A Study on the Extraction Method and The Stability of Blue Pigment from Various Natural Source for Food Coloring

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Abstract*--The demand for natural food colouration keep on increasing annually after the benefits of natural colour which are not only for food colouring but also have abundance of health effect have been revealed. However, there is a major factor that prevent these natural colour from being commercialize which is its stability. Natural colour such as anthocyanin have weakness in heat, light, storage time and many other external factors that usually done in industry. Therefore, this experiment was done to determine the better extraction method and to test the extracted pigment stability in food product. The natural colour sources come from Clitoria ternatea and Melastoma malabathricum, fruits which gives blue colour. The solvent used for the extraction are deionized water and ethanol. The stability test will be conducted on the encapsulated liquid, the encapsulated powder and on the muffins, that contain these encapsulated dye pigment. For the extraction procedure, the pigment that extract using solvent show higher absorbance compared to others. For the stability on encapsulated solution and on powder, higher temperature affects more on colour degradation compared to lower temperature while for storage time, longer time also should effect on colour degradation on powder also muffin. For *C. ternatea,* the solution extract with ethanol 20% have higher reading on spectrophotometer which is 2.807 compared when extract with deionize water which is 1.69 at 600nm. While for *M. malabathricum*, the solution extract with ethanol 20% also have higher reading on spectrophotometer which is 2.05 compared when extract with deionize water which is 1.706 at 800nm. In powder test, *C. ternatea* with water extract shows highest reading in chromameter for lightness test. For coordinate a^*, *M. malabathricum* extract with ethanol 20% shows highest reading while coordinate b^* *M. malabathricum* with deionize water extract have highest reading. The time for testing the sample are at days (0,7,14,21 and 28). The different temperature used are room temperature (25^{o}C) and refrigerator (4^{o}C) while the presence of light is tested by wrapping and unwrapping the sample using aluminum foil. Therefore, it is observed that temperature, storage time and light gives impact on the stability of anthocyanin blue colour.

***Keywords*— anthocyanin, pigment, encapsulated, colour degradation**

1. INTRODUCTION

During the past years, most of the colourant that used in industry came from unnatural sources such as synthetic dye. After a few years, researcher have found that animal can become alternative sources for dye industries because some animals can produce unique dye and also have high stability. The highly stable colourants that are permitted to be used as food colourants comes from synthetic dye or animal-origin (e.g. carmine) (Müller-Maatsch et al, 2016). However, these types of colourant gives more harm than good to human if it is consumed in food products. McCann et al, (2007), stated that several synthetic dyes have been associated with adverse effects on children. Synthetic dye also cause increase in environmental pollution and health hazard (Rajendran et al, 2012). Due to these disadvantages, people tend to change their source of colourant to natural source.

Our environment contains variety of plant that have its own colour and it makes them very beautiful to see. These different colours comes from different chemicals that can be found in them. For example, there are many colour of flowers in this world. These flowers actually contain pigments that give them color which are chlorophyll, anthocyanins, and carotenoids. Chlorophyll is a chemical that give green colour for leaf, anthocyanin gives colour of red, blue and purple for fruits and flowers while carotenoid is a chemical that give yellow, orange and brown pigments mostly for fruits (Chadde, 2012).

One of the most common pigment from natural source that used in food industry is from anthocyanin. These chemical from plant is more attractive than others where it contains blue, red and purple colour. Furthermore, this natural colour have many nutritional values and can treat disease. Anthocyanin for example, it can reduce cancer cell proliferation and inhibit tumor formation (Lila, 2004). However, when comes to food industry, it becomes a challenge because production of food product needs to get through heating process which is a weakness for anthocyanin. According to Sui, (2016), heat treatment and storage temperature plays an important role for the color and stability of thermally treated anthocyanin aqueous solution where it can result with loss of color and degradation of nutrients. Thermal processing is one of the most common processes in the food industry, which helps to prolong their shelf-life, destroy harmful pathogen, and enhance their functional properties. Thermal processing can be classified as pasteurization (63–100 ^{o}C), sterilization (100–130 ^{o}C), and ultra-high-temperature (UHT) treatment (130–160 ^{o}C). Usually, the colour that is extracted from flowers, leaves or fruits will be used as colourant for food because it is expensive and the production is very limited. It is a waste if this colour goes to textile or paint industry. With the heat limitation of anthocyanin, the most suitable method to counter this problem is by encapsulation. Encapsulation of anthocyanin can increase thermal stability and photo stability (Zhang et al, 2014). The polymer that used for encapsulation process also important because different polymer gives different result of encapsulation. This is approved by Tantituvanont, (2008) statement where the polymer type should be carefully selected as it affects the stability of loaded color.

Nowadays, the cost of energy source and time for every operation are the vital criteria to be considered in operation of industry. The lower the production time, the higher product can be produced. One of the time-consuming process in dye industry from

natural source is at extraction process. In order to fully extract dye pigment from plant, the solvent used and extraction method need to be chosen wisely. The extraction can be easily done by putting the colour source into stirring solvent such as distilled water or ethanol and the colour will be extracted by following the concentration gradient. However, this conventional method is very slow and takes long time to extract the colour pigment. One of non-conventional method suggested is using microwave. It is not only give dramatic reduction in reaction time but also environment friendly. This method has been proved by Sinha et al, (2012) experiment where the extraction time of *Clitoria ternatea* blue pigment was reduced from 3 hours to 2 minute for the same amount of dye extraction by using distilled water.

In this time, the demand of natural colour in food and beverage industry keep increasing every year but the source and the durability of natural colour have its own limitation. It is beneficial for food and beverage industry if there are variety of natural sources come from their own country that can be used as food colouring. In this study, the natural source of blue pigment that need to be extracted are from *Clitoria Ternatea* and *Melastoma malabathricum* fruits. The solvent used to extract the blue pigment are deionize water and ethanol. The stability test will be conducted on the encapsulated blue pigment or anthocyanin and also on the food product. Maltodextrin will be used as encapsulating agents.

Figure 1: *Melastoma malabathricum* fruit

Figure 2: *Clitoria ternatea* flower

In this study, the natural blue pigment source comes from *Melastoma malabathricum* fruit locally known as “buah pokok senduduk” and *Clitoria ternatea* locally known as “bunga telang”. *Melastoma malabathricum* fruit blue pigment is extracted by deionize water and ethanol as solvent while *Clitoria ternatea* blue pigment is extracted using microwave by deionize water as solvent. That three samples of extract solution then will be encapsulated using maltodextrin. All three sample will undergo stability test on storage time (every 7 days for 2 months) and presence of light (open area at room temperature, unwrapped at refrigerator 4ºC, wrapped at room temperature and wrapped at refrigerator 4ºC) for liquid dye extract, while stability test on storage time (7,14,21 and 28 days) for powder dye extract after spray drying process. The research also focus on food product where the powders from spray dried are used as colourant for muffin. The stability of blue colour muffin is studied with the effect of temperature (at room temperature, oven (60^0C) and refrigerator (4^0C)), light (dark place and open place), taste and colour (dark place and open place) using sensory receptor all after (7 days for 2 weeks).

2. METHODOLOGY

2.1 SUMMARY OF METHODOLOGY

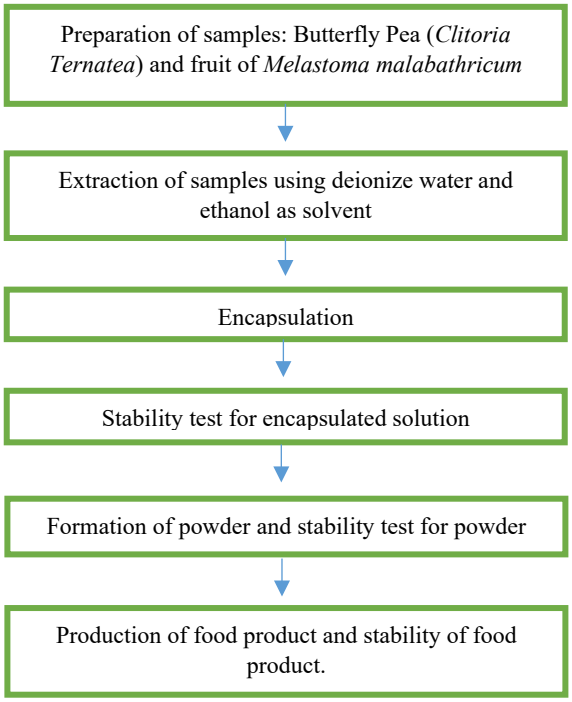


Figure 3: Summary of methodology

2.2 CHEMICALS AND MATERIALS

Deionize water, ethanol, maltodextrin, Butterfly Pea (*Clitoria Ternatea*), fruit of *Melastoma malabathricum*, butter, sugar, egg, milk and flour.

2.3 APPARATUS

Erlenmeyer flask, measuring cylinder, cheesecloth, aluminum foil, beaker, bowl, baking tray, muffin cup, sample bottle with screw cap, filter paper, petri dish, seal tape, magnetic stirrer and spoon.

2.4 EQUIPMENT

Spectrophotometer, spray dryer, oven, refrigerator, incubator, blender, chroma meter, hot plate, electronic weighing scale and centrifuge machine.

2.5 PREPARATION OF COLOUR EXTRACT

2.5.1 Preparation of Clitoria ternatea Colour Extract

This extraction method was modified from Tantituvanont, (2008). *Clitoria ternatea* flower was collected from different houses in Shah Alam area. The flower was washed properly using tap water. Forty-five grams of fresh butterfly pea petals were blended with volume, V of 1.5 liter of deionize water that act as solvent. The mixture then was filtered twice through 4-layers of cheesecloth. The sample was tested for absorbance at wavelength, λ 600 nm. The procedure was repeated using ethanol 20%. The extracted solutions were stored in refrigerator for next test.

2.5.2 Preparation for Fruit of Melastoma malabathricum Colour Extract

Fruit of *Melastoma malabathricum* was collected from bushes in different area and kept in refrigerator at 4ºC. Based on Aziz, (2012) and Singh, (2014), the method that was used in this extraction was modified a bit to match with different amount of raw materials. The fruit was washed properly and then dried properly in open air for 1 hour. The mass, *M* of 200 grams of fresh fruit was dissolved with 1.5 liter of deionize water that act as solvent. The mixture was stirred at room temperature, *T* for 3 hours using magnetic stirrer on a hot plate. The supernatant liquids were then filtered using filter paper. The sample was tested for absorbance at wavelength, λ 800 nm. The procedure was repeated using ethanol 20% concentration. The extract solution was stored in refrigerator for next test.

2.6 MICROENCAPSULATION PROCESS FOR DYE EXTRACT

This method was adopted from (Tantituvanont, 2008) and (Selim, 2008). A liter of dye extract solution from every samples that are butterfly pea colour extract, fruit *of Melastoma malabathricum* colour extract using deionize water and using ethanol were prepared in 3 separate beakers. For each beaker, 5% of maltodextrin (50 grams) was added and stirred continuously. All the mixtures were divided into two, for stability test (20%) and for powder formation (80%) using spray dryer. 800 mL of sample undergo spray dry method for next stability test. The powder from spray dried procedure would undergo chromameter analysis. The condition of spray dryer was the inlet temperature is set 130 ^{0}C and feed rate is 5 mL/min which is speed 3. The summary for this procedure is at table 1.

Table 1: Summarization of procedure for producing powder form of sample

No.	Sample	Solvent extract	Total solid polymer added (grams)	Liquid formation (Liter)	For powder formation (Liter)
1	*C. Ternatea*	Deionize water	50	0.2	0.8
2	*C. Ternatea*	Ethanol	50	0.2	0.8
3	*M. malabathricum*	Deionize water	60	0.2	0.8
4	*M. malabathricum*	Ethanol	60	0.2	0.8

Note: The extraction was done by using 1 L of solvent extract and then divided 20% for liquid stability test and 80% for powder stability test after encapsulation.

2.6.1 Stability Study for Encapsulated Dye Extract Solution

2.6.1.1 Study for presence of light for 30 days

4 sample bottles were prepared and filled with 25 mL of sample each. The bottles were placed in open area with presence of light and kept at room temperature, wrapped with aluminum foil and kept at room temperature, wrapped with aluminum foil and kept in the refrigerator at temperature 4^{0}C, and lastly unwrapped and kept in the refrigerator at temperature 4^{0}C respectively, adopted from (Tantituvanont, 2008). The sample is tested with spectrophotometer at absorbance 600 nm at days (0,7,14,21 and 28). The picture of the result was taken and data was recorded.

2.6.1.2 Study for storage time

2 sample bottles which contain 50 mL of sample each were capped and placed in open area with or without presence of light at room temperature. The presence of anthocyanin is tested with spectrophotometer at absorbance 600 nm at days (0,7,14,21 and 28). The picture of the result was taken and data was recorded.

2.6.2 Stability Study for Powder Encapsulated Sample

2.6.2.1 Study for storage time

5 grams of powder sample was placed in petri dish and sealed. The sample was placed at open area with or without presence of light at room. The presence of anthocyanin is tested with Chroma meter at days (0,7,14,21 and 28). The picture of the result was taken and data was recorded. The summary can be seen on table 2.2.

2.7 PREPARATION OF MUFFIN WITH EXTRACTED POWDER AS COLORANT

The muffin cake preparation method based on BBC Food, (2016). The oven was preheated to 180 ºC and muffin cups were prepared in the hole of muffin tin. The butter and sugar were creamed together in a bowl until pale. The eggs were beat in a little at a time and the vanilla extract was stirred in. the flour was folded in using a large metal spoon, a little milk and dye powder were added until the mixture was dropping consistency. The mixture was spooned into the muffin cup until they are half full and baked in the oven for 10-15 minutes, or until golden-brown on top. A skewer was inserted into one of the cakes to check if it were fully cooked. The muffins were set aside to cool for 10 minutes, then removed from the tin and cool on a wire rack.

2.7.1 Stability Test for Coloured Muffin

The summary can be seen on table 2.

2.7.1.1 Study for temperature

3 covered muffins were placed at room temperature, oven 60^{0}C and refrigerator 4^{0}C. The changes were observed after 7 days for 2 weeks. The image and results were recorded.

2.7.1.2 Study for presence of light

2 covered muffins were placed in a room temperature at different places. 1 at dark place and 1 at open place. The changes were observed after 7 days for 2 weeks. The image and results were recorded.

2.7.1.3 Study for taste and colour

4 covered muffins were placed in a room temperature at two different places. 2 muffins at dark place and 2 muffins at open place. The changes were observed after 7 days for 2 weeks. The image and results were recorded.

Table 2: Stability test for muffin

No.	Type of stability test	Area to be tested	Day being tested (days)
1	Temperature	Room temperature 25 °C	0
			7
			14
		Oven 60 °C	0
			7
			14
		Refrigerator 4°C	0
			7
			14
2	Presence of light	Room temperature 25 °C	0
			7
			14
		Wrapped at room temperature 25 °C	0
			7
			14
3	Taste and colour	Room temperature 25 °C	0
			7
			14
		Wrapped at room temperature 25 °C	0
			7
			14

Note: The muffin made with the pigments extracted from *C. ternatea* and *M. malabathricum*.

3. RESULTS AND DISCUSSION

The graph shows the reading in UV Spectrophotometer and chromameter analysis where almost all the data is decreasing in value. For the liquid samples, they are labelled with A (unwrapped, 35^0C), B (wrapped, 35^0C), C (unwrapped, 4^0C) and D (wrapped, 4^0C) in every sets of studies conducted. For the powder samples, they are labelled with CW (*C. ternatea* with deionize water), CE (*C. ternatea* with 20% ethanol), MW (*M. malabathricum* with deionize water) and ME (*M. malabathricum* with 20% ethanol). Below are the results.

3.1 Colour stability of C. ternatea and M. malabathricum extract solution for light, temperature and storage time.

In the figure 4 and figure 7 some of the samples show increasing at 7th day because there is formation of microorganism's growth on the samples.

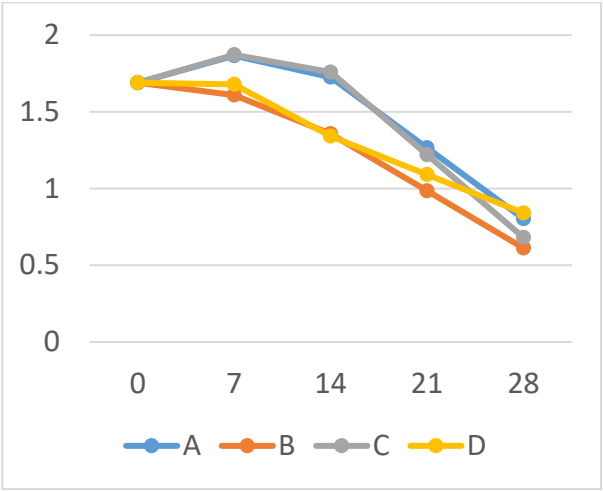


Figure 4: *C. ternatea* with deionize water at absorbance 600 nm.

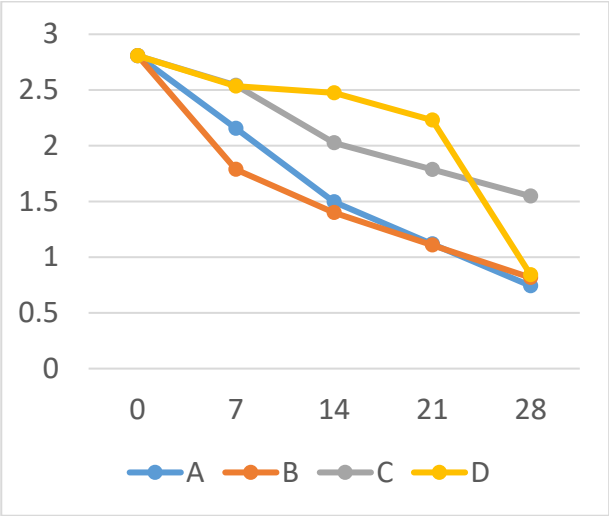


Figure 5: *C. ternatea* with 20% ethanol at absorbance 600 nm.

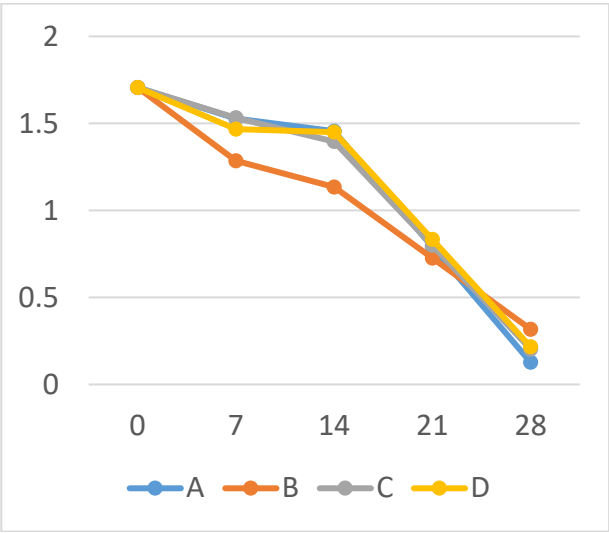


Figure 6: *M. malabathricum* with deionize water at absorbance 800 nm.

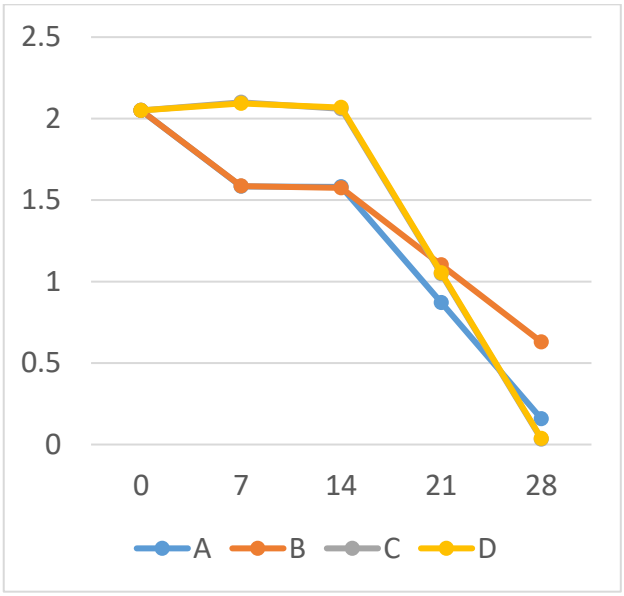


Figure 7: *M. malabathricum* with 20% ethanol at absorbance 800 nm.

From all the graph shown, light, temperature and storage time can degrade the colour of the sample if it stored in liquid form. Some chemical need to be added to preserve the sample from being contaminated by microorganism.

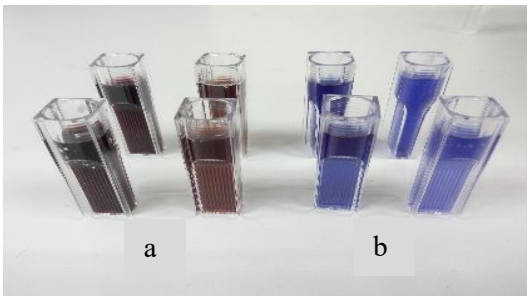


Figure 8: the extracted solution at day 0 where a) *M. malabathricum* extract solution b) *C. ternatea* extract solution

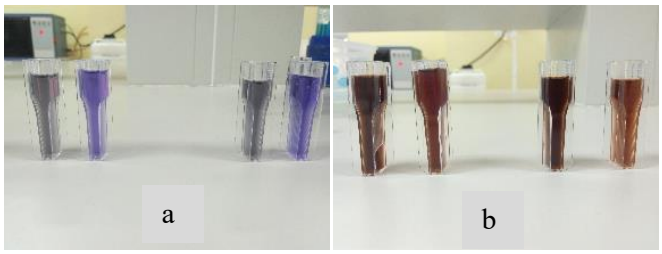
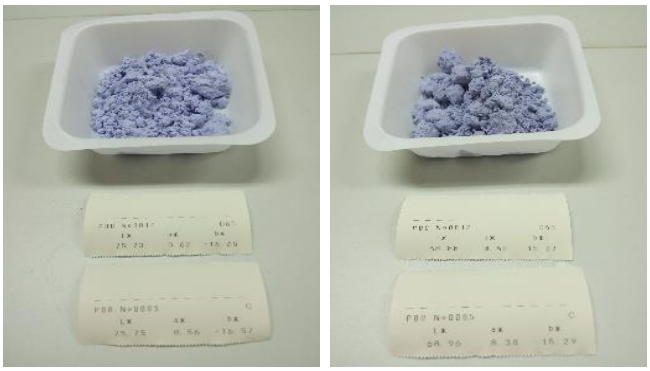


Figure 9: the extracted solution at day 28 where a) *M. malabathricum* extract solution b) *C. ternatea* extract solution

The reading was done using spectrophotometer and the absorbance for *C. ternatea* extract solution was 600 nm while for *M. malabathricum* extract solution was 800 nm. The absorbance different because the viscosity of *M. malabathricum* extract solution was high and the penetration of light through it might affect the reading. From figure 8 and figure 9 it is proved that the colour undergo degradation.

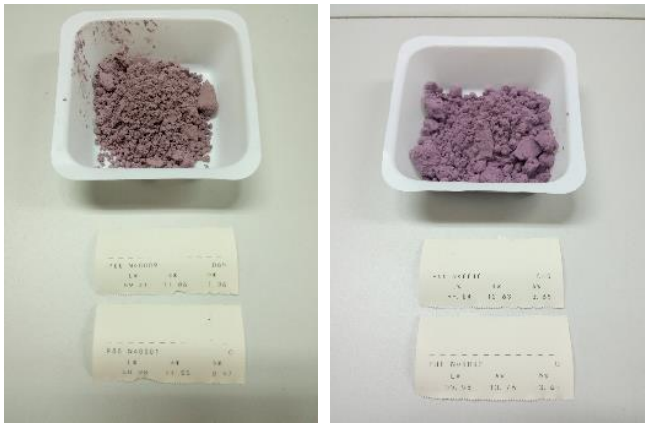
3.2 Colour stability on C. ternatea and M. malabathricum powder for storage time and application on muffin.

The results of chromameter of the four samples all shows decreasing in value. That means even they are stored in powder form, the degradation still occurs by time.



(a) (b)

Figure 10: the powder of *C. ternatea* extract where a) using deionize water b) using ethanol 20%



(a) (b)

Figure 11: the powder of *M. malabathricum* extract where a) using deionize water b) using ethanol 20%

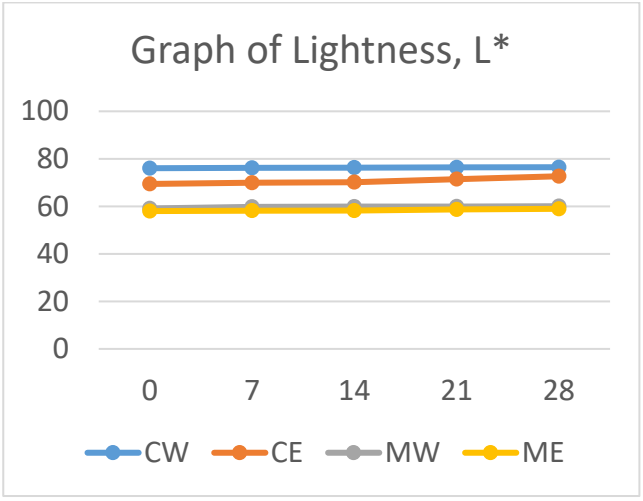


Figure 12: Reading of the L value in 28 days.

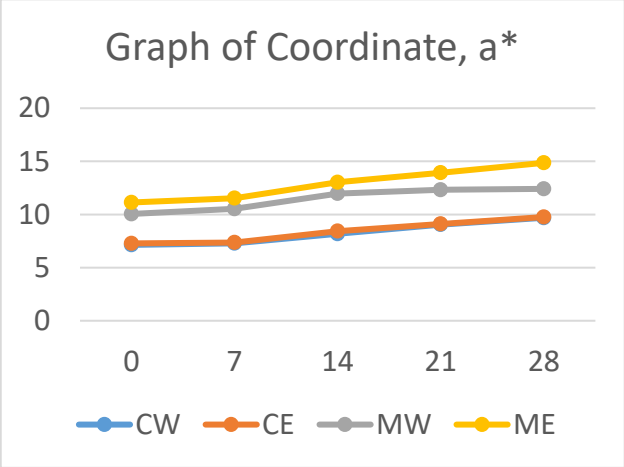


Figure 13: Reading of the coordinate a* in 28 days.

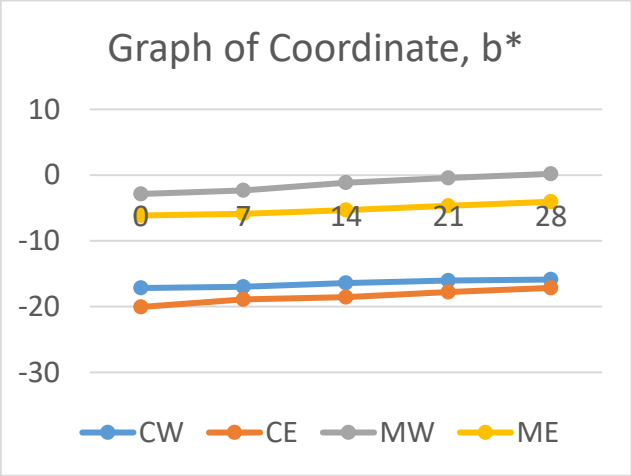


Figure 14: Reading of the coordinate b* in 28 days.

From figure 8 and figure 9 we can see the difference of colour when extracted using deionize water and ethanol 20%. After reading was taken using chromameter, the graph was plotted and different results was shown on graph of lightness, coordinate a* and b*. For graph of lightness *C. ternatea* extract with deionize water get highest reading while *M. malabathricum* extract with ethanol 20% get lowest. For graph of coordinate a*, M. *malabathricum* extract with ethanol 20% get highest reading *C. ternatea* extract with deionize water get lowest. While for graph of coordinate b*, M. *malabathricum* extract with deionize water get highest reading *C. ternatea* extract with ethanol 20% get lowest.

Figure 15: Muffin after bake and after 2 weeks stored.

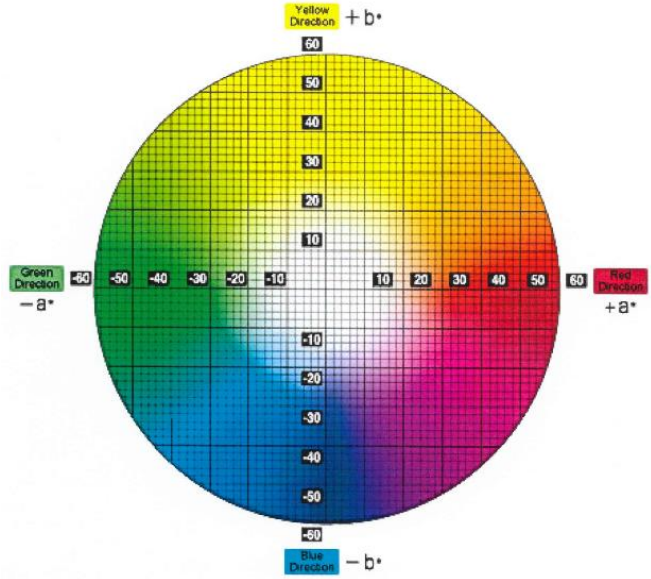


Figure 16: Chromameter colour graph.

Graph of L shows lightness axis, graph of coordinate a* shows red to green axis while, graph of coordinate b* shows yellow to blue axis. From graph of L we can see the reading is increasing but very little that means the colour have been degrade because more light can penetrate through the sample. From graph of coordinate a*, the values were all positive that means all reading shows more to red. Lastly, graph of coordinate b*, the positive value means the reading shows more to yellow while the negative value shows the reading more to blue axis. From the value, we can see the colour on figure 16. The sample in powder form also undergo degradation by time. That means something must be added to preserve the colour. However, when the powder was used in food product, the colour did not degrade much. It is because the colour is stable when it is used in food product. From the observation on muffin, when the powder is added to them, the colour does not change but only mold grow on them as shown in figure 15. This can be corrected by adding some natural preservatives such as honey. The muffin is stored in refrigerator 4°C and open area at 25°C.

4. CONCLUSION

There are many factors of degradation of colour especially natural colour. This paper study on *C. ternatea* and *M. malabathricum* in the form of liquid and powder after the colour has been extract from the samples using deionize water and ethanol 20%. From the results, it is proved that light, temperature and storage time affect the degradation of colour.

The addition of maltodextrin also helps to protect the colour from being over degrade. Maltodextrin also help to enhance the colour when it is put into muffin.

However, the presence of growing microorganism on the sample effect the reading of the results. It is suggested to add anti-microbial substance if it want to be commercialize for future used.

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