A Study On the Extraction and Stability of Red and Purple Pigment from Roselle and Dragon Fruit for Food Coloring

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***Abstract*— In this research, *Hibiscus Sabdariffa*, Roselle and Dragon fruit used as natural sources for red and purple food colorant which being compared with artificial of red and purple colorant. Natural food colorant contains less toxicity and carcinogenic effect towards human body and provides abundance of benefits to human health. There is less production for natural food colorant gives highly in market demand due to increasing awareness about the harmful effect usage of artificial color which highly chemical content. Several extraction methods apply consist of supercritical fluid extraction (SFE), hydro distillation and ultrasonic homogenizer. SFE is carried out for extraction of natural product with a solvent of CO_2. The research study has conducted to investigate the effect of extracted natural colorant on different condition of storage, toxicity, temperature and light in compared with artificial color. The stability of natural color powder form from spray dryer is measured by using Chroma meter for 7 days. Absorbance reading for red and purple color measured by spectrophotometer at wavelength of 700 nm and 420 nm. The scopes of the research project are based on the stability study of natural and artificial or artificial food coloring in different temperature, light and storage time, determination of chemical elements in food coloring through ICP and analysis of anthocyanin stability is conducted through a storage test, muffin and toxicology study. Stability test for temperature is conducted at temperature 25, 30, 40, 50, 60 and 70 °c. In addition, usage of spray dryer must be used at optimum condition of 130°C and 4 rpm to avoid from color degradation and less flavor taste. As a conclusion, usage of SFE , hydro distillation and ultrasonic homogenizer does not suitable to carry out the oil extraction from sample of purple dragon fruit and red Roselle because there is no oil content in both samples.**

I. Introduction

According to US Food & Drug Administration (FDA) in 2003, a color additive, as defined by regulation, is any dye or pigment that can impart color to food, drug or cosmetic. Color additives are important components in many products to make them attractive, appealing, appetizing, and informative (Lakshmi G., 2014). The U.S. Food and Drug Administration's (FDA) roles is to ensure that color additives are safely and convenient used (Barrows & Arthur L. Lipman, 2003).

Color additives are classified as straight colors, lakes, and mixtures. Straight colors are color additives that have not been mixed or chemically reacted with any other substance. Lakes are formed by chemically reacting straight colors with precipitants and substrate. Lakes for food use must be made from certified batches of straight colors. Mixtures are color additives formed by mixing one color additive with one or more other color additives or non-colored diluents, without a chemical reaction (for example, purple color).

Food coloring is a type of food additives that use to maintain and improve safety, freshness, nutritional value, taste, texture and appearance (Martins, Roriz, Morales, Barros, & Ferreira, 2016). It is a form of pigment or dye which can be found in the form of powder, liquid and gel that change the color when it is added into food. In addition, it helps to prevent the color loss of food due to exposure of light, air, temperature, moisture and storage condition. In Malaysia's industry, it is commonly used a synthetic food color compare to natural food color due to lower cost.

By 1900, many foods, drugs, and cosmetics available in the U.S. were artificially colored. A detail analysis of the chemicals used for coloring foods found that many poisonous and dangerous materials consist of heavy metal such as lead, arsenic, and mercury being added. In some cases, the toxicities content in materials for synthesizing coloring agents were well known and could be toxins, irritants, sensitizers, or carcinogens.

By 1931, there were 15 straight colors of artificial color approved for use in food, including six of the seven in use today: FD&C Blue No. 1 (Brilliant Blue FCF), FD&C Blue No. 2 (Indigotine), FD&C Green No. 3 (Fast Green FCF), FD&C Red No. 3 (Erythrosine), FD&C Yellow No. 5 (Tartrazine), and FD&C Yellow No. 6 (Sunset Yellow).

A natural way lifestyle will give a positive effect to human health, which lead to a better lifestyle by restoring and maintaining health with anything available in nature is an old-age practice. Nutrition in food plays a vital role in strengthening our body immune system. A person with strong immune system will keep the doctors away.

The natural sources for red and purple colorant were from *Hibiscus Sabdariffa*, Roselle and purple Dragon fruit. Roselle rich in anthocyanin and could be used as good source for producing red colorant to many products. Dragon fruit is a good natural source which has some of phytonutrients that rich in antioxidants, vitamin C, polyunsaturated (good) fatty acids, and several B vitamins for carbohydrate metabolism. Natural color contains an anthocyanin which is a group of plant molecule carry the color from variety fruits and flowers that protect the plant from damage due to UV

radiation (Cid-Ortega & Guerrero-Beltran, 2014). The anthocyanin derive from aglycon (anthocyanidin) and general structure of (flavylium cation). The most common anthocyanin found are cyanidin, delphinidine, pelargonidin, peonidin and petunidin.

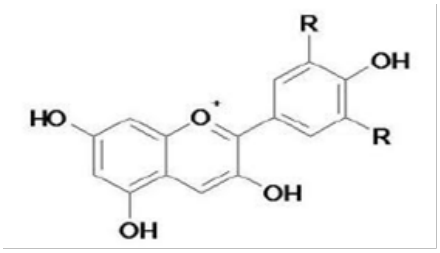

Fig. 1: structure of anthocyanin content in natural color

II. METHODOLOGY

A. Plant Materials

Fruits were collected from plants of dragon fruit *(Hylocereus polyrhizus)* and calyces of flower were collected from plants of Roselle *(Hibiscus Sabdariffa)* growing in field of MARDI. Artificial color of blue and red in liquid form.

i. Sample preparation for hydro distillation

A 300 g of fresh samples were placed in a blender with 500 ml of water and blended for 30 s. After that, another 500 ml of water was added to mix and blended it for an additional 10 s. Next, the slurry mixture was transferred to hydro - distillation (Clevenger – type) flask, resulting in total mixture 1500 ml of total water and 300 g of sample mix with ratio of water to sample (5 : 1) (Mouahid, Dufour, & Badens, 2017).

ii. Sample preparation for SFE

For dragon fruit sample, it was dried in microwave for 20 minutes 400 ˚c and sun-dried for one-whole day to ensure the fruit flesh totally dried with only 9 % water content. For Roselle calyces sample, it only being dried through sun-dried in one-whole day. After that, each samples were grind and weighted to achieve 10 g to pour it into a sachet like “tea bag”.

B. Extraction through hydro distillation

The slurry mixture was pour into boiling flask and put above the Clevenger – Flask. Then, the boiling flask connected with a Clevenger apparatus and clamp by using a retort stand. All apparatus was wrapped with aluminum foil to avoid from heat loss. Connected the pipe of water in and out to has a condensation process for a steam. The mixture was boiled at temperature of 110 ˚c for 3 hours.

C. Extraction through SFE

Approximately 10 g of dried Roselle and dragon fruit in a sachet were introduced into the basket of the extraction vessel. The extraction vessel including the basket of Roselle and Dragon fruit were purged with CO_2 for 2 min to remove any moisture or impurities from the pump filter. After purging, the exit valve was closed and the water batch heated to the required temperature using the thermostat, and then pressure set to the desired value using the control panel of the SF10 pump. Time was recorded upon reaching the desired temperature and pressure (T_c = 31.3 ℃ and P_c = 73.8 atm), where the extraction took place.

D. Extraction through Ultrasonic Homogenizer

Each samples were blend for 3 s to has a smaller size. The slurry mixture was pour into a beaker and set it up into the ultrasonic homogenizer. The beaker was placed under the titanium tip. Extraction could be done whether in auto or manual process. In auto process, power could not exceed limit of 50 Hz as precaution step due to increase heat in beaker and at titanium tip. Manual process could exceed limit 50 Hz till 80 Hz for process within 15 minutes. After used, clean titanium tip with distilled water and paper tissue.

E. Spray drying

80 g of Maltodextrin was added into the mixture of 800 ml contain sample. The mixture was boiled in a beaker by placing it onto the hotplate until there was no more clump and it mixed well with Maltodextrin. Then, the spray dryer was set up at temperature 130 ˚c and 4 rpm with a process time almost one hour (Shakiba, Mansouri, Selomulya, & Woo, 2016).

F. Stability test for natural color in powder form

Each samples of natural red and purple color produce from spray dryer in powder form was measured with a Chroma meter. This method was repeated for each week in a month to test the color stability.

G. Stability test with effect of storage time

Two sample tubes which containing 10 ml of each sample were capped and stored in open area with or without presence of light at room temperature. The presence of anthocyanin was tested with spectrophotometer at wavelength 700 nm and 420 nm for red (Jabeur et al., 2017) and purple color at days (0,7,14,21 and 28). The picture of the result was taken and data was recorded.

H. Stability test for different temperature

The crude extract from fresh sample of Roselle and dragon fruit and also artificial red and purple colors were exposed to several temperatures of 25℃, 30℃, 40℃, 50℃, 60℃ and 70℃ respectively. Each tube was pour with 10 ml of sample. Effect of different temperature on color intensity of the sample extracts were measured by reading the absorbance at wavelength 700 nm and 420 nm for red and purple color. The picture of the result was taken and data was recorded.

I. Stability test for light

Four bottles sample were prepared and filled with 10 ml of each sample. The bottles were placed at different condition such as in open area with presence of light and kept at room temperature, wrapped with aluminum foil and also kept at room temperature, wrapped with aluminum foil and kept in refrigerator at temperature 4℃ and lastly unwrapped and kept in refrigerator at temperature 4℃ respectively. Those samples were tested with spectrophotometer at wavelength of 700 nm and 420 nm for red and purple color at days (0,7,21 and 28). The picture of the result was taken and absorbance value was recorded.

J. Stability test for muffin

Baking process was followed from common process to bake muffin (Abdel-Moemin, 2016). Firstly, the oven was preheated to 180℃. Mixed butter and sugar together in a bowl until it become one. Eggs were added later and beat it until the mixture achieve it consistency. Vanilla extract was then added into the mixture and mixed well. Baking flour and milk were added into the mixture while mixing until it well mixed and achieved a desired consistency. The color dye sample was added at the last and mixed until the texture shown a consistent color. The mixture was added with artificial and natural color and pour into each paper cup by using a spoon until they were half full. The tray was placed in heated oven and baked for 10 to 15 minutes or until the top of muffin is already golden-brown. The tray was taken out and placed at room temperature for 10 minutes to cool down before being removed. The muffin was observed for 7 days and measured the color by using Chroma meter.

K. Run ICP for toxicology study

10 ml sample from Roselle and Dragon fruit in liquid state were prepared to run an ICP analysis to trace heavy metal. The ICP analyze heavy metals consist of Arsenic, Barium, Calcium,

Cadmium, Cobalt, Copper, Iron, Potassium, Lithium, Magnesium, Manganese, Sodium, Nickel, Lead, Strontium, Vanadium and Zinc with specific element wavelength 189.04, 455.40, 393.37, 228.80, 228.62, 324.75, 259.94, 766.49, 670.78, 279.55, 257.61, 589.59, 221.65, 220.35, 407.77, 309.31 and 213.86 nm. The ICP need to be run for one day for each sample.

III. RESULTS AND DISCUSSION

A. *Supercritical fluid extraction*

First and foremost, a supercritical fluid is a substance which is condition above its critical point ($T > T_c$, $P > P_c$, or $T > T_c$, $\rho > \rho_c$). Supercritical fluid behaves as a dense gas phase and has a surface tension or enthalpy (heat) of vaporization while used as an extraction solvent to separate many flavors, spices and aroma from natural sources (M.M.R. de Melo, A.J.D. Silvestre, 2014). It does practically used for flavor extraction through supercritical CO_2. Carbon dioxide is the most commonly used due to safety, availability and low cost.

Based on the observation, there was only one drop of oil form after 3 hours extraction time. The sample preparation for each sample were quite different from other types of equipment due to it must be in a dry form with a water content less than 5 %. Both samples consist of dragon fruit and Roselle were blend it to reduce the size and make it easy for oil extraction.

(Smith, Inomata, & Peters, 2013) stated that CO_2 gas at extraction temperature and pressure enters the extraction vessel where the gas in contact with natural source. Above the critical temperature, carbon dioxide will behave as a fluid that could not be condensed at any pressure. Dissolution of substance into CO_2 depends on particle size of natural materials and their characteristics, extraction time, flow condition, temperature and pressure.

B. *Hydro distillation*

The researchers of (Grosso, Coelho, Urieta, Palavra, & Barroso, 2010), said that extraction of essential oil from aromatic plant only can be done through process of distillation. After boiling the sample from Dragon fruit for almost 18 hours, only one tiny droplet of oil was formed, but after several days keep in refrigerator the oil droplet become vaporize. Due to longer boiling time in Clevenger, it destroys the structure of anthocyanin which is burn the pigment make changes in natural colour from purple to brown with a pungent smell.

Another test was carried out to extract oil through hydro distillation from natural sources by reducing the extraction time to 3 hours and lowering the temperature to 110 ̊c. After 3 hours extraction, the result obtained was different which there is no presence of oil but the natural colour of a mixture does not change to brown colour. It maintains with red and purple colour due to lower temperature. It is due to extraction of anthocyanin should be carried out under lower temperature or at cold condition using methanol or ethanol that contains a little quantity of acid in order to obtain the formation of flavylium cation, which is red and stable in acidic medium (V H et al., 2016).

Fig. 2: Shown the tiny droplet oil form after 18 hours extraction but change the natural colour solution and result different after extraction time reduce to 3 hours.

C. *Ultrasonic homogenizer*

The ultra-sonication used a principle of ultrasound gentle, induced an aggregation followed by enhanced the sedimentation to produce Nano emulsion through high energy method. Nanoemulsions are nano size members of colloidal dispersions and can be formed by dispersing immiscible oil phase in the aqueous phase. It provides stability against gravitational separation, easy handling, promising product appearance and flavor (E. Akbas, Soyler, & Oztop, 2018). The longitudinal vibration generated by transducer are amplifies by titanium tip and transmitted into liquid as ultrasonic waves, consisting of alternate expansion and compression. The pressure fluctuation gives a forming to microscopic bubble which expand during the negative pressure excursion and implode violently during positive excursion. When the bubbles reach a certain size, they collapse violently during a high-pressure cycle. During this implosion very high pressures and high speed liquid jets are generated locally.

The resulting currents and turbulences disrupt particle agglomerates and lead to violent collisions between individual particles. It is a mechanical process to reduce small particles in a liquid so that they become uniformly small and evenly distributed (H. Z. Akbas, Aydin, Yilmaz, & Turgut, 2017). This ultrasonic create a layer formation of sedimentation, liquid and some oil at the surface. Due to there is no oil content in dragon fruit flesh and Roselle, the sample only being separated between sedimentation or biomass/ solid and liquid.

D. *Spray drying*

The natural coloring solution of purple from dragon fruit and red from Roselle extracted from those three method extraction were carried out through spray dryer to change liquid phase to powder form. The spray dry was operated at speed of 4 rpm and temperature of 170 ℃ (González-Palomares, Estarrón-Espinosa, Gómez-Leyva, & Andrade-González, 2008) and 130 ℃ to compare the result. Spray dry of liquid process was added with 70 g of maltodextrin for each 500 ml sample to increase the volume of powder. Maltodextrin 20 DE is commonly used to provide a high preservation for the encapsulated pigments such as roselle anthocyanin because if does not use it, the roselle calyces will collapse rapidly during storage and it becomes more powerful barrier to oxygen permeation (Selim, K. A. Khalil, K. E., Abdel-Bary, M. S., and Abdel-Azeim, 2006).

E. *Stability test for natural color in powder form*

The different color intensity form due to different operating temperature. It shown red powder form with a slightly pink due to operating temperature at 170 ℃ which is high. The red powder does not form a dark red because of color degradation affected by temperature. A dark purple colorant operated at 130 ℃ which is an optimum condition to operate with natural colorant and prevent from color degradation.

Purple powder after 3 weeks stored at room temperature. The powder itself become agglomerates and cannot longer be used. The agglomeration phenomenon is a natural process to the powder when there is a moisture content. This only resulting for purple

powder and not happening to red powder because of using a dragon fruit flesh which is highly water content.

Chroma meter had been used to measure the color intensity in all sample. Value of L*, a* and b* were obtained which indicates lightness and brightness color. **Figure 3** shows the Chroma meter reading scale to measure the color changes whether increasing in brightness or become faded due to color degraded.

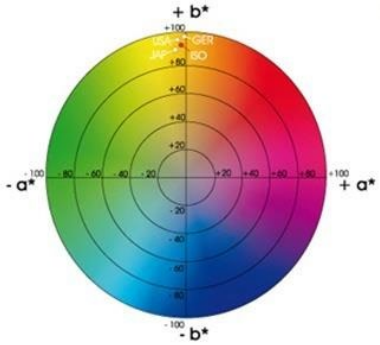

Fig. 3 : Chroma meter reading scale (Ballat, 2014)

Table 1: Chroma meter result for powder from natural sources

Week	Red (Roselle)			Purple (Dragon fruit)		
	L*	a*	b*	L*	a*	b*
1	68.34	23.39	1.78	63.77	32.97	-7.25
2	52.99	14.10	1.18	46.91	19.82	- 3.58
3	52.68	14.01	0.93	40.58	18.77	- 1.15
4	52.56	16.40	- 0.41	45.26	23.40	- 3.20

Based on the chroma meter reading shown in table 1, it shows that the natural color powder become more faded and changed after several weeks. The color degraded due to storage factor.

F. Stability test with effect of storage time

Spectrophotometer had been used to measure the absorbance of all sample. Absorbance value that has been obtained present the stability in each sample by comparing initial absorbance value. Figure 4 and 5 refer to the condition of presence and without presence of light which affect the anthocyanin color stability.

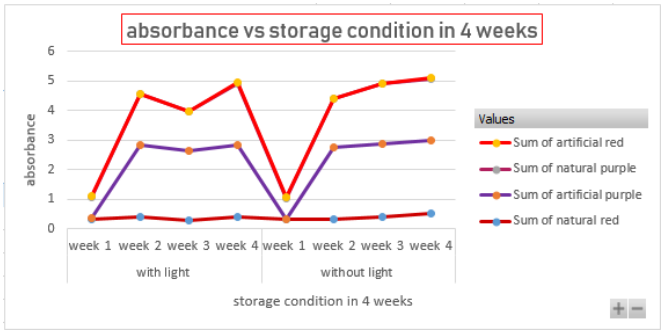

Fig. 4: Absorbance vs storage condition in 4 weeks

G. Stability test for different temperature

Based on observation, it shows that anthocyanin of natural colour cannot be sustain in temperature between 25 to 70 ℃ for a month. In third weeks, there are a white spot presence at the surface of the sample due to microorganism growth. The anthocyanin cannot be sustained in certain condition of temperature because temperature is one of the factor affecting encourage microorganism to growth (Sui, Bary, & Zhou, 2016). Those temperatures would not kill the microorganism, because it is the optimal condition for microbial growth (D'ans, Gottlieb, & Kokotovic, 1972), only the boiling temperature of 100 ℃ will kill them and ensure the food coloring can be safe and last longer.

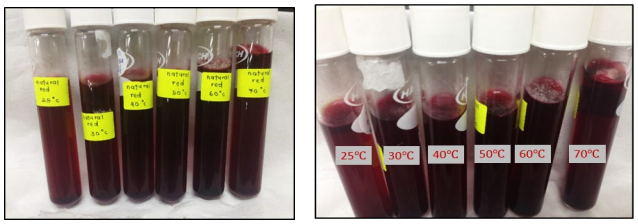

Fig. 5: stability test at different temperature for natural red colorant

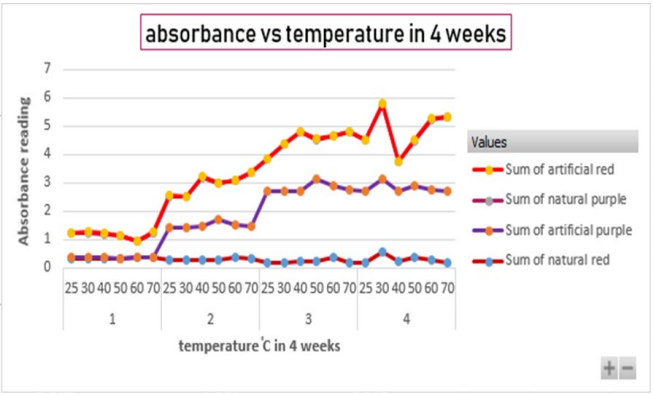

Fig. 6 : absorbance vs different temperature in 4 weeks

H. Stability test for light

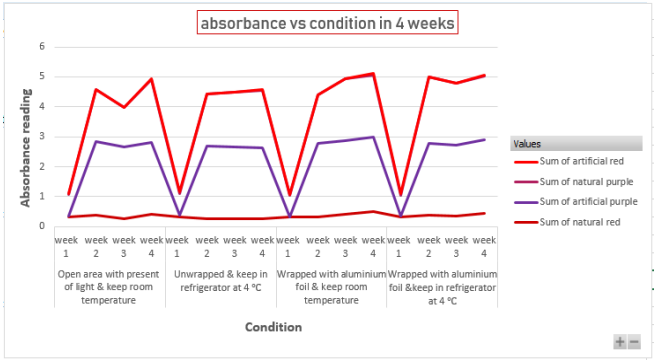

Fig. 7: absorbance vs condition in 4 weeks

Figure 4.23 shows the absorbance reading increase through 4 weeks due to less solute concentration. It is because the anthocyanin pigment is being destroyed with presence of light. It shows that exposure of pigments to light speed up their destruction. Absorbance value can be obtained only with a specific wavelength which is red in range of 700 – 720 nm and purple in range of 400 – 450 nm. Unsuitable wavelength used in spectrophotometer will lead to a negative result of absorbance reading. From figure 4.17, it shows increasing of absorbance value in a month due to concentration effects the absorbance. If the concentration of solution is increased, then there are more molecules for the light to hit when it passes through. As the concentration increases, there are more molecules in the solution, and more light is being blocked.

I. Stability test for muffin

Muffin is produce to act as indicator to measure the color intensity through observation and by using a Chroma meter with a certain amount of artificial and natural color powder added. It is being observed for 7 days to observe the color degradation and microorganism growth.

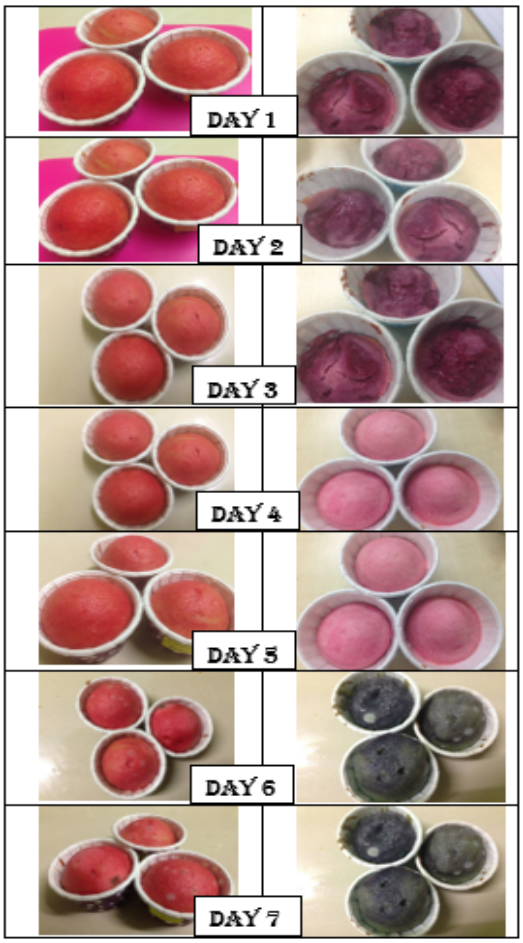

Fig. 8 : Observation Of Muffin Cake For 7 Days

Fig. 9: muffin cake for natural red and purple color

Figure 9 shown the result after being bake at 180℃ for 15 minutes. Muffin added with natural colorant shows a brighter color compare to artificial. The observation shows at fourth day, the color of muffins starts to change and microorganism start to growth. Taste of the muffin reduces with increasing of storage time.

Table 2: Chroma meter reading for muffin cake from natural source for red and purple color

Day	Red (Roselle)			Purple (Dragon fruit)		
	L*	a*	b*	L*	a*	b*
1	27.48	18.59	2.79	45.74	16.95	12.97
2	39.04	20.10	2.73	31.15	14.62	12.58
3	38.17	19.76	2.54	40.57	16.06	12.25
4	36.40	13.63	1.57	29.36	12.46	12.04
5	32.63	12.56	1.41	28.17	11.74	11.71
6	28.91	10.10	1.33	27.77	11.58	11.68
7	26.35	8.03	0.67	27.10	11.50	11.61

Lightness is one of the important aspect in measuring colors. Color can be separated into bright and dark color. Maximum measurement for lightness, L* = 100 (white) and minimum L*= 0 (black). From figure 10, it shows that both colorant of anthocyanin only more stable at 2-3 days after that it become faded and unstable.

Table 3: Chroma meter reading for muffin cake from artificial color for red and purple color

Day	Red(Roselle)			Purple(Dragon fruit)		
	L*	a*	b*	L*	a*	b*
1	53.58	25.26	7.17	16.96	11.11	0.76
2	48.30	29.02	6.27	21.65	12.08	0.32
3	51.04	25.24	5.89	21.15	13.92	0.21
4	50.02	23.49	6.45	18.79	12.91	0.41
5	50.18	8.14	14.01	15.05	11.81	1.14
6	45.87	7.35	12.75	14.82	14.41	1.39
7	45.13	7.51	12.51	16.40	15.31	1.30

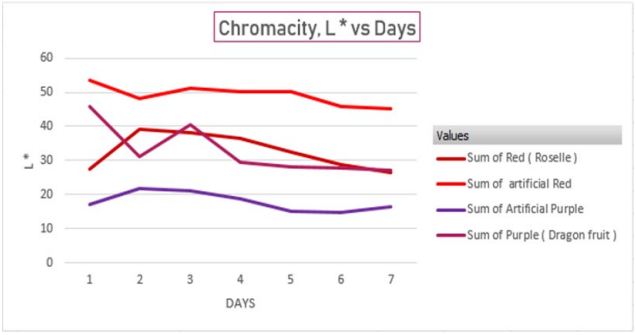

Fig. 10 : Chromacity vs day for muffin cake from artificial color

J. Run ICP for toxicology study

Inductively coupled plasma mass spectroscopy (ICPMS) is one of the most powerful methods for qualitative and quantitative trace element detection. According to World Health Organization (1989), they declares the maximum allowable level of Arsenic (As) , Cd and Pb amount in food are 1.0, 0.3 and 10 mg/kg (Başgel & Erdemoğlu, 2006) . A high consumption of Cu will lead to liver damage.

Based on table 4, In Roselle, highly content of Potassium and Calcium which is good to human body. Otherwise, highly content of Potassium in Dragon Fruit is double times with Roselle. The different of metal content in both are Dragon Fruit has a little amount of Arsenic while Roselle has a zinc substance. Even though Dragon Fruit content Arsenic which is may affect to human health, but the concentration is only 0.4 which allowable level from FDA is 1.0 mg/kg . Presence of zinc in Roselle also can affect human immune function and level of high density lipoproteins.

Table 4: Heavy Metal Content in Roselle and Dragon Fruit

NO	TYPE OF HEAVY METAL	ELEMENT WAVELENGTH (NM)	CONCENTRATION IN DRAGON FRUIT (PPM)	CONCENTRATION IN ROSELLE (PPM)
1	Arsenic	189.04	0.407	0.000

NO	TYPE OF HEAVY METAL	ELEMENT WAVELENGTH (NM)	CONCENTRATION IN DRAGON FRUIT (PPM)	CONCENTRATION IN ROSELLE (PPM)
2	Calcium	393.37	12.397	133.001
3	Iron	259.94	2.558	5.357
4	Potassium	766.49	459.093	279.321
5	Magnesium	279.55	41.748	40.874
6	Manganese	257.61	0.187	3.526
7	Sodium	589.59	2.185	20.473
8	Lead	220.35	1.171	7.240
9	Zinc	213.86	0.000	8.567

I. Conclusion

As a conclusion, those three method of extraction consist of SFE, hydro distillation and ultrasonic homogenizer do not suitable to has an oil extraction with a sample from Dragon fruit and Roselle due to lack of oil content. Those factor of storage time, temperature and presence of light effect the stability of natural colorant to be sustain.

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