judgements like immaturity/ripe/overripe, burnt/uncooked, safe /unsafe, fresh/ spoiled, taste good/ bad are made. (K. Kandansamy et al., 2012). Colour become the crucial than any other factor that influences the acceptance of products by consumers. Colour is often seen as the same with quality and is used as a strong display of product safety and value. Foods with characteristic appeal and colour are generally preferred. Based on James C. Griffiths, 2005, studies has been done to show that colour predetermines our expectations of flavor and taste. Consumers recognize that yellow colour always goes with "lemon" and pink colour goes with "grapefruit." The opinion of consumers will changes if the colours being reversed. Consumers either wrong interpreted yellow tangerine flavor and orange raspberry flavor or judge them low quality to the right match.

Besides, colour also affects the apparent level of sweetness. For example, a strongly red-coloured strawberry-flavored drink will be sweeter than a less strongly coloured version based on consumer's logic. Colour is an important part in enhancing foodstuffs, constituting one of the major dietary additives. The food industry has consequently used to enhancing or restoring the colour of foods to increase consumer interest and acceptability of the foods. Subsequently, over hundred years ago, colour has come to play a noticeable role in things important to humans, food, medicine and physical appearance. (A. Shamina et al., 2007).

HISTORY OF FOOD COLOURING

The ancient history of the use of natural dyes written ages back to 2600 BC in China and addition of colourants to foods is reported in Europe during the Bronze Age. Around 1500 BC in Egyptian cities, it is also reported that candy makers used to add natural extracts and wine to improve the appearance of the candies. (Chaitanya Lakshmi, G., 2014). Besides, Indus Valley era has used natural dye as early as 2500 BC. Saffron and Henna was used even before 2500 BC as stated in the Bible. The ancient populations of the island of Great Britain were the first to use the blue dye known as woad, which might have in wild conditions originated from Palestine. While dyes have been discovered out of the blue, their use has become a part of human's customs in the modern world and the art of dyeing has spread widely with the expansion of cultivation. (A. Shamina et al., 2007).

There are some of the well-known dyes used in prehistoric times such as a red dye obtained from the roots of Rubia tinctorium L. or known as dyer's madder; blue indigo dye from the leaves of indigo or as known as its specific name Indigofera tinctoria L.; and yellow dye which have two sources which is from the stigma of saffron (Crocus sativus L.) and from the rhizomes of Curcuma longa L. which known as turmeric (A. Shamina et al., 2007). The first artificial organic chemical colour dye mauvine was discovered by Sir William Henry Perkin way back in 1856. Mauvine also known as aniline purple and Perkin's mauve. The beginning of the 1900th years, it was remarked for the mass of production and recovery of synthetic colors from the petroleum derived products like aniline, therefore they were called 'coal-tar' colours because coal is the starting materials for this products. (Chaitanya Lakshmi, G., 2014)

SYNTHETHIC FOOD COLOURING

Synthetic colours remain the most popular type of food colourings because they give better brighter colours, more uniform, better morphology, and of greater dyeing strength, include a broader range of shades, and are much cheaper than natural color derived from nature sources. The disadvantage to working exclusively with synthetic colours is use restrictions based on amounts added via good manufacturing practices and the recognized "baggage" the synthetics add to an ingredient label. In addition, the synthetic colourant also has adverse effect to human health in food industry such as toxicity and carcinogenity. The example of synthetic pigment is tartrazine (E102) and sudan red. (K. K. Wo et al., 2011).

To ensure health of public protected from toxic impurities, each batch must be strictly inspected and certified. It is restricted to be used in foods and it must be stored separately from certified batches while waiting for certification to complete. Upon certification, the Food and Drug Administration issues a certificate with a unique lot number and allows the industrial or common colour name to be changed to the mandatory Federal Food, Drug, and Cosmetic Act designation, such as FD&C Red No. 40. (Griffiths, 2005)

Both domestic and foreign manufacturers applies to certification, every "lot" of colour necessary to be submitted as a batch sample to FDA and extensively analyzed by advance procedures to certify that it meets required specifications for residual contaminants, heavy metals, pesticides, and unreacted contaminants. However, if a colour requiring certification has not been certified and is used in food, the food will be considered as contaminated and the food need to be removed and disposed.

Based on Griffiths, 2005, over 17.2 million pound and more of synthetic food colours were certified in 2004 (FDA, 2004). FD&C Red No. 40 and FD&C Yellow No. 5

are the most popular certified food colour. The acronym FD&C, D&C and Ext. D&C shows different meaning. For FD&C acronym, it is for use in colouring foods, drugs, and cosmetics which is approved by FDA. Colours in a more limited category, D&C, are considered only safe to use with drugs and cosmetics. Lastly, colours in a third category, Ext. D&C, are only considered for external use which can't to be taken orally or applied to mucous membranes. It is only certified in drugs and cosmetics on external use.

NATURAL FOOD COLOURING

Natural food colour can be defined as any dye, pigment or any other substance found or extracted from plant, animal, insect, algae, mineral or other sources that have ability of colouring food, drug, and cosmetics. By conventional methods, the colours can be extracted from a variation of sources such as seeds, fruits, bark, leaf, root, stem, wood flower and whole plant. The natural colour is being biological, it is also called as "biocolours". (A. Shamina et al., 2007)

The presence of natural pigments such as carotenoids, chlorophylls, myoglobins and anthocyanins are the results of the natural colour of foods and modification of chemical during the processing of natural constituents of foods. (A. Shamina et al., 2007). A natural colour can be advanced by manipulation of certain suitable factors such as pH, heat, light, storage and other ingredients. In food and beverages, the spectrophotometers range used are 10-500ppm. (A. Shamina et al., 2007). Natural colours need to be used at higher levels than artificial colours. This mean that natural colours need to fight with the strong and steady characteristics of synthetic colours. Some of disadvantages, they may all of a sudden change the texture, taste or smell of the food. Besides, they are less stable and less steady. In addition, appearance of natural colours are often duller, more pastel, and slightly change of pH, salts, vitamins, flavors, and other factors can easily affected the food matrix. Besides, natural colours are also tend to be polluted with unwanted trace metals, insecticides, herbicides, and bacteria.

However, natural colours are recognize by the consumer as being less harmful to health than the petroleum synthetics which derived from coal tar, examples like tartrazine, indigotine, and erythrosine. Natural colours add a class to food products marketed as "natural", "save" and "organic" that the increasing people of health-conscious consumers search for. (Griffiths, J. C., 2005). From A. Shamina et al., 2007, the natural colourant area can be divided into anthocyanins, betalains, chlorophylls, carotenoids, flavonoids, polyphenols, Monascus, haems, quinones, biliproteins, safflower, turmeric and miscellaneous. All are in different groups of chemical compounds, which may be used directly as colourants, or may be chemically modified to produce different colour or increased stability. All method usually involve of collection, extraction, purification, stabilization and formulation. A variation of colours can be obtained varying from green to yellow, orange red, blue and violet, depending on the source of colourant.

ANTHOCYANINS

Anthocyanins are come from the Greek words which Anthos means flower and Kyanos means dark blue. (F. Delgado-Vargas et al., 2000). Anthocyanins are water-soluble vacuolar pigments that if according to pH, the pigments may look like red, purple, or blue. According to Naz, n.d, they belong to a parent class of molecules called flavonoids, and their characteristics are odorless and nearly tasteless, which contributing to taste as a moderately astringent sensation. All tissues of higher plants, including stems, leaves, flowers, roots and fruits is the place where anthocyanins occur.

Based on Delgado-Vargas, (2000), there are more than 5000 flavonoids have been chemically characterised, and new flavonoids structures are being continually labelled. The anthocyanins are the most important pigments colouring flower parts in the flavonoid family. Some parts of the flowers such as petals, leaves, fruits, leaves, stems and even roots act as protection against predators and excess of light, and also important roles in growth and reproduction.

Colour from anthocyanin are depends on pH. If the pH in acidic, colour of anthocyanins turns red whereas in pH of alkali, the colour will turn in blue. As the pH in neutral, anthocyanins will appear in purple colour.

STRUCTURE OF ANTHOCYANINS

Anthocyanin has basic C6-C3-C6 structure, it is the source of an infinity of colours produced by its chemical combination with glycosides and/or acyl groups and by its interaction with other molecules and/or media situations.

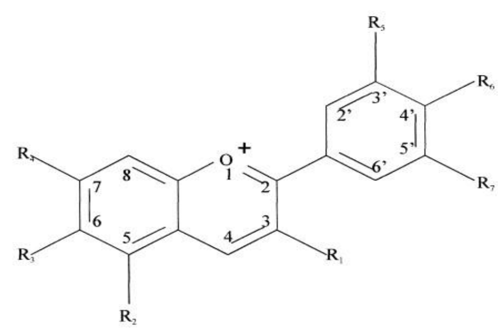

Figure 1: Basic structure of anthocyanin pigments in which Rx could be H, OH, or OCH3 depending on what pigment required. The basic structure show the most common accepted nomenclature for numbering carbons. (F. Delgado-Vargas et al., 2000)

Based on structural point of view in Figure 2.2, two aromatic rings C6-C6 also known as benzene joined by a 3-carbon bridge in the middle, which in the form of a heterocycling ring will formed an anthocyanins. Around ring C has variations in lateral chains give result in the major flavonoid classes. (Solymosi et al., 2015) Hence, Anthocyanins are substituted glucosides of the anthocyanidin basic skeleton.

FUNCTIONS OF ANTHOCYANINS

Bright reds and purples in flowers functions for attracting pollinators. Besides, the colourful skins in fruits can attract the attention of animals, which disperse the seeds everywhere by eat the fruits. In leave and stems tissue, where the photosynthetic process occur, anthocyanins have an ability to act as a "sunscreen" where it protecting the cells from high-light damage by absorbing the UV light and also blue-green light. Thus, anthocyanins can act to protecting the tissues against photo inhibition, or high-light stress. It has been proved to occur during the winter season, red juvenile leaves, autumn leaves and broad-leaved evergreen leaves turn into red. This is because, the red colour of the leaves can let them to hide or camouflage from herbivores that blind to red colours, or unappealing signal, since synthesis of anthocyanin synthesis often tally with synthesis of unpleasant phenolic compounds. Besides, they also act in plant resistance mechanisms in response to attack of pest that can destroy the plants.

Furthermore, Anthocyanins are also used for antioxidant agent and food colouring agent. The example of food colouring agent used is in fruit fillings, dairy product such as yogurt, in candies, and for making cakes. The important of regular consumption of antioxidants is good for vital organ and vital processes because they have an ability to scavenge free radicals and can improve and repair cell defense mechanisms.

DRAGON FRUITS

Dragon fruits is a Hylocereus species and also known as red pitaya or pitahaya in Latin America which meaning is "the scaly fruit". Dragon fruits belong to the cacti family which is originated in Mexico, North, Central, and South America. Plantations of dragon fruits are now well establish to provide fruit for the local and export markets in North America, Europe and also Asian. About 1 century ago, the French brought *Hylocereus sp.* into Vietnam exclusively for the king and it is also grown exclusively in Vietnam. After few years later, it became well known in the rich families throughout the country in Asia. Therefore, dragon fruits has been established widely in Asia where it has plantation plant which can provide the fruit to the local and export markets in Europe.

Figure 2.1 Red Dragon Fruits. (Google image 2, 2016)

Dragon fruits are widely growth and well distributed, such as in tropical rainforests, from coastal areas and also in high mountains. Dragon fruits has many types, thus each of appearance of it's also differ to each types. For example, the size and the flesh colors.

This dragon fruits are in the cactus species, which they are highly adaptable to a new environment. It has an ability to tolerate with heat, drought, cold condition and also poor soil. Examples of the ability are the stem modification to be place to store the water, the absence of leaves and waxy surfaces on the leaves, and night-time opening of the tissues for carbon dioxide uptake. These adaptations are enough to survive in hot and dry conditions, and can plant above-ground only. (L. Luders et al., 2006)

Figure 2.2 Dragon Fruit Tree (Google Image 3, 2016)

ROSELLES

Crown Roselle flower also known as its specific name Hibiscus sabdariffa L, it is has not many commercially used and only discarded as waste. But in the red petals, it is already widely used as natural colorant in a food coloring (Siti Nuryanti et al., 2012). Chen et al., (2013) reported that the use of Roselle petals known as calyx of Roselle as a drug can lowering blood cholesterol levels, blood pressure. Besides, it is also can lowering blood sugar levels for diabetics and as detoxification which can neutralizing poison in body.

Amor and Allaf (2009) has stated that part of Roselle which is calyx are used for coloring food, prevent the growth of cancer cell, maintain stamina, lowering the level of fat that were clump in the liver, balancing the weight and helps to lower the heat inside the body. Besides, advantage of calyx Roselle is helping recover from drug addiction, reducing headache (migraine), treating coughs and diarrhea (Farombi et al., 2005). In the calyx Roselle contains cyanidin-3-rutinoside and delphinidin-3-glucoxyloside can be found in the calyx Roselle which act as the major anthocyanin

3. METHODOLOGY

3.1 SUMMARY OF METHODOLOGY

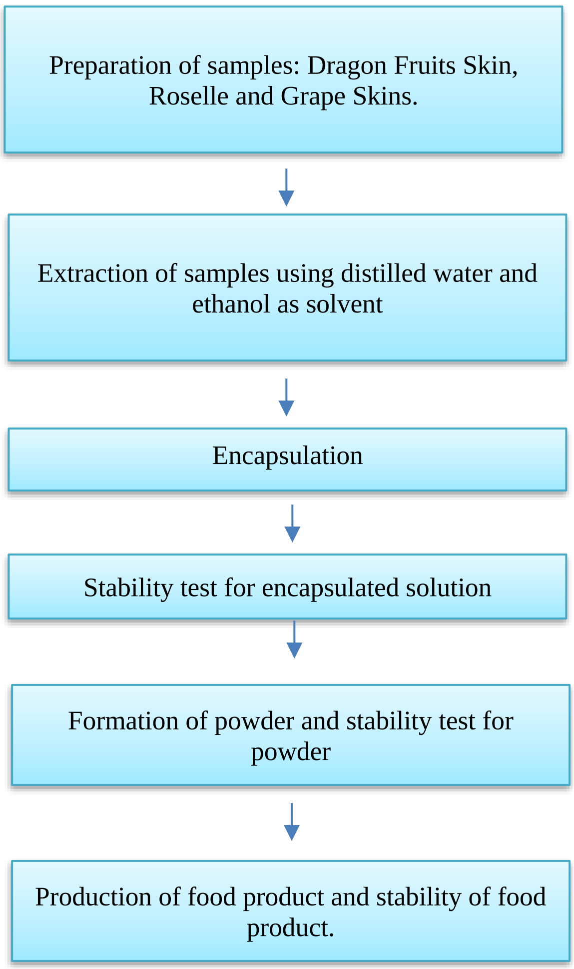

Figure 3 Summary of methodology

3.2 Solvent Extraction of Anthocyanin in Red Dragon Fruits

Anthocyanins was extracted using reflux method based on Sri Priatni et al, (2015). This method was combined with method used by Tang, C.S. et al (2007) to suit with the pulp used as the sample. 250mL round-bottom flask with a three-necked top connected to a condenser, a magnetic stirrer and a boiler. The reflux extractor contained peel component, only 80 % acetone and 80 % methanol were used for pigment extraction. In pigment extraction, 1 part of the peel and 2 part of solvent was shaken for a few minutes. Then, the mixture was allowed to stand for 15 min. Next, seeds, raw fibres, mesocarp fibres and mucilagenous material were separated from the pigment extract by centrifugation at 3500 rpm for 15 min and filtration. The excess of material was extracted again by using selected solvents and by using same procedures. Both filtrate was combined with that obtained earlier and the filtrated was stored at -20 ^{0}C prior to anthocyanin pigments quantification

3.3 Solvent Extraction of Anthocyanin in Roselle

The evaluated solvents were: ethanol acidified with 1.5N HCl (85:15, V/V). Extraction of pigments was carried out according to the procedures same as Red Dragon Fruits.

3.4 Solvent Extraction of Anthocyanin in Grape

Firstly, grape skin was weighed accurately. Next, small amount of extraction solvent was added to ground into a paste. Extraction solution was made up with 70% of ethanol and pH was adjusted to 3.0. After that, the extracted solution was speedy freeze under certain temperature for period of time. Next, the solution was filtered and the value of supernatatant was measured at OD value of 516nm. (Wrolstad et al., 2005).

3.5 STABILITY TESTS ON COLOURED MUFFIN

3.5.1 TEMPERATURE TEST

This stability test was adopted from Tantitunovant (2008) with slight modification to suit with the incorporation of the muffin in the test. Three muffins were covered with a packaging as protection purpose and placed in room condition which is at room temperature, in oven at 30°C and in refrigerator at 4°C respectively. The changes were observed and the images of these muffins were captured every 7 days for 2 weeks.

3.5.2 PRESENCE OF LIGHT TEST

This stability test was adopted from Tantitunovant (2008) with slight modification to suit with the incorporation of the muffin in the test. Two muffins were covered with a packaging as protection purpose and placed in dark and open place respectively at room temperature. The changes were observed and the images of these muffins were captured every 7 days for 2 weeks.

3.5.3 TASTE AND COLOUR TEST

This stability test was adopted from Tantitunovant (2008) with slight modification to suit with the incorporation of the muffin in the test. Four muffins were covered with a packaging as protection purpose and placed in two different places at room temperature. Two of the muffins were placed in dark place while the other two were placed in open place. The changes were observed, tasted and the images of these muffins were captured every 7 days for 2 weeks.

4. RESULTS AND DISCUSSION

4.1 Extraction of Red Colors

From Figure 4, the colors of roselle with distilled water is much greater compare to the roselle with 20% of ethanol.

Roselle with distilled water has 22.1 g of powder whereas roselle with ethanol has 6.1 g only after spray drying process.

Figure 4 Roselles red extraction in powder form.

Next, in Figure 5 below, Grape skins with solvent gave more red in colors compare to distilled water. But the quantity of the powder of roselle with distilled water in greater than in roselle with ethanol which is 21 g and 0.73 g respectively. This is because the solution were high in ethanol. Thus, when maltodextrin were added into the solution, it become clumped at the bottom. The red pigment from grape skins are not fully encapsulated because the maltodextrin are not dissolved completely in the solution.

Figure 5 Extraction of red colour from grape skin in powder form

In Figure 6 below, colors on left side is yellowish and right side is more to pinkish red. The quantity of the powder is also comparable where left is 14.5 g and right is 1.77 g. For dragon fruit skin with distilled water, at the beginning of the extraction of colour, it gave red colours. But after several hours, the red colour degrade to orange and then turn into yellow. This may due to anthocyanin content in Dragon fruit skin are unstable. Anthocyanin has the ability to regenerate by recondensation of hydrolysis products associated with a colour regain. (Rebecca et al., 2008)

Anthocyanin content was obtained from peels which extracted by methanol and ethanol is higher than anthocyanin content in distilled water. (Sri Priatni, 2015). This show that the colour of powder obtained below where DFS with solvent is red compare to DFS with distilled water is yellow.

Figure 6 Extraction of red colour from dragon fruit skin in powder form

4.2 Effect of Light Exposure and Temperature on Stability of Chlorophyll Encapsulated Dye Extract Solution

The graph below shows the reading obtained from UV Spectrophotometer and chromameter analysis. For the liquid samples, they are labelled with A (unwrapped, 35^{0}C), B (wrapped, 35^{0}C), C (unwrapped, 4^{0}C) and D (wrapped, 4^{0}C) in every steps of experiment conducted.

Graph A in Figure 7 showed all the four curves plotted in increasing trend. However it can be seen that curve A and B at week 3 has a slightly increase in reading of absorbance. This is may due to the growth of mold in the sample.

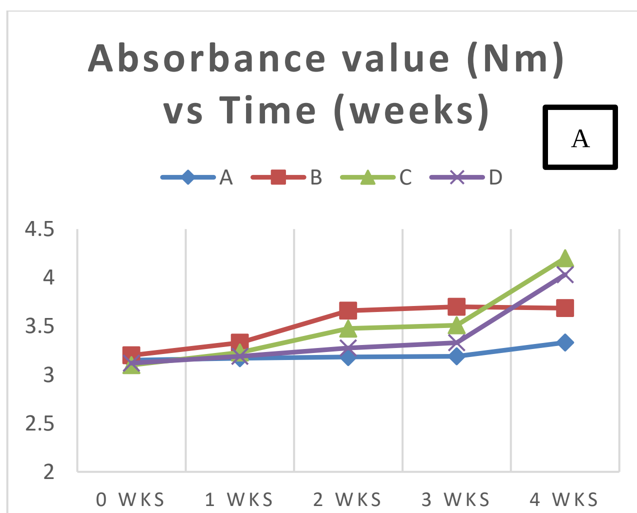

Figure 7 Anthocyanins pigments present in roselle-DW extract stored for 4 weeks

But for bottle C & D which stored at 4^{o}C, the value are higher than bottle A & B. This may due to the color in cold temperature become thicker. Thus, the light is difficult to penetrate through the sample.

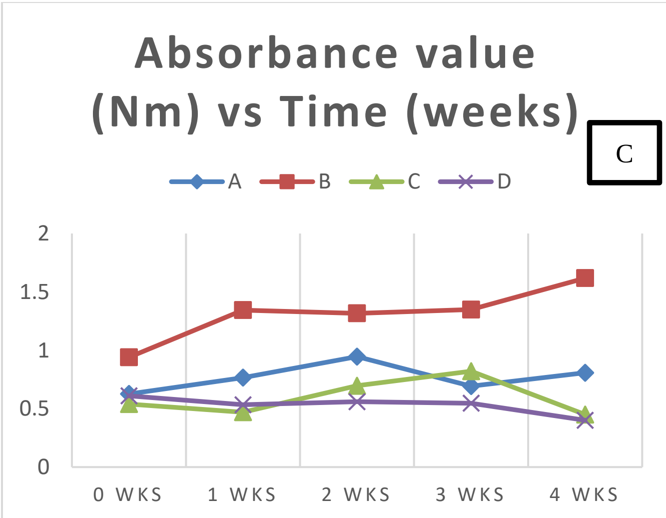

Figure 8 Anthocyanins pigment present in grape skin-DW extract stored for 4 weeks

Graph C in Figure 4.1 showing the different patterns form by each curve. Curve A and C show inconsistency from the beginning. Both A and C are exposed to light. But sample C in weeks 3, the reading are dropped whereas reading in sample A is increased. Next, in sample C and D, both reading at weeks 4 drop at the same point. This can be proved that grape skin with distilled water are sensitive to cold temperature 4ºC.

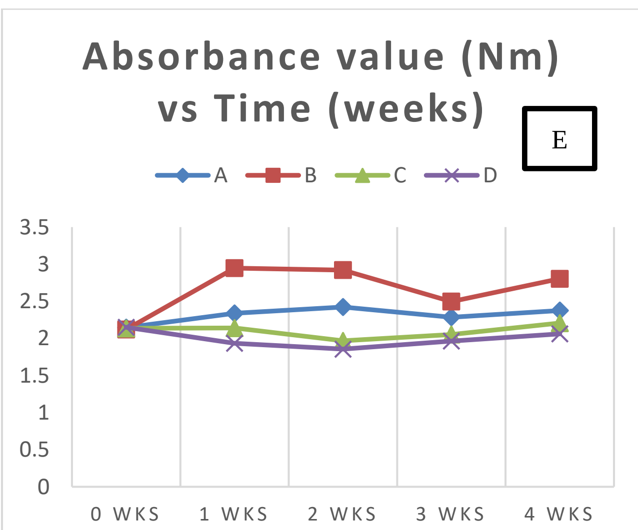

Figure 9 Anthocyanins pigment present in dragon fruits skin-DW extract stored for 4 weeks

Graph E showed better form of curve and almost similar in weeks 4. But in sample B, the curve at weeks 1 in increasing highly compare to others. Sample B is wrapped and place at room temperature. Thus it is sensitive to dark place.

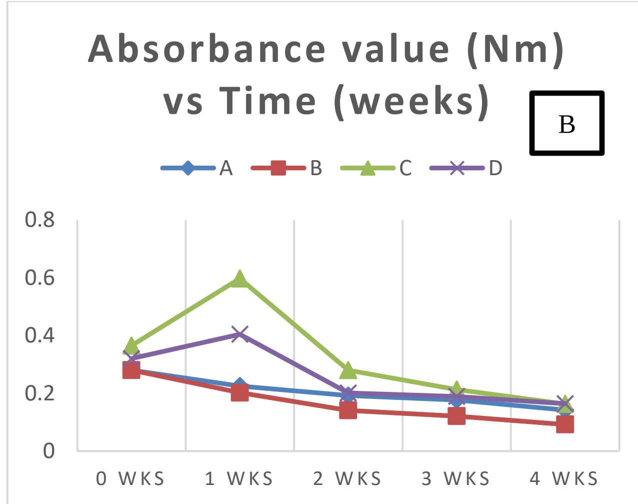

Figure 10 Anthocyanins pigments present in roselle-ethanol extract stored for 4 weeks

In Graph B, absorbance value had an increased on Bottle C & D on week 1 due to cold condition. But then the following weeks, it decreased uniformly like others. Colours on A & B also degraded into faded red.

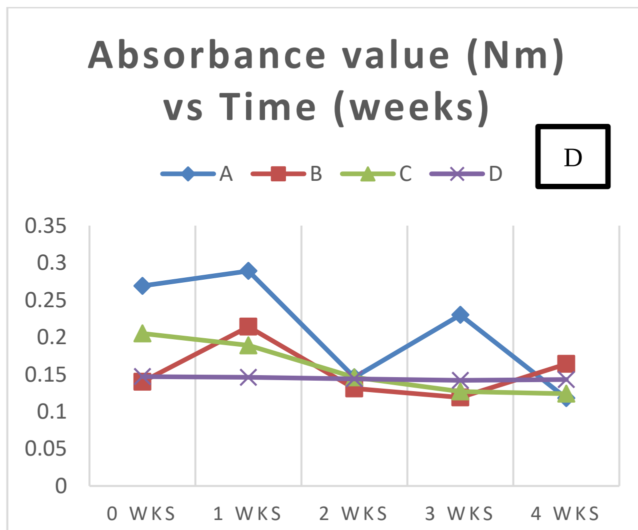

Figure 11 Anthocyanin pigment present in grape skin-ethanol extract stored for 4 weeks

In curve A and B, the pattern is quite similar to each other but had a slightly different at week 3 to week 4 where curve A is decreasing and curve B is increasing. In sample A, the sample A is sensitive to light because the it is exposed to light and room temperature, whereas sample B, it is wrapped so it does not exposed to light.

In curve C and D, the curve is showing decreasing in graph but in stable curve. This show that grape skin with ethanol extract pigment can be controlled by placed it in the cold place.

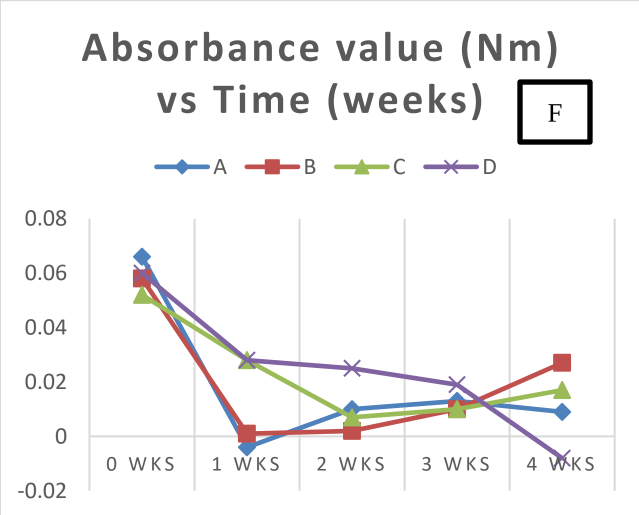

Figure 12 Anthocyanins pigment present in dragon fruits skin-solvent extract stored for 4 weeks

Graph F in Figure 4.1 on the other hand showing the vigorous curves with different patterns form by each curve. Curve D shows the unstable line where it unable to retains its color.

4.3 Colour stability on Roselle, Grape skins and Red Dragon Fruits powder for storage time and application on muffin.

For the powder samples, they are labelled with DFDW (Dragon fruit with distilled water), DFS (Dragon fruits with 80% ethanol & methanol), GDW (Grape skin with distilled water) and GS (Grape skin with 60% ethanol), and RDW (Roselle with distilled water) and RS (Roselle with 20% of ethanol) Below are the results.

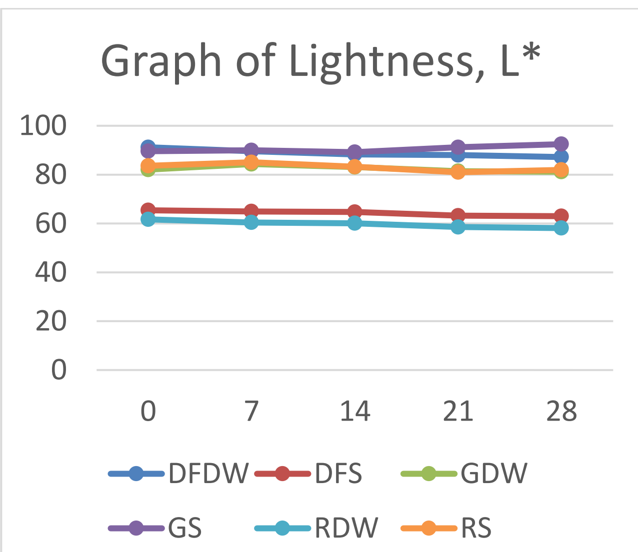

Figure 13: Reading of the coordinate L* in 28 days

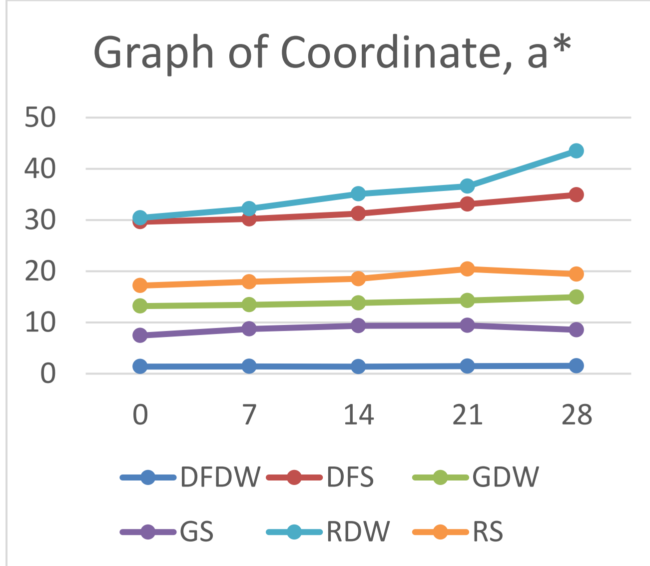

Figure 14: Reading of the coordinate a* in 28 days.

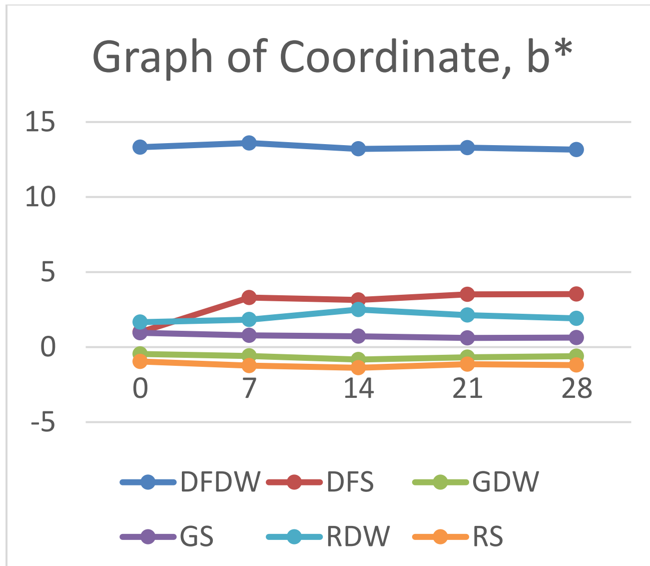

Figure 15: Reading of the coordinate b* in 28 days.

The sample in powder form also undergo degradation by time. This is can be proved when the powder were used in food product, the colour are not turning into red, but instead became yellow and grey in colour. It is because the colour is unstable when it is used in food product.

4.4 Stability tests on Colored Muffin.

4.4.1 Temperature Test

Week 1

Dragon Fruits Skin (DFS)

Based on Figure 16 & 17 below, there are both muffin were tested on temperature test on week 1. On room temperature, there are mould appeared on the surface of the muffin. But the muffin in the cold temperature, the muffin is perfectly looked without growth of mould.

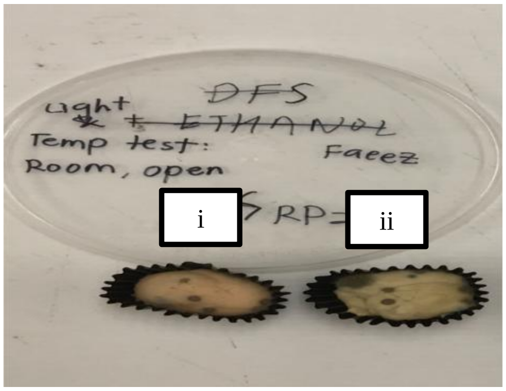

Figure 16 i) DFS with Distilled Water ii) DFS with solvent on 1 weeks at room temperature.

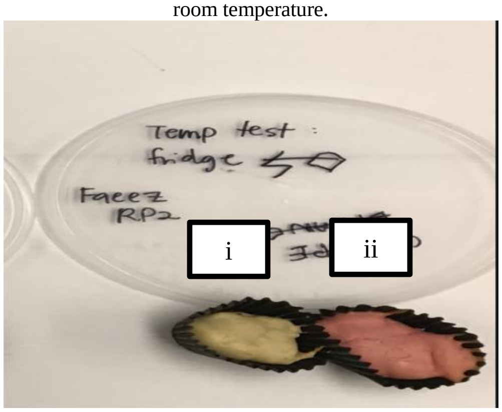

Figure 17 i) DFS with Distilled Water ii) DFS with solvent on 1 weeks at cold temperature.

Roselle

From figure 18 below, both roselle are growth with mold after 1 weeks were stored at room temperature. But roselle with solvent give more microorganism in surface of the muffin such as red and yellowish fungi.

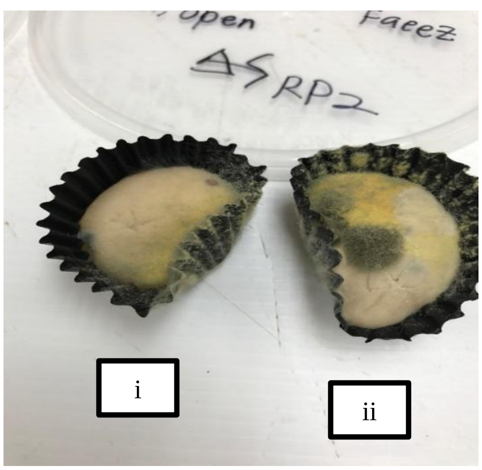

Figure 18 i) Roselle with Distilled Water ii) Roselle with solvent on 1 weeks at room temperature.

But in Figure 19, both muffin are well preserved with none of it had the growth of microorganism.

Figure 19 Roselle with Distilled Water ii) Roselle with solvent on 1 weeks at cold temperature.

Grape Skins

In Figure 20, both muffin were growth with microorganism after 1 weeks stored at room temperature.

Figure 20 i) Grape Skins with Distilled Water ii) Grape skins with solvent on 1 weeks at room temperature.

In Figure 21, both muffin were well preserved with none of the microorganism growth on the surface.

Figure 21 i) Grape skin with Distilled Water ii) Grape skin with solvent on 1 weeks at cold temperature

Week 2

In week 2, as showed below in Figure 22, the muffin are growth with colonies and the muffins itself were wet. But in Figure 23, there are no sign of the growth of colonies on the surface of the muffins.

The red coloured muffin stand to last longer at 4ºC environment compared to 30ºC.

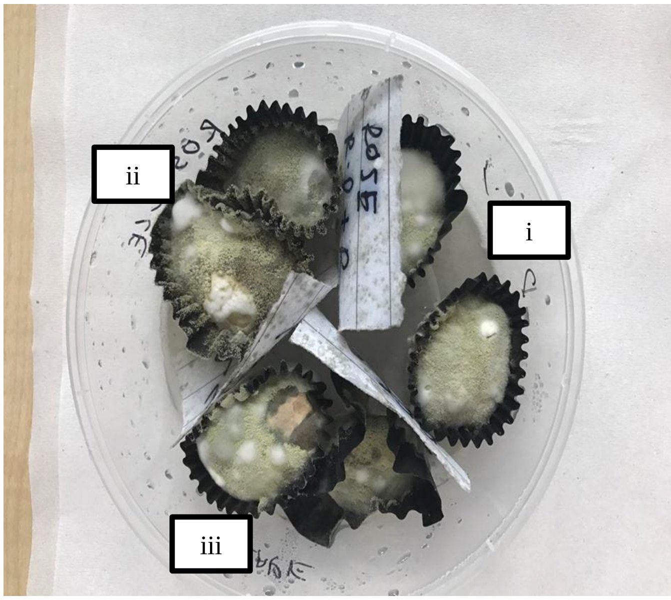

Figure 22 i) DFS ii) Roselle iii) Grape on 2 weeks on room temperature.

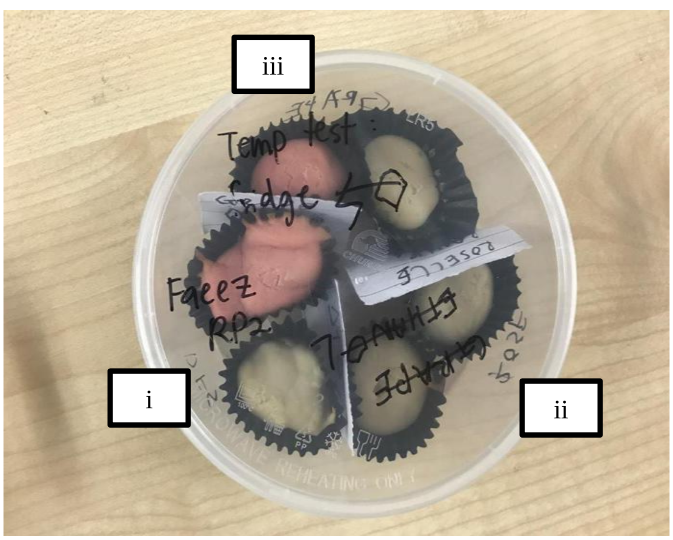

Figure 23 i) DFS ii) Roselle iii) Grape on 2 weeks on cold temperature.

4.4.2 Presence of Light test

For light test, there are two set of muffin were placed at two different parameter. Figure 24, 26, and 28 showed that the muffin were placed at dark place in 1 week. There are sign of mould growth on the surface of the muffins. Same goes to Figure 25, 27, and 29 mould are growth on the surface of the muffins, but not as much as the one that place on the dark place.

Figure 24 i) DFS with distilled water ii) DFS with solvent wrap with aluminium foiled and place in dark place at room temperature

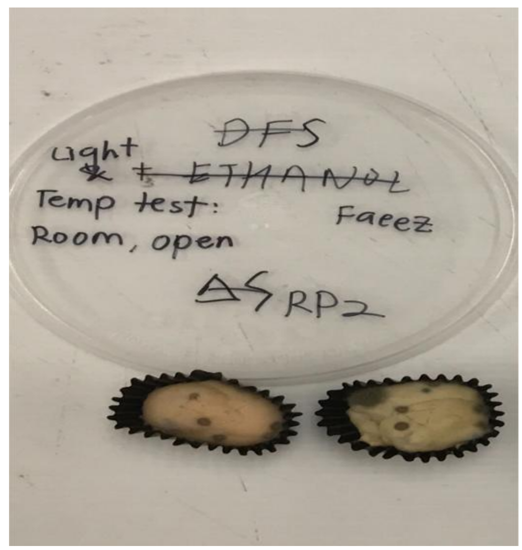

Figure 25 i) DFS with distilled water ii) DFS with solvent wrap with aluminium foiled and place in open place at room temperature

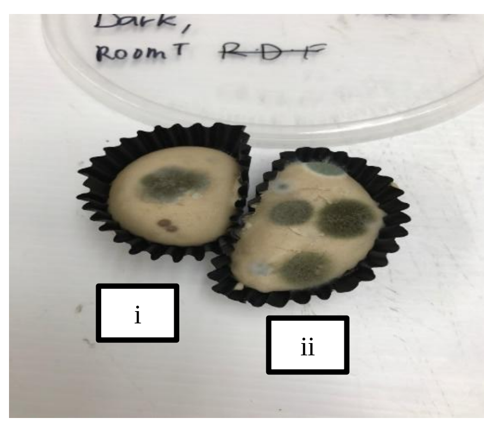

Figure 26 i) Roselle with distilled water ii) roselle with solvent wrap with aluminium foiled and place in dark place at room temperature

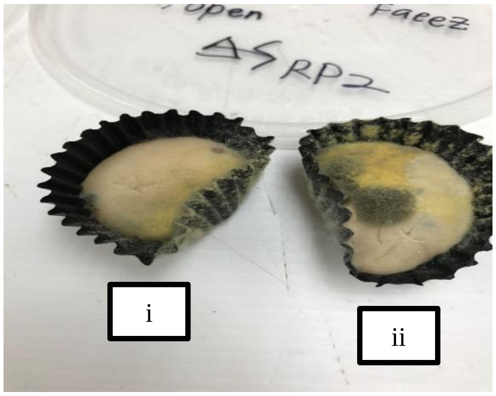

Figure 27 i) Roselle with distilled water ii) roselle with solvent wrap with aluminium foiled and place in room temperature.

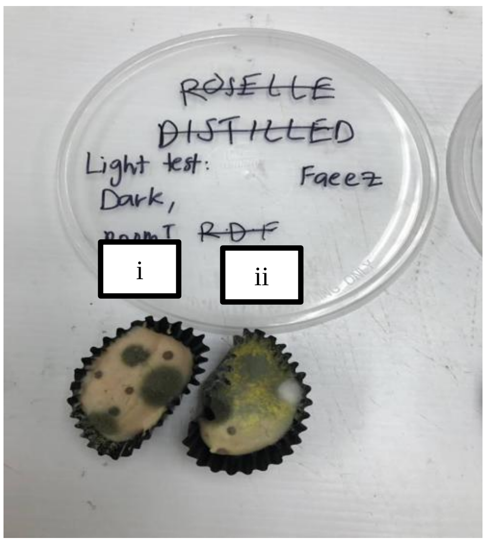

Figure 28 i) Grape with distilled water ii) Grape with solvent wrap with aluminium foiled and place in dark place at room temperature

Figure 29 i) Grape with distilled water ii) Grape with solvent wrap with aluminium foiled and place in room temperature.

Week 2

In 2 weeks later, the muffins that were placed on dark place were presented with colonies. And it has unpleasant smell. For the muffin were placed on open place, the muffin also were growth with colonies but not too much as the one place at dark place.

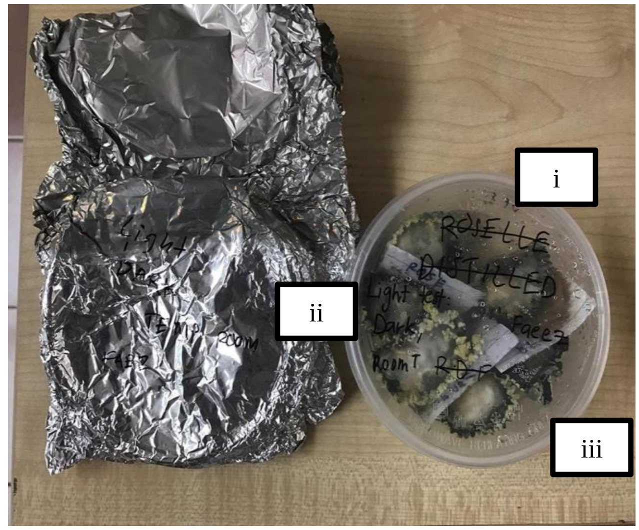

Figure 30 i) DFS ii) Roselle and iii) Grape on dark place in room temperature.

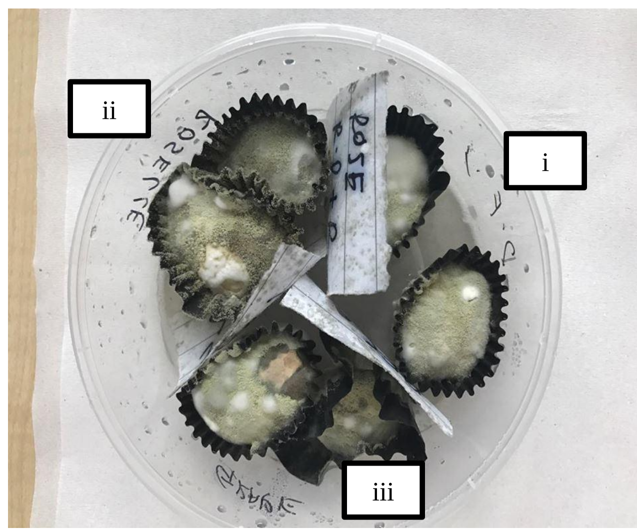

Figure 31 Figure 30 i) DFS ii) Roselle and iii) Grape on dark place in room temperature.

4.4.3 Taste and colour test

In Figure 32 and 33, for taste test, both set of muffins gave the same result. For week 1, the taste of muffin with DFS with distilled water bitter, and taste of DFS with solvent are bland taste. While for colour test, both DFS with distilled water the colour is pale yellow whereas for DFS with solvent, the colour is red color.

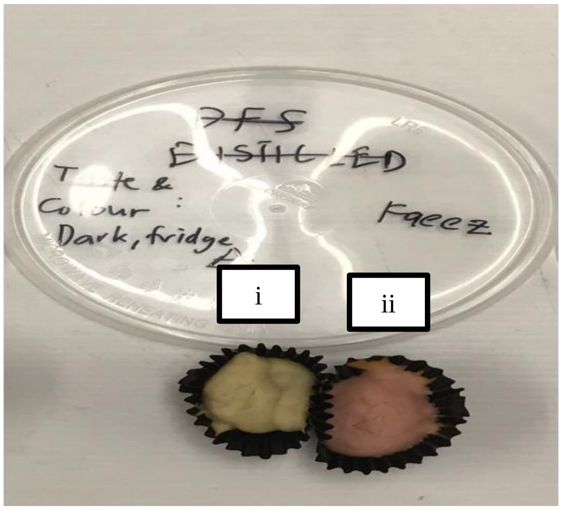

Figure 32 i) DFS with distilled water ii) DFS with solvent on 1 weeks in fridge and wrapped.

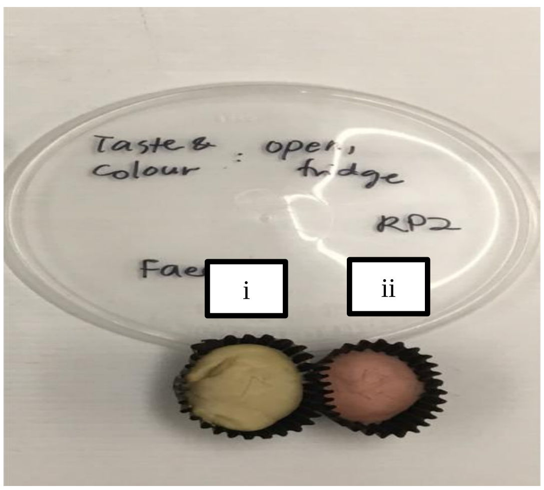

Figure 33 i) DFS with distilled water ii) DFS with solvent on 1 weeks in fridge and open.

In Figure 34 and 35, for taste test, both set of muffins gave the same result. For week 1, the taste of muffin with roselle with distilled water is sweet, and taste of roselle with solvent are bland taste. While for colour test, both roselle with distilled water the colour is yellow whereas for roselle with solvent, the colour is grey color.

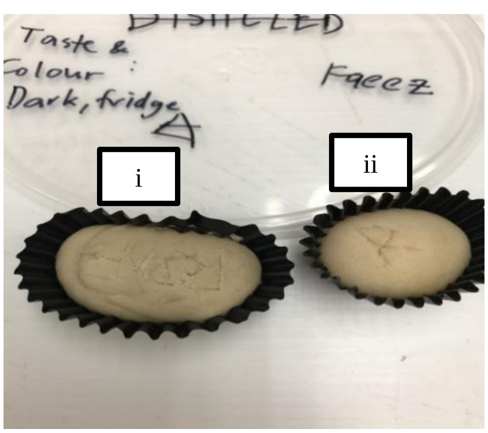

Figure 34 i) Roselle with distilled water ii) Roselle with solvent on 1 weeks in fridge and wrapped.

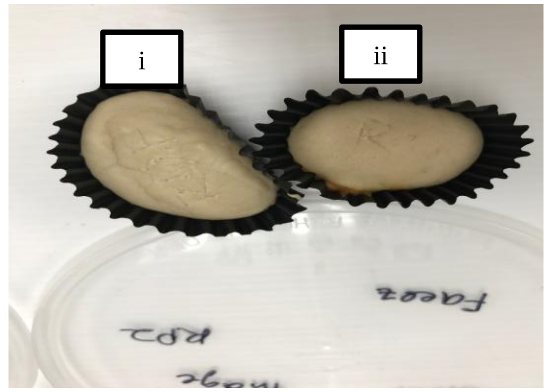

Figure 35 i) Roselle with distilled water ii) Roselle with solvent on 1 weeks in fridge and open.

In Figure 36 and 37, for taste test, both set of muffins gave the same result. For week 1, the taste of muffin with grape with distilled water is sweet, and taste of grape with solvent are bland taste. While for colour test, both grape with distilled water the colour is red whereas for grape with solvent, the colour is yellow color.

Figure 36 i) Grape with distilled water ii) Grape with solvent on 1 weeks in fridge and wrapped.

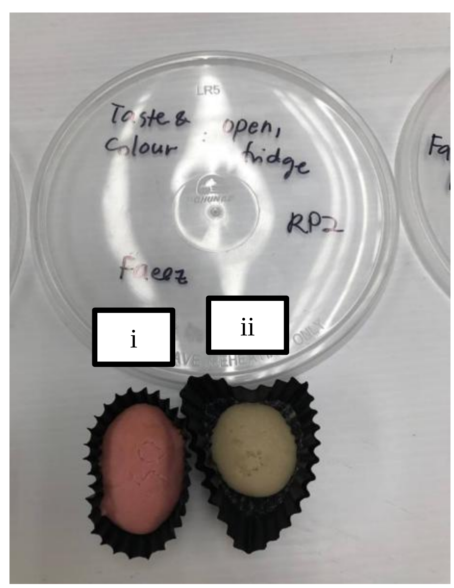

Figure 37 i) Grape with distilled water ii) grape with solvent on 1 weeks in fridge and open.

Week 2

For week 2, the colour of the muffins are have a slightly changed from week 1. In figure 38 and 39, Color of muffin from DFS with distilled water turn into more soft yellow color. For the taste test, both have a same result from week 1. The taste of muffin with DFS with distilled water are bitter, and taste of DFS with solvent are bland taste.

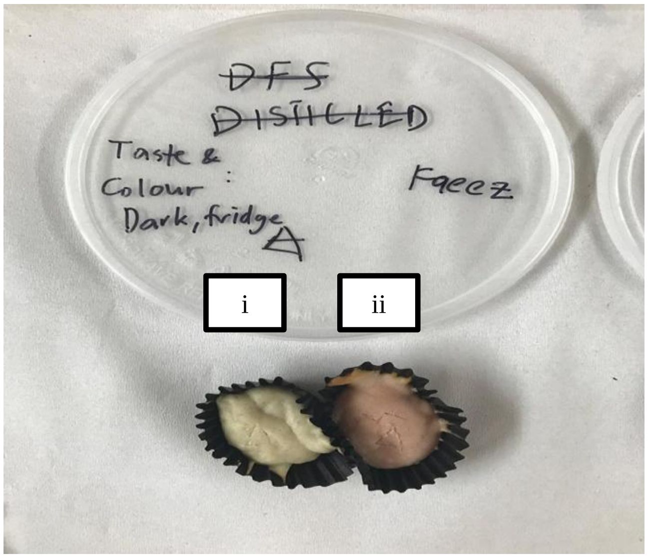

Figure 38 i) DFS with distilled water ii) DFS with solvent on 2 weeks in fridge and wrapped.

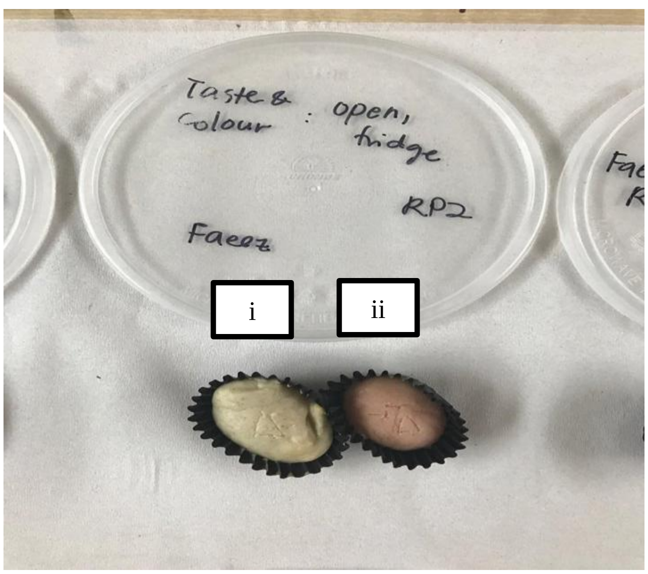

Figure 39 i) DFS with distilled water ii) DFS with solvent on 1 weeks in fridge and open.

In figure 40 and 41, the colour of the muffins are still same with week 1. For the taste test, both have a same result from week 1. The taste of muffin with DFS with distilled water are bitter, and taste of DFS with solvent are bland taste.

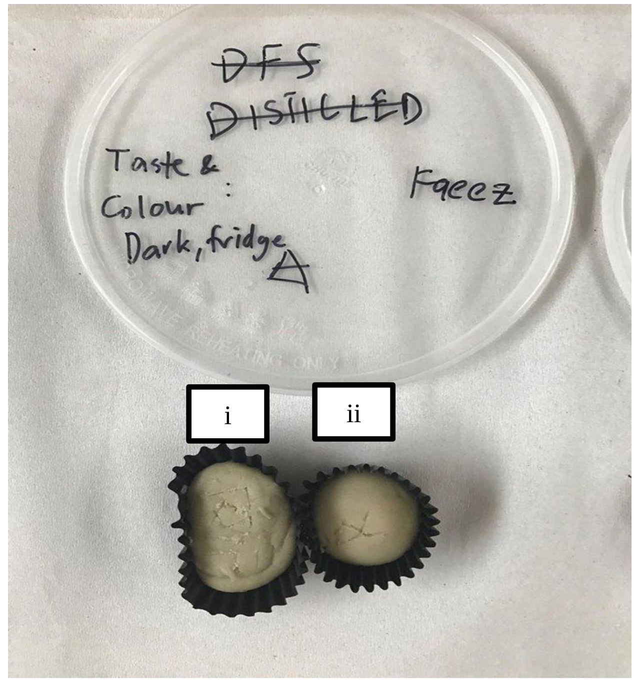

Figure 40 i) Roselle with distilled water ii) Roselle with solvent on 1 weeks in fridge and wrapped.

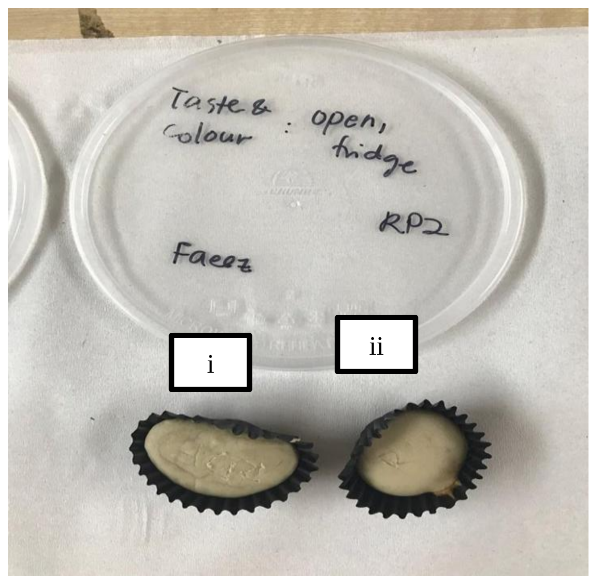

Figure 41 i) Roselle with distilled water ii) Roselle with solvent on 1 weeks in fridge and open.

In figure 42 and 43, the colour of the muffins are still same with week 1. For the taste test, both have a same result from week 1. The taste of muffin with DFS with distilled water are bitter, and taste of DFS with solvent are bland taste.

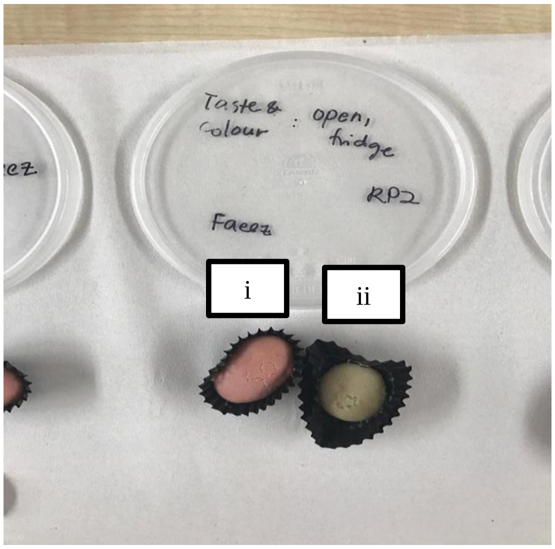

Figure 42 i) Grape with distilled water ii) Grape with solvent on 1 weeks in fridge and wrapped.

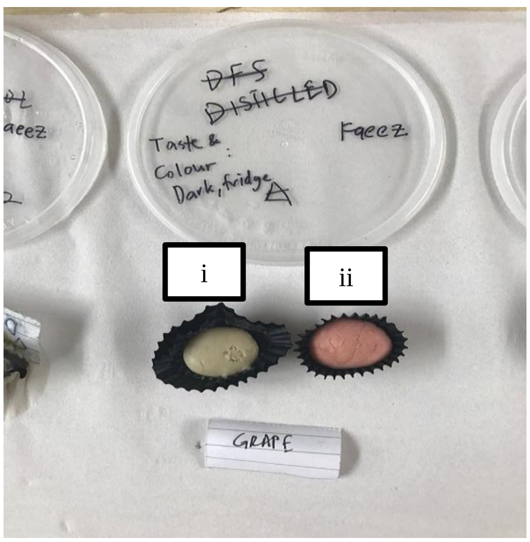

Figure 43 i) Grape with distilled water ii) grape with solvent on 1 weeks in fridge and open.

5. CONCLUSION

Based on results from this experiment shows that the extraction of red colouring from Dragon Fruits Skin are not successful in both liquid and solid extraction. Initially, the extraction of pigment process gave a red colour in liquid, but the colour degrade from red colour to yellow pale colour. These may due to the anthocyanin content in the Dragon Fruit Skin are unstable that can easily degrade. Thus, for further study of extraction of red colour from DFS, new extraction method should be conducted.

However, the extraction of red colour from roselles and grape skin are near to success. Both roselles and grape skin extracted with distilled water and designated solvent showed a visible red colour for liquid but not solid product. The solid product of designated solvent of roselles did not give a red colours whereas for grape skins, the solid product of extraction with distilled water gave a pale pink colour. Roselles have a more stable anthocyanins in term of red colours and also the absorbance value if compare to grape skins which have unstable absorbance value.

For stability test on muffins, the contamination and growth of mould occur to the sample that were place at temperature 35°C and no sign of contamination at all when the sample were place at temperature 4°C. The growth of mould in dark place in room temperature is higher. This also cause the container become watery like. However, the problem of food that place in room temperature can be prevented by addition of preservative such as antioxidant and also the used of microbial packaging.

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