A Study on the Extraction and Stability of Blue and Green Pigment from Clitoria ternatea and Pandanus amaryllifolius for Food Colouring.

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Abstract—**The purpose of this project is to evaluate the extraction and stability of colour pigment from butterfly pea pandan leaves, Blue and green artificial colourant for food colouring. The natural colourant was extracted using Hydrodistillation, Supercritical Fluid Extraction and also Ultrasonic Homogenizer. The sample preparation was done by washing, cut into pieces, mixed with water and blend to achieve high surface area for the pigment to be extracted. The stability studies was done by the observation of the product (making of muffin), difference range of temperature, light condition, stored at the presence of light and absence of light and also storage. The analysis was done for 3-4 weeks for all tests. Besides, toxicology studies been done by inductive-coupled plasma spectroscopy (ICP-S). The findings are the artificial colourant showed better stability than natural one but natural blue colourant showed good stability result compared to natural green colourant. Blue colourant showed the higher stability in temperature analysis. The colour for both artificial and natural colourant faded towards time but the most significant instability was the pandan extract. The limitations were, the extraction using Hydrodistillation and Supercritical Fluid Extraction was not suitable for colour extraction since the purpose of using those method is for oil extraction. The colour of untreated samples quickly faded during heating and storage at different temperature. Colour intensity was the best at lower temperature if it is been stored for a long time. Further improvements may fine the method to make the sample's colour intensity last long The results indicate the possibility of using Ultrasonic Homogenizer as an extraction method for natural colourant gives better stability results.**

Keywords— Butterfly pea, Food colourants, Hydrodistillation, Pandan leaves, Ultrasonic Homogenizer, Stability

I. INTRODUCTION

The doubt about safety of synthetic food colourants which is believed to cause health problem to consumer lead to demand of natural colourants for food colouring. Scientific research based on toxicity and stability of natural colourants still on progress to make sure the optimum stability and less toxic before it can be commercialize.

Colours from common natural sources include blue from Clitoria ternatea (butterfly pea) and Pandanus amaryllifolius (Pandan leaves). Clitoria ternatea is a climber plant belongs to Leguminosae family [1]. It originates in Southeast Asia and been broadly conveyed to numerous tropical and subtropical nations where it has gotten to be naturalized [2]. It is known to gather ternatins, a group of (poly) acylated anthocyanins, in its petals. The primary anthocyanins in butterfly pea are delphinidin glycoside which traits to their blue colour [1].

Pandan leaves with its Latin name Pandanus amaryllifolius had been utilized broadly in cooking and conventional home grown treatment in South East Asia Nations. It give a refreshing, fragrant flavour and as natural food colorant to South East Asia dishes [3] The chlorophyll content attributes to their green colour. Pandan leaves comprise aroma compounds which is 2-Acetyl-1-Pyroline (ACPY) were also contain in several aromatic rice such as Jasmine and also Basmathi rice (Schreiner,et.al., 2016). However, these two sources, Butterfly pea and Pandan leaves have been found to be weak under stability to be used commercially.

To increase the stability of natural colorants is by the way of extraction of these compounds and there has been intensive research regarding the extraction process of natural source colorants. The initiative to extract colour pigment of butterfly pea and pandan leaves is with Supercritical Fluid Extraction (SFE) Hydro distillation and Ultrasonic Homogenizer.

The main purpose of this research is to study the stability of natural food colour by comparing with synthetic one. spray dryer is use to obtain the microencapsulation for dye extract to be use in the experimental procedure [4]. On top of that, the parameters that will be study are the effect of various temperature, light exposure, storage time and toxicity for both natural and synthetic colorant by those extraction techniques that help to investigate the stability studies.

The objectives of this study is to identify the extraction methods to obtain green and blue colour pigment from Pandanus amaryllifolius and Clitoria ternatea by Supercritical Fluid Extraction, Hydro distillation, and Ultrasonic Homogenizer and also to explore the effects of extracted colour and the synthetic colour on different condition of storage, temperature, light, toxicity and product.

II. METHODOLOGY

A. Materials

i) Pandan leaves

The pandan leaves was obtained or purchased from any local markets that are available. The sharp part of the leaves were removed with stainless–steel knife and washed properly with tap water. The leaves were cut into small pieces around 500g and scattered on tray before drying. The pieces were put into the oven for drying at 40°C or sun-dried until the moisture content at 9%. The dried pieces were grind using grinder to achieve desired particle size which is 600μm.

ii) Butterfly pea

The fresh butterfly pea was obtained at seksyen 7 area. The flowers were washed properly with tap water and were cut into small pieces around 500g and scattered on tray before drying. The pieces were put into the oven for drying at 40°C or sun-dried until the moisture content at 8%. The dried pieces were grind using grinder to achieve desired particle size which is 600µm.

B. Methods

Colour Extraction by Supercritical Fluid Extraction

Approximately 50 g of dried pandan leaves and butterfly pea flowers respectively were introduced into the basket of the extraction vessel. The extraction vessel including the basket of leaves and flowers is purged with CO_2 for 2 min to remove any moisture or impurities from the pump filter. After purging, the exit valve is closed and the water batch is heated to the required temperature using the thermostat, and then pressure is set to the desired value using the control panel of the SF10 pump. Time is recorded upon reaching the desired temperature and pressure, where the extraction can take place.

Colour Extraction by Hydrodistillation method (Clenvenger)

300 g of 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 hydrodistillation (Clenvenger – 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). Collect the mixture of sample with water that had been heated in the Hydrodistillation extraction method to proceed the next process which is filter and spray dry.

Colour Extraction by Ultrasonic Homogenizer

100 g of samples were blend with 300 ml of distilled water and blend for several seconds. The mixture than being put into 200 ml beaker. The beaker than been put into the Ultrasonic Homogenizer and set 80 Hz for power with pulse power of 3 for 15 minutes. The mixture then being filter and ready for next process.

Stability Test

Stability test of each colour blue and green from natural and synthetic sources were investigated in a time period. The stability test includes effect of storage time, temperature, light, food product and toxicity by specific method that suits the characteristics. The result was taken by the reading of spectrophotometer and naked eye for observation of the changes.

Storage Time

2 sample bottles of each natural and synthetic source contain 50 ml each was placed in petri dish and seal. The sample then placed in open area with and without presence of light at room temperature. The presence of anthocyanin was tested with chroma meter at absorbance 600 nm at days (0,7,14,21 and 28). The picture of the result was taken and data was recorded.

Temperature

The crude extract from butterfly pea and pandan leaves and also synthetic blue and green colour were exposed to different temperatures at 20°C 25°C 30°C, 37°C, 40°C, 50°C, 60°C, 70°C and 80°C respectively. Effect of different temperatures on the colour intensity of the extracts was measured by reading the absorbance at 520 nm [5].

Light

4 samples bottles were prepared and filled with 25 ml of sample. The bottle then were placed in open area with presence of light and kept at room temperature, wrapped with aluminium foil and kept in the refrigerator at temperature 4°C, and lastly unwrapped and kept in the refrigerator at temperature 4°C respectively. The sample is tested with chromameter at days (0,7,14,21 and 28). The picture of the result was taken and data was recorded.

Food product

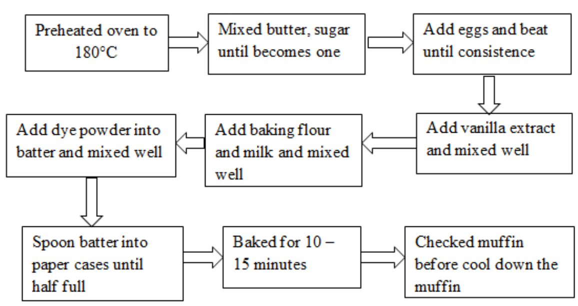

Figure 1: Flowchart preparation of muffin

Toxicity

<u>Sample preparation</u>

Butterfly pea and Pandan leaves were prepared. Weighed 50 g each sample and wash it thoroughly. Put the sample in the blender and add 100 ml of water. Blend each sample respectively for several seconds. The sample mixture then put into the 200 ml beaker before proceed to another process.

<u>Instrumentation</u>

The toxicology study analysis is by using Inductive Coupled Plasma Spectroscopy where it can detect multiple element that content in the sample. An inductively coupled plasma spectrometer is a tool for trace detection of metals in solution, in which a liquid sample is injected into argon gas plasma contained by a strong magnetic field. The elements in the sample become excited and the electrons emit energy at a characteristic wavelength as they return to ground state. The emitted light is then measured by optical spectrometry. This method, known as inductively coupled plasma atomic emission spectrometry (ICP-AES) or inductively coupled optical emission spectrometry (ICP-OES), is a very sensitive technique for identification and quantification of elements in a sample.

CP Spectrometers can be used for the analysis of environmental samples, contaminants in food or water, metalloproteins in biological samples, and similar studies. Most ICP-AES instruments are designed to detect a single wavelength at a time (monochromator). Since an element can emit at multiple wavelengths, it is sometimes desirable to detect more than one wavelength at a time. This can be done by sequential scanning or by using a spectrometer that is designed to capture emissions of several wavelengths simultaneously (polychromator). Detection limits typically range from parts per million (ppm) to parts per billion (ppb), although depending on the element and instrument, can sometimes achieve less than ppb detection [6].

III. RESULTS AND DISCUSSION

A. Hydrodistillation

Colour extraction process by using Hydrodistillation is not efficient since the principle of Hydrodistillation is to extract essential oil for quality control. Those two samples have small amount of oil, therefore a small droplets of oil was appear. In addition, the intention for this process is not the oil, but the colour pigments from the samples. In this case, we conclude that Hydrodistillation process is not suitable in extract colour pigments from the samples.

Figure 2: Set up of Hydrodistillation process

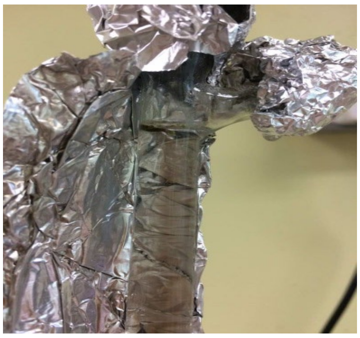

Figure 3: Oil formed from Pandan Leaves

Figure 4: Butterfly pea mixed with water for Hydrodistillation process

B. Supercritical Fluid Extraction

The principle of Supercritical Fluid Extraction also to get the essential oil from the samples. Therefore, small amount of oil droplets was appeared. So we conclude that using Supercritical Fluid Extraction is not the best way and not suitable in getting colour pigments from the sample.

C. Ultrasonic Homogenizer

Since these two processes Hydrodistillation and SFE is not efficient, we propose a new way to extract the colour pigment which is Ultrasonic Homogenizer. Ultrasonic Homogenizer break tissues and cells through cavitation and ultrasonic waves. It creates shear and shock waves which disrupts cells and particles since ultrasonic homogenizer has a tip that rapidly vibrates and causing bubbles in the solution to rapidly form and collapse. Therefore, this method is suitable in getting colour pigments from the sample.

D. Spray Dry

By using spray dry at suitable temperature and flow rate for both samples, we had managed convert the liquid samples into solid (powder).

Figure 5: Powder form of Pandan leaves after spray dry

Figure 6: Powder form of Butterfly pea

E. Colour measurement by using Chromameter

Chromameter had been used to measure colour intensities in all samples. In chromameter, there are value of L*, a* and b* which indicates lightness, redness and yellowish colour according to CIELAB method. In this project, chromameter had been used to investigate colour intensities in synthesis of muffin, temperature, light and also storage.

F. Investigation on Muffin

Figure 7: Artificial Green Colour

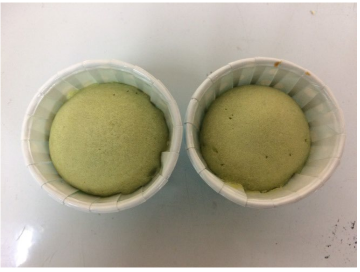

Figure 8: Natural Green Colour

Figure 9: Artificial Blue Colour

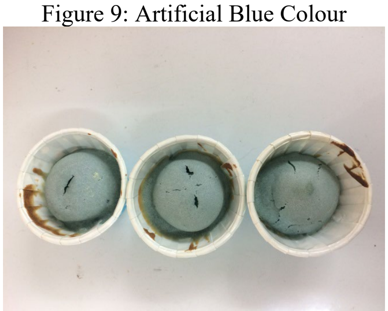

Figure 10: Natural Blue Colour

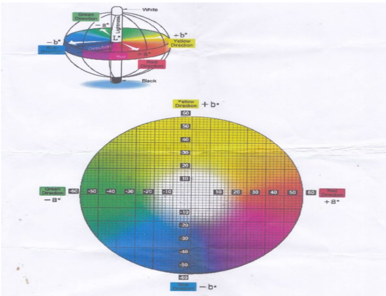

Figure 11: Chromameter Chart

Table 1: Result of colour intensities of muffin on week 1

Chroma meter	Artificial Blue	Natural Blue	Artificial Green	Natural Green
L*	44.49	53.25	52.45	54.57
a*	-21.77	-4.73	-20.11	-7.65
b*	-5.69	1.03	38.55	21.31

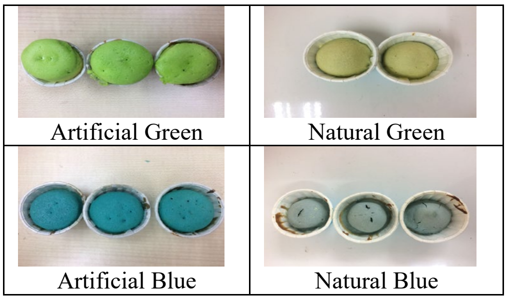

Figure 12: Colour Intensity Week 1

Table 2: Result of colour intensities of muffin on week 2

Chroma meter	Artificial Blue	Natural Blue	Artificial Green	Natural Green
L*	37.6	37.43	49.57	40.21
a*	-20.08	-2.35	-16.69	-2.58
b*	-5.79	7.84	37.03	20.75

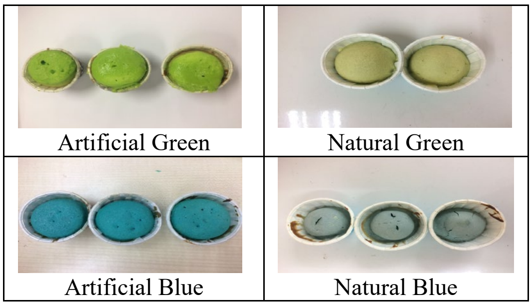

Figure 13: Colour Intensity Week 2

Table 3: Result of colour intensities of muffin on week 3

Chroma meter	Artificial Blue	Natural Blue	Artificial Green	Natural Green
L*	32.35	35.43	35.17	30.22
a*	-9.68	-2.20	-8.20	-2.49
b*	-5.34	5.20	20.14	18.98

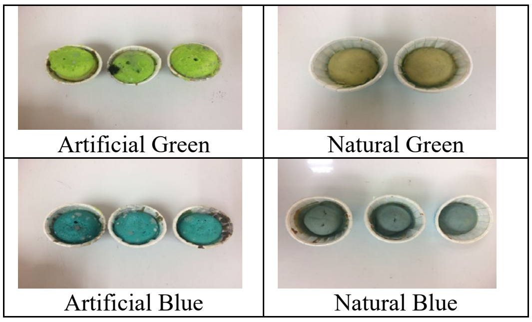

Figure 14: Colour Intensity Week 3

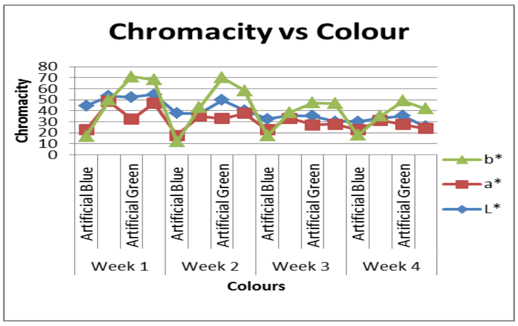

Figure 4.12: Chart of chromacity vs colour

Analysis on the investigation of muffin showed the difference of chromameter reading which L* indicate lightness, a* indicates green direction and b* indicates blue direction. From the results, the readings from week 1 to week 4 show difference in L*, a* and b* value. For L* value, from week 1 to week 4, shows decreasing trend especially for natural colour, where it shows greater change while for artificial colours show insignificant changes. It indicates that the colour was faded every week.

For a* value, both artificial and natural blue colours shows increasing in value but in insignificant condition. It shows positive trends since it become away from the green direction. Colour to the direction of blue b*, shows increasing trends where the colour faded for artificial and also natural blue colours.

From the results, natural colours were less stable compared to artificial as the values changes were quite large. Between natural blue and green colour itself, blue colourant shows better stability as the chromameter value were changed slightly.

G. *Temperature*

Table 5: Result of effect of temperature to colour intensities

Temperature	Artificial Blue	Natural Blue	Artificial Green	Natural Green
25°C	0.343	2.839	0.109	2.583
30°C	0.362	2.76	0.109	2.317
40°C	0.350	2.61	0.104	2.726
50°C	0.367	2.8	0.104	2.734
60°C	0.368	3.082	0.100	2.683
70°C	0.371	3.178	0.103	2.652

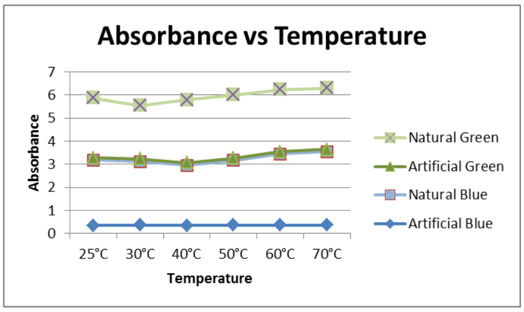

Figure 15: Absorbance vs Temperature

The thermal treatment shows no changes in absorbance for artificial blue for all temperature from 25°C to 70°C. Stability of artificial blue colourant does not based on the temperature change. Natural green colourant better stability at 30°C as the absorbance value is lesser at that point and it continually increases as the temperature increase. For artificial green and natural blue, show almost the same trend where less absorbance at 40°C and the highest at 70°C. From the result proved that butterfly pea colour extract has the potential to be used as natural colourant for food products as the stabilization period lengthened from 27 to 37°C.

H. Light

Table 6: Result of effect of light for colour absorbance (Week 0)

	Artificial Blue	Natural Blue	Artificial Green	Natural Green
Room temperature, a	0.407	2.817	0.111	2.690
Without light and refrigerate, b	0.421	3.008	0.121	2.648
With Light and Refrigerate, c	0.374	3.313	0.118	2.718

Table 7: Result of effect of light for colour absorbance (Week 1)

	Artificial Blue	Natural Blue	Artificial Green	Natural Green
Room temperature, a	0.413	2.820	0.113	2.707
Without light and refrigerate, b	0.429	3.011	0.123	2.652
With Light and Refrigerate, c	0.381	3.332	0.120	2.729

Table 8: Result of effect of light for colour absorbance (Week 2)

	Artificial Blue	Natural Blue	Artificial Green	Natural Green
Room temperature, a	0.418	2.832	0.118	2.717
Without light and refrigerate, b	0.432	3.019	0.125	2.663
With Light and Refrigerate, c	0.383	3.338	0.122	2.738

Table 9: Result of effect of light for colour absorbance (Week 3)

	Artificial Blue	Natural Blue	Artificial Green	Natural Green
Room temperature, a	0.420	2.854	0.119	2.728
Without light and refrigerate, b	0.434	3.032	0.128	2.675
With Light and Refrigerate, c	0.386	3.432	0.129	2.740

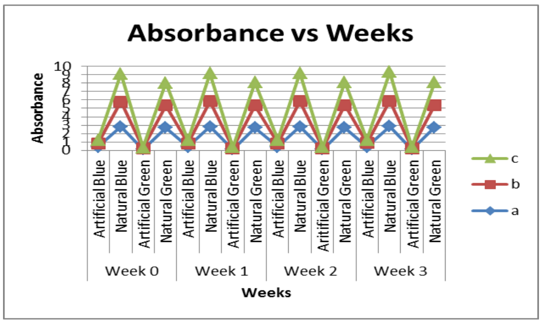

Figure 4.13: Chart of absorbance vs light condition

I. Storage

Table 10: Result of effect of storage for colour intensities (week 0)

Colour/ Light	Artificial Blue	Natural Blue	Artificial Green	Natural Green
Light	0.361	2.958	0.110	2.757
Without Light	0.346	2.909	0.108	2.688

Table 11: Result of effect of storage for colour intensities (Week 1)

	Artificial Blue	Natural Blue	Artificial Green	Natural Green
Light	0.365	2.679	0.123	2.776
Without Light	0.348	2.914	0.118	2.693

Table 12: Result of effect of storage for colour intensities (Week 2)

	Artificial Blue	Natural Blue	Artificial Green	Natural Green
Light	0.369	2.682	0.125	2.790
Without Light	0.350	2.916	0.121	2.701

Table 13: Result of effect of storage for colour intensities (Week 3)

	Artificial Blue	Natural Blue	Artificial Green	Natural Green
Light	0.371	2.689	0.128	2.797
Without Light	0.352	2.923	0.126	2.711

Table 14: Result of effect of storage for colour intensities (Week 4)

	Artificial Blue	Natural Blue	Artificial Green	Natural Green
Light	0.373	2.691	0.129	2.810
Without Light	0.354	2.932	0.128	2.729

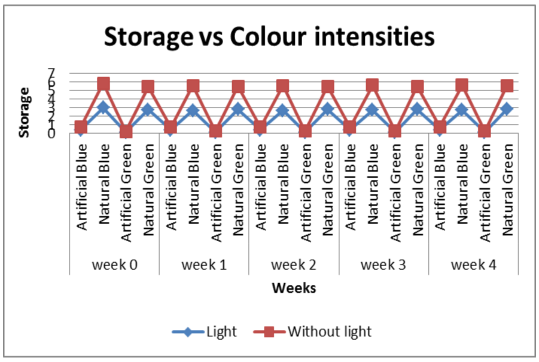

Figure 4.13: Chart of storage vs colour intensities

The sample stored in dark condition shows less degradation compared to being exposed to light. Colour changes for sample in present of light is more rapid for natural colourant while artificial colourant almost no change.

J. *Toxicology Study*

Sample Name	Acquisition Date	Correction Factor
pandan	5/23/2018 9:39:18AM	50.00

Concentration

Element/Wavelength	**As1890**	**Ba4554**	**Ca3933**	**Cd2288**	**Co2286**
Units:	ppm	ppm	ppm	ppm	ppm
Avg. of Repeats:	-0.307938	0.0820737	27.7895	-0.0311202	-0.0268827
Std Dev:	0.0707774	0.00399802	0.552381	0.00157306	0.00368212
%RSD:	22.9843	4.87125	1.98773	5.05479	13.697
Repeat: 1	-0.23757	0.0774616	27.1674	-0.0295741	-0.0251577
Repeat: 2	-0.379118	0.0842064	28.2223	-0.0310676	-0.0243798
Repeat: 3	-0.307127	0.0845532	27.9788	-0.0327189	-0.0311107
Element/Wavelength	**Cu3247**	**Fe2599**	**K_7664**	**Li6707**	**Mg2795**
Units:	ppm	ppm	ppm	ppm	ppm
Avg. of Repeats:	0.0149088	0.208926	179.206	-0.0221084	14.3874
Std Dev:	0.00930852	0.00529309	0.986747	0.000407918	0.0940394
%RSD:	62.4363	2.53348	0.55062	1.84509	0.653624
Repeat: 1	0.0252046	0.204957	180.335	-0.0219176	14.4287
Repeat: 2	0.0124342	0.214935	178.776	-0.0218307	14.4537
Repeat: 3	0.00708765	0.206885	178.508	-0.0225767	14.2798
Element/Wavelength	**Mn2576**	**Na5895**	**Ni2216**	**Pb2203**	**Sr4077**
Units:	ppm	ppm	ppm	ppm	ppm
Avg. of Repeats:	0.301446	15.3738	-0.0658734	0.0307781	0.0265939
Std Dev:	0.00625821	0.179713	0.00189048	0.0211032	0.000514237
%RSD:	2.07606	1.16896	2.86987	68.5657	1.93367
Repeat: 1	0.294249	15.2768	-0.0679793	0.0551428	0.0269411
Repeat: 2	0.304487	15.2634	-0.0653181	0.0189397	0.0268374
Repeat: 3	0.305603	15.5812	-0.0643228	0.0182519	0.0260031

Figure 4.14: Elemental data for Pandan

Sample Name	Acquisition Date	Correction Factor
telang1	5/23/2018 9:45:44AM	50.00

Concentration

Element/Wavelength	**As1890**	**Ba4554**	**Ca3933**	**Cd2288**	**Co2286**
Units:	ppm	ppm	ppm	ppm	ppm
Avg. of Repeats:	-0.350212	-0.00666807	5.53235	-0.0247092	-0.0261668
Std Dev:	0.0610945	0.00606692	0.0148745	0.0111009	0.00647101
%RSD:	17.445	90.9845	0.268864	44.9263	24.7299
Repeat: 1	-0.287242	0.000280139	5.53957	-0.0126541	-0.0218802
Repeat: 2	-0.354156	-0.00936808	5.54223	-0.0269636	-0.0336104
Repeat: 3	-0.40924	-0.0109163	5.51524	-0.0345099	-0.0230098
Element/Wavelength	**Cu3247**	**Fe2599**	**K_7664**	**Li6707**	**Mg2795**
Units:	ppm	ppm	ppm	ppm	ppm
Avg. of Repeats:	0.05257	0.440653	140.141	-0.0232704	5.52212
Std Dev:	0.0117708	0.0258282	1.43709	0.000439508	0.0136989
%RSD:	22.3906	5.86134	1.02546	1.8887	0.248074
Repeat: 1	0.0660745	0.468239	141.783	-0.0234205	5.51964
Repeat: 2	0.0471491	0.436675	139.531	-0.0227755	5.53689
Repeat: 3	0.0444865	0.417044	139.11	-0.0236152	5.50983
Element/Wavelength	**Mn2576**	**Na5895**	**Ni2216**	**Pb2203**	**Sr4077**
Units:	ppm	ppm	ppm	ppm	ppm
Avg. of Repeats:	0.0192942	2.52412	-0.0591072	0.160053	-0.0183627
Std Dev:	0.00280594	0.019833	0.00318683	0.0508041	0.00117652
%RSD:	14.5429	0.78574	5.39161	31.7421	6.40712
Repeat: 1	0.0223351	2.54691	-0.0559639	0.218367	-0.0170204
Repeat: 2	0.0187422	2.51078	-0.0623358	0.12536	-0.0188526
Repeat: 3	0.0168053	2.51467	-0.0590218	0.136432	-0.0192151

Figure 4.15: Elemental data for Butterfly pea

Based on the result from the toxicology study by using Inductive Coupled Plasma-Mass Spectroscopy ICP-MS, where it can detect several elements in the samples. Result shown that for both samples Clitoria ternatea which is Butterfly pea and also Pandan leaves, their element contents are under permissible for food and drugs material. Permissible levels for arsenic (As) is 1.0 mg/kg while Cadmium is 0.3 mg/kg and lead 10 mg/kg. This is by World Health organization. The average amount Arsenic for pandan and butterfly pea are -0.307938 ppm and -0.350212 respectively which equivalent to mg/kg. Average amount of Cadmium for both pandan and butterfly pea are -0.0311202 ppm and -0.0247092 while average amount of lead for pandan is 0.0307781 ppm while butterfly pea is 0.160053 ppm based on figure 4.22 and 4.33 above.

IV. Conclusion

In conclusion, Ultrasonic Homogenizer was the best method to extract the colourant compared to Supercritical Fluid Extraction and Hydrodistillation. For analysis, artificial colourant still shows the best stability compared to natural colourant, but by doing all of the method involves, it can be conclude that the colour stability for natural colourant is not that bad. Talk about the colour itself, not much change in colour for butterfly pea colour extract for weeks but a little bit difference for pandan extract where the colour change faster in just several days. So do to the live longevity. Artificial colours and also natural colourant from butterfly pea does not have a big problem compared to pandan extract, it is does not last long. The results for all analysis were lead to the same findings which are the more stable colourant for artificial and leading behind it was butterfly pea extract which shows better stability also. Therefore, that analysis can lead to further investigation for butterfly pea extraction to find the best way to make it more stable in conjunction with artificial colourant. These can be achieved by using Ultrasonic Homogenizer as a method to extract the colour from the sample with proper handling and criteria specification. For green colourant, green tea can be test for further stability analysis as it is an antioxidant plant. Other test that could be applied in future is the taste analysis. Butterfly pea colour extract was more stable by using Ultrasonic Homogenizer as a method for colour extraction. Proper method for different sample must be taken care of to achieve higher stability. Not all the sample is suitable for some method. Lastly, it is important to make sure the suitableness of the sample to go through what method.

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