CHARACTERIZATION OF EXTRACTED NATURAL COLOUR FROM CAPSICUM AND ITS APPLICATION IN FOOD PRODUCT

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Abstract

Natural colour, a kind of high-quality natural dye, has a great interest as an alternative colourant to use in food products. This work aims to design a strategy for the preparation of free-flowing colour powder from dried capsicum by encapsulating the capsanthin (colour compound) with the coating material. In this work, firstly, 72.63% of capsaicin (piquancy) was eliminated using 0.1 M sodium hydroxide solution. The piquancy-eliminated capsanthin colour compound was extracted under various conditions to optimize the extraction process. The colour value and capsanthin content were calculated by the ASTA analytical method and Beer-Lambert Law respectively, using the absorbance measured at the wavelength 460 nm throughout this research. Additionally, the microencapsulation process of oily-capsicum paste was implemented by coating with gum arabic (GA) on the oily-capsicum particles to improve the appearance and ease of handling. The colour value and capsanthin content of encapsulated powder are 143.17±3.46 and 22.89±0.44 mg g^{-1}. The solubility of encapsulated powder was found 100% in water. The resultant data have proved that this colour could have a natural colouring material giving an added value to the various food products. Therefore, this capsicum colour can be used in the food industry as an alternative to synthetic dye.

Keywords: capsicum, food colour, capsaicin, capsanthin, encapsulation, gum arabic.

Introduction

The development of products with attractive colour and appearance is an essential sensory characteristic in food and cosmetic industry since the colour and appearance impart the first impression to consumers for a rapid judgment of quality (Chan, 2015). Natural dyes have many properties such as little side effects, high safety, biodegradable, green environmental protection. Besides, food colourants can be classified as natural colours, synthetic colours (Rodriguez-Amaya, 2016), there are two types of food colourants approved for industrial use: certified and uncertified. Certified food colourants are synthetic dyes for which testing and approval are processed by the Food and Drug Administration (FDA), whereas uncertified food colourants are natural colours that do not need batch approval by the FDA (Chan, 2015).

Capsicum is the round-shaped chilli that has the non- or mild-pungency. Capsicum colour extracted from the dry capsicum is a high-quality natural dye that has anticancer food additives and colouring properties. Sweet and hot capsicum are widely consumed as vegetables and are used as food colourants because they are a good source of the red carotenoid pigment: capsanthin ($C_{40}H_{56}O_3$) and capsorubin ($C_{40}H_{56}O_4$). As carotenoid compounds are lipophilic compounds, they are usually extracted with acetone or ethanol (Tanaka, 2009; Arimboor, 2015). The capsicum extracts involve bioactive compounds, such as polyphenols, carotenoids, capsaicinoids, and ascorbic acid.

The pungency of capsicum depends on the concentration of capsaicinoids, particularly capsaicin ($C_{18}H_{27}NO_3$). Despite the pungency could be attractive food ingredients in spicy, it is

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unavailable for use in food industry. To address this limitation, the capsaicin content should be eliminated to give a higher attractive ingredient as colouring of various products (Zhao & Chen, 2018). Capsicum extract contains colouring carotenoids predominantly capsanthin and capsaicinoids. Besides the pigments, chemical entities such as flavours, essences, vitamins and fatty oil are also present in the capsicum extract (Rafajlovska, 2011).

Microencapsulation is a process by which solid, liquid or gas is enclosed in microscopic particles of wall material thin coatings around the substances (Ribeiro, 2010). For example, the entrapment of oily colour paste within coating material can protect them from environmental factors such as moisture, air or light. The structure, formed by the microencapsulating agent around the substance to be encapsulated (core), is called the wall (Gupta, 2015). The wall protects the core against deterioration and releases under desired conditions that is a key functionality provided by microencapsulation (Aguiar, 2016).

Materials and Methods

Raw Materials

The dried capsicum was collected from Sin Phyu Kyoon Township, Magwe Region. 95% ethanol, acetone, sodium hydroxide, gum arabic were purchased from Academy Chemical group, 28th Street, Pabedan Township, Yangon Region. All chemicals are of analytical grades and gum arabic is of food grade.

Methods

Preparation of capsicum colour extract from capsicum powder

Firstly, as shown in Figure 1, dried capsicum (50 g) was washed, dried and ground into fine powder (about -30+60 mesh). To eliminate the capsaicin (piquancy) content, the capsicum powder was immersed into NaOH solution at R.T for 12 h. After elimination of capsaicin, the capsaicin-eliminated capsicum was washed with water and dried again. Since the oily capsicum red colour was soluble only in the solvent, the capsicum red colour was extracted with the solvent (acetone and ethanol) for different time (0.5, 1, 2, 4, 6, 8 h) at the temperature range (15, 20, 25, 30, 40, 50 ºC). After the solvent was recovered by distillation, the capsicum colour extract was evaporated by sun-drying to remove the residual solvent. The dried capsicum colour extract is an oily phase so that it cannot be dried into the powder form.

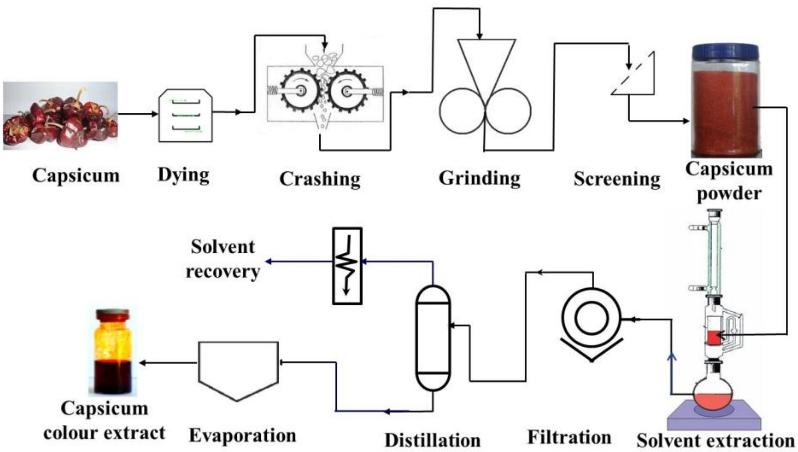

Figure 1 Schematic diagram of the extraction of capsicum colour from dried capsicum.

Preparation of microencapsulated capsicum colour powder

Since the capsicum colour extract is an oily and insoluble in water, oil-in-water emulsion containing capsicum extract as dispersed oil droplet was prepared to carry out the microencapsulation process. For preparing capsicum extract-loaded emulsion (Figure 2), the dried capsicum paste was dispersed with 10% gum arabic (GA) solution to give it in the forms of fine droplets. The GA solution was prepared by vigorous shaking with distilled water before the capsicum extract was added into it. Stirring was continued until the good emulsion was obtained. During emulsification, the oily capsicum extract was surrounded by GA molecules as the coating material. After this encapsulated or entrapment was successfully done, the water was removed by sun-drying and the resultant encapsulated capsicum was ground into fine powder.

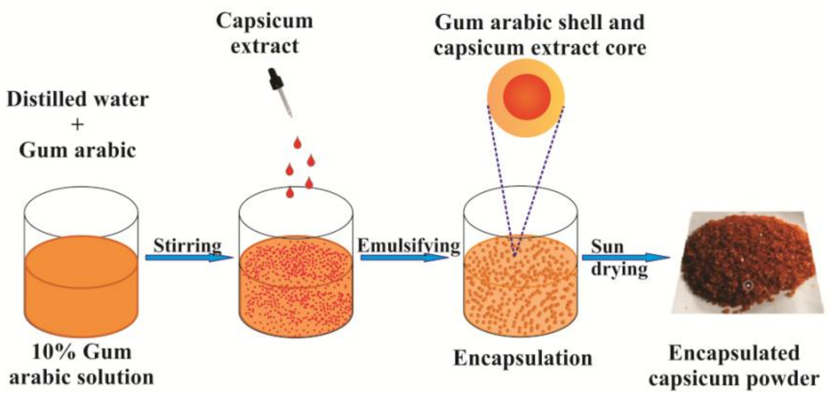

Figure 2 Schematic diagram of capsicum colour powder encapsulated with gum arabic solution.

Characterization of capsicum powder and capsicum colour extract

The characteristics of capsicum powder were studied by phytochemical investigations: polyphenol, flavonoid, glycoside, phenolic, sugar, tannin and saponin (Harborne, 1973). For capsicum powder, colour extract and encapsulated colour powder, physicochemical properties in terms of colour value, capsaicin content and capsanthin content were calculated from resultant absorbance values determined by UV-Vis spectrophotometer (UVmini-1240), respectively. From each sample, a mean value of three readings was recorded to avoid any miscalculation. The possible functional groups for characteristic absorption peak were examined with Fourier Transform Infrared (FT-IR) spectroscopy (Kopec, 2012).

Determination of capsaicin (piquancy) content

The capsaicin content of capsicum was investigated to eliminate the piquancy of capsicum. The concentration of capsaicin was estimated from the standard calibration curve for capsaicin given as follows (Rafajlovska, 2011):

$$y = 9.64\ x + 0.005 \qquad R^2 = 0.9909$$

where x = µg capsaicin/mL of extract, y = absorbance at 281 nm.

Determination of colour value

According to ASTA (American Spice Trade Association) analytical method (Method 20.1), colour values were evaluated throughout this research by the absorbance at 460 nm (AOAC official method 971.26) (Frick, 2003). The colour value was calculated as follow:

ASTA colour value for capsicum= {($A_{extract}$ at 460 nm} x (16.4 *I_f)}/g of sample

where, 16.4 is the conversion factor, $I_f = 0.0600/A_s$ (A_s is the absorbance of standard solution) and $A_{extract}$ is the absorbance values of extract determined by UV-Vis spectrophotometer (UVmini-1240) (Mı´nguez-Mosquera, 2001).

Determination of capsanthin content

The capsanthin content of capsicum colour was calculated by Beer-Lambert Law in this study. The extinction coefficient of the major pigment capsanthin ($1\%E^{460nm} = 2300$) in acetone (Hornero-Méndez et al., 2000).

$$A = \varepsilon cl$$

where, A = Absorbance at 460 nm, c = concentration of capsanthin, mmol mL^{-1}, l = length of cuvette, cm and ε = molar extinction coefficient, L mol^{-1} cm^{-1}

Determination of pH stability

The capsicum colour powder was dissolved into the different pH solution which is prepared using HCl or NaOH. And then absorbance values at 460 nm were measured.

Results and Discussion

To prepare the capsaicin-eliminated capsicum colour extract that can use in food and cosmetics, we have developed the capsicum free-flowing powder prepared by the encapsulation process. In this study, the mild-heat capsicum was chosen because it can give a high intensity of colour. The physicochemical characteristic of capsicum powder: the contents of moisture, ash, crude fiber, total solids; percentages of capsaicin and capsanthin; and then pH and colour value were determined and compared with the literature values. It was observed that the resultant data meets with the literature values as shown in Table 1.

Table 1 Physicochemical characteristics of raw capsicum powder

Sr. No.	Characteristics	Experimental values	Literature values (Krithika, 2014)
1	Moisture content, (%w/w)	8.46	8-14
2	Ash content, (%w/w)	4.19	4-10
3	Crude fibre content, (%w/w)	25.74	15-40
4	Total solids content, (%w/w)	91.54	-
5	pH	4.0	3.00 -5.00
6	Colour value	104.09	>100
7	Capsaicin content, (%w/w)	0.036	0.01-0.5
8	Capsanthin content, (%w/w)	0.28	>0.1

In the phytochemical examination by AOAC method (AOAC, 1990), it has colouring compounds such as polyphenol, flavanoid, phenolic and sugar in capsicum powder and no glycoside, tannin and saponin compounds as presented in Table 2.

Table 2 Phytochemical characteristics of raw capsicum powder

Sr. No.	Tests	Extract	Reagents	Observation	Inference
1	Polyphenols	EtOH	1% $FeCl_3$+1%K_4{Fe(CN) }	Greenish blue colour	+
2	Flavonoids	EtOH	H_2SO_4(conc:) + Mg turning	Pink colour	+
3	Glycosides	H_2O	10% $FeCl_3$	Purple colour	-
4	Phenolics	H_2O	10% $FeCl_3$	Blue-black colour	+
5	Sugar	H_2O	Benedict's solution	Red ppt	+
6	Tannins	H_2O	2% NaCl+ 1% $FeCl_3$	Deep blue ppt	-
7	Saponin	H_2O	1% $NaHCO_3$	Froth	-

+ = present, - = absent

Table 3 Effect of sodium hydroxide concentration on the percent removal of capsaicin (piquency) in capsicum extract

Immersion temperature = 28-32 ºC
Immersion time = 12 h
Ratio of capsicum to NaOH solution (w/v) = 1:10

Sr. No.	Concentration of NaOH solution (M)	Capsicum extract			
		Absorbance (281 nm)	Residual capsaicin (μg g^{-1})	Residual capsaicin (%w/w)	Eliminated capsaicin (%w/w)
1	0	1.81	36.19	100.00	0.00
2	0.01	1.57	31.38	86.70	13.30
3	0.05	0.83	16.67	46.07	53.93
4	0.1*	0.50	9.91	27.37	72.63
5	0.2	0.48	9.65	26.65	73.35
6	0.4	0.48	9.56	26.41	73.59

* The most suitable NaOH concentration

Although the capsaicin (piquancy) is one of the active compounds in capsicum and it has benefits as an antioxidant and anti-fungus actions, it is unsuitable as a colourant (dye) for food and cosmetics. The capsaicin content can be removed by the treatment with suitable NaOH concentration at room temperature for 12 h while stirring. To eliminate the capsaicin present in capsicum powder, different concentrations of NaOH (0.01, 0.05, 0.1, 0.2 and 0.4 M) were used. The capsaicin content was calculated by using the absorbance at the wavelength of 281 nm (Chen, 2009). It was found that 0.1 M NaOH is the minimum concentration to eliminate the maximum amount of capsaicin (72.62%) as shown in Table 3 and Figure 3a. The small absorbance peak at 281 nm is observed in Figure 3b.

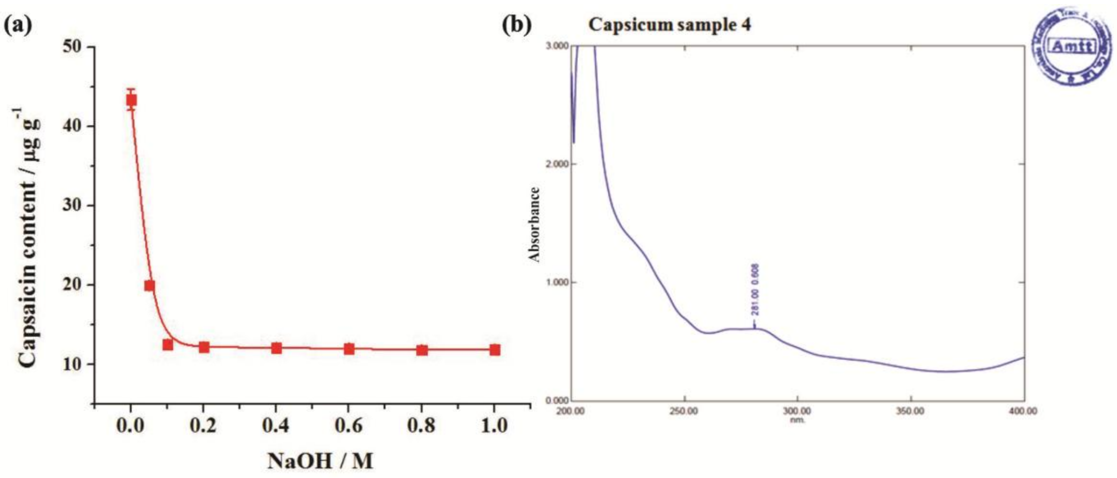

Figure 3 Capsaicin content analysis (a) changes in capsaicin content with the concentrations of NaOH and (b) UV-Vis spectrum of capsaicin (72.62%) eliminated capsicum colour extract treated with 0.1 M NaOH solution.

To explore the optimal extraction and purification process of capsicum colour extract from dry capsicum, the effect of material to solvent ratio, extraction temperature, and extraction time on the yield percent, colour value and capsanthin content in extracted capsicum colour were investigated. The effect of capsicum powder to solvent (acetone and ethanol) ratio on the yield percent, colour value and capsanthin content of extracted colour are presented in Table 4. It was observed that the highest yield percent (4.2% w/w), colour value (159.04) and capsanthin content (25.42 mg g^{-1}) of extracted capsicum colour was obtained from the 1:15 ratio of capsicum powder to acetone solvent when the extraction temperature at 28-32 ºC for 6 h.

Table 4 Effect of capsicum-solvent ratio on the yield percent, colour value and capsanthin content

Capsicum colour extraction temperature = 28-32 ºC
Capsicum colour extraction time = 6 h

Sr. No.	Capsicum : solvent	Capsicum colour extract (Acetone)				Capsicum colour extract (Ethanol)			
		Yield (%w/w)	Abs	Colour value	Capsanthin (mg g^{-1})	Yield (%w/w)	Abs	Colour value	Capsanthin (mg g^{-1})
1	1:5	2.2	0.79	125.28	20.02	2.2	0.70	111.14	17.76
2	1:10	3.8	0.99	157.71	25.21	2.1	0.70	111.00	17.74
3	1:15*	4.2	1.00	159.04	25.42	2.5	0.71	112.59	18.00
4	1:20	4.1	0.97	154.53	24.70	2.8	0.72	114.58	18.32
5	1:25	3.8	0.98	155.85	24.91	2.7	0.79	125.32	20.03

* The most suitable solvent is acetone and capsicum to acetone ratio is 1:15.
* Abs = absorbance

The effect of extraction temperature and time on the yield percent, colour value and capsanthin content of extracted capsicum colour was examined based on the different extraction temperature (15, 20, 25, 30, 40, 50 ºC) and different extraction times (0.5, 1, 2, 4, 6, 8 h) in capsicum colour extraction. The absorbance was measured both for different extraction temperatures and extraction times and then the colour value and capsanthin content were calculated with the absorbance at the wavelength 460 nm (Arimboor, 2015). As shown in Table 5, the highest yield, colour value and capsanthin content of extracted capsicum colour are indicated when the extraction temperature was 30 ºC. The extraction time that gave the maximum yield, colour value and capsanthin content is 4 h, as expressed in Table 6.

Table 5 Effect of extraction temperature on the yield percent, colour value and capsanthin content

Capsaicin-eliminated capsicum powder = 50 g
Volume of acetone = 750 mL
Capsicum colour extraction time = 6 h

Sr. No.	Temperature (ºC)	Capsicum colour extract			
		Yield (%w/w)	Absorbance	Colour value	Capsanthin (mg g^{-1})
1	15	2.0	0.59	107.66	14.90
2	20	2.3	0.67	123.07	17.04
3	25	2.7	0.82	151.03	20.91
4	30*	3.9	1.02	187.05	25.89
5	40	4.2	1.00	183.83	25.45
6	50	4.1	0.97	178.78	24.75

* The most favourable temperature

Table 6 Effect of extraction time on the yield percent, colour value and capsanthin content

Capsaicin-eliminated capsicum powder = 50 g
Volume of acetone = 750 mL
Capsicum colour extraction temperature = 28-32 ºC

Sr. No.	Time (h)	Capsicum colour extract			
		Yield (%w/w)	Absorbance	Colour value	Capsanthin (mg g^{-1})
1	0.5	1.5	0.46	85.21	11.80
2	1	1.8	0.58	105.62	14.62
3	2	2.3	0.61	112.86	15.62
4	4*	4.1	1.02	187.56	25.97
5	6	4.1	1.01	184.75	25.58
6	8	4.1	0.99	182.15	25.22

* The most favourable time

The optimal capsicum colour extract was characterized by UV-Vis spectroscopy at 460 nm and Fourier Transform Infrared (FT-IR) spectroscopy. Figure 4a indicates the large colour peak in the UV-Vis spectrum at 460 nm. In Figure 4b, the characteristic absorption peak of capsicum extract is as follows: the peak of cyclopentane and cyclohexane is at 2950 -2800cm^{-1}, the stretching absorption peak of carbonyl (C=O) is at 1720-1710 cm^{-1}, absorption peaks of methylene (CH_2) and methyl (CH_3) are at 1465 cm^{-1},and the stretching absorption peak of methoxy (C-O) is at 1170-1150 cm^{-1} (Sun, 2008).

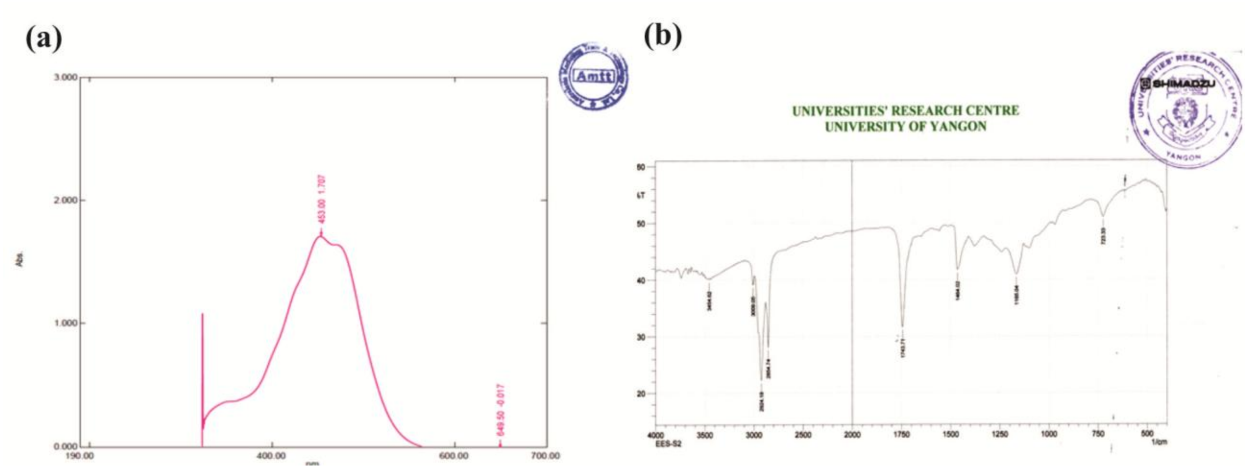

Figure 4 Characterization of capsicum colour extract by (a) UV-Vis spectrum at 460 nm and (b) FT-IR spectrum.

Table 7 Effect of ratio of capsicum extract to gum arabic (GA) on the colour value and capsanthin content of encapsulated capsicum extract

Gum arabic solution = 10% in distilled water
Temperature of sun-drying = 30-40 ºC

Sr. No.	Extract : GA	Encapsulated capsicum colour powder					
		Yield (%w/w)	Absorbance	Colour value	Capsanthin (mg g^{-1})	Organoleptic properties	
						Colour	Phase
1	1:1	2.2	1.01	160.85	25.71	chilli red	oily paste
2	1:5	3.6	0.99	156.91	25.08	chilli red	oily soft film
3	1:10*	4.1	0.9	143.17	22.89	chilli red	non-oily red powder
4	1:25	5.0	0.68	107.78	17.23	yellowish red	slightly hard powder
5	1:50	6.5	0.57	89.88	14.37	yellowish brown	hard powder

* The most suitable ratio of capsicum extract to gum arabic solution.

To develop the encapsulated colour powder, the dispersion of capsicum extract in 10% gum arabic solution was done by shaking to be thoroughly homogeneous phase. The microencapsulated capsicum colour powder was obtained by a cost-effective sun-drying method instead of spray-drying and freeze-drying. The effect of different ratios of capsicum extract to gum arabic (1:1, 1:5, 1:10, 1:25, 1:50) on the characteristics of capsicum powder were studied. As shown in Table 7, 1:1 and 1:5 ratios were found to be oily film manner but 1:25 and 1:50 ratios impart the hard texture and the brownish colour. The 1:10 ratio gave a good appearance and colour as well as free-moving manner on the colour powder.

The stability test of encapsulated capsicum colour powder was conducted in different pH solutions in Table 8. The results show that the colour was not stable in pH 1 because the absorbance value is declined to 0.29. At the pH range of 2-10, it was found that absorbance values slightly decreased when the pH is lower or greater than 7. It was revealed that capsicum colour can be stable in food products.

As regards the colour values were calculated, the colour value of raw capsicum powder was found to be 104.09±7.06, capsaicin-eliminated capsicum extract was 187.56±2.53 and encapsulated capsicum colour powder was 143.17±3.46 (Table 9). It was found that the capsanthin, one of the carotenoids, content is about 2.6 % of capsicum extract and 2.3 % of encapsulated capsicum powder. These results indicate that it has anticancer properties and free-radical scavenging.

Table 8 The pH stability test for encapsulated capsicum colour powder

pH	pH 1	pH 2	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Absorbance	0.29	0.62	0.81	0.84	0.83	0.88	0.92	0.90	0.89	0.85

Table 9 Physicochemical characteristics of capsaicin-eliminated capsicum extract and encapsulated capsicum colour powder

Sr. No.	Characteristics	Raw capsicum powder	Capsaicin-eliminated capsicum extract	Encapsulated capsicum colour powder
1	Absorbance	0.57±0.12	1.02±0.3	0.90±0.05
2	ASTA Colour value	104.09±7.06	187.56±2.53	143.17±3.46
3	Capsaicin content, $\mu g\ g^{-1}$	36.19±1.34	9.91±0.15	5.14±0.12
4	Capsanthin content, $mg\ g^{-1}$	2.88±0.90	25.97±0.32	22.89±0.44

* For the absorbance measurement, all samples were diluted 100 times with acetone. These errors indicate the standard deviation of triplicates.

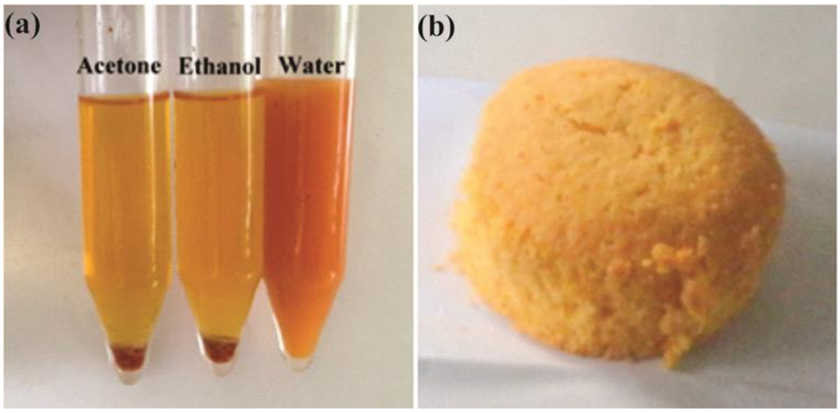

Figure 5 (a) The solubility test of encapsulated capsicum powder diluted with 20 times each of acetone, ethanol and water. It was found 100% solubility in water. (b) Cake with encapsulated capsicum colour powder.

The solubility of encapsulated capsicum colour powder was examined with different solvent; acetone, ethanol and water. Although the capsicum extract is insoluble in water, the encapsulated capsicum powder was observed that it is absolutely soluble in water (100%). It has about 50% solubility in acetone and ethanol as observed in Figure 5a. The encapsulated capsicum colour was applied in the cake baking as shown in Figure 5b. The encapsulated colour solution (1%, 1 mL) as the colour additive and the other common ingredients in cake baking were used as presented in Table 10. The cake photo reveals that encapsulated capsicum powder gives attractive colour used in confectionery. In a consequence of these results, this encapsulated capsicum colour powder will give many benefits such as appearance, stability, antioxidant, etc., in the food products.

Table 10 Composition of the ingredients used in the cake baking

Sr.No	Ingredients	Amount
1	Flour (g)	150
2	Sugar (g)	120
3	Butter (g)	120
4	Egg (no.)	2
5	Baking powder (g)	0.5
6	Vanilla essence (mL)	0.1
7	Natural colour solution (mL)	1

* 1% colour solution in distilled water

Conclusion

To improve the cost-effective method, the extracted capsicum colour was encapsulated with the optimum ratio of extract to wall material (1:10) by sun-drying method. The capsaicin (pungency) in capsicum powder was eliminated using 0.1 M NaOH solution. The suitable condition for the extraction of colour from capsicum was found to be 1:15 ratios of capsicum to acetone at 28-32 ^{o}C for 4 h. The entrapment of oily-capsicum particles within 10% gum arabic coating not only can protect them from environmental factors such as moisture, air and light but also can transform the oily-capsicum paste into the free-moving particles. Moreover, the lipophilic oily-capsicum extract was changed into the hydrophilicity after microencapsulation. The resultant values in terms of colour value and capsanthin content of capsicum might reveal to use as an alternative colour in food industry. Thus, microencapsulation enhanced the solubility and bioavailability of capsicum colour extract as well as the ease of handling in food and cosmetic industry.

Acknowledgements

We would like to express my gratitude to the members of Myanmar Academy of Arts and Science for allowing us to submit this article. The authors would like to acknowledge Dr. Khin Hla Mon, Professor and Head of Industrial Chemistry Department, Dagon University for her kind permission to submit this article at Research Paper Reading Session organized by Myanmar Academy of Arts and Science.

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