Effect of Total Solids Content in Feed Emulsion on the Physico-Chemical Properties and Thermal Stability of Freeze-Dried Roselle Extract

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Abstract: ***Roselle anthocyanin has some potentials in the development of natural food colorants and as a source of antioxidant. Nevertheless, during processing or storage, some factors affects its stability and leads to the degradation. The purpose of this work is to analyze the effect of total solids content on the physico-chemical properties of freeze-dried roselle extract (FDRE) and compare the thermal stability with roselle extract under controlled temperature (80°C and 126°C) and period (0, 20, 40, 60, 80 min). Physico-chemical properties were done from the form of roselle extract, roselle pre-mix solution, and FDRE while thermal stability was done on roselle extract and FDRE following the first order of degradation kinetics. FDRE with 17%, 23%, and 28% total solids content (TSC) were prepared. Results proved that freeze-drying improved the physico-chemical properties and thermal stability of FDRE. Encapsulation efficiency represented that TSC of 28% was the best formulation among others and it affected the half-life of FDRE. At 80°C, TSC of 28% (277.26 min) had the half life 4 times longer than TSC 17% (67.96 min) while at 126°C, the half life of 28% TSC (154.03 min) was 2 times longer than 17% TSC (70.73 min).***

I. INTRODUCTION

Roselle (Hibiscus sabdariffa L.) is a tropical plant belonging to the family of Malvaceae and cultivated for the edible external part of flower calyces. Brilliant red colorant from roselle anthocyanin extract has been processed into various food products. As the most important quality attribute, there has been much interest in the development of natural food colorants following the safety concerns over continued usage of synthetic food colorants [1]. In addition, many studies have found the relation between anthocyanin and antioxidant properties, included: anti-inflammatory, anticarcinogenic, protection against heart disease and cancers, reduction of the risk of diabetes, and cognitive dysfunction [2]. Nevertheless, the stability of anthocyanin is affected by structure of anthocyanin, pH, temperature, oxygen, light, and water activity, which leads to colour degradation [3] .

Due to low molecular weight sugars and high acidic content in roselle, it has a low glass transition temperature, leading to the stickiness problem and low product yield if it proceeds further [4,5] Hence, it is important to encapsulate anthocyanin prior to its usage in foods or beverages to limit its degradation during processing or storage.

Embodiment might be characterized as a procedure to entangle and ensure modest particles or beads of dynamic specialists inside another divider materials from light, temperature, oxygen, dampness, and their collaborations with different substances [6]. Epitome of dynamic specialists can veil their organoleptic properties, similar to shading, flavor, and taste along these lines it will add to an augmentation of the items time span of usability and advance less demanding taking care of [7].According to Shukla [8], freeze drying is the most reliable method nowadays for thermo-labile, especially anthocyanin. The wall material used is either a carbohydrate or a protein which are food grade nutrient supplements. Maltodextrin as a polysaccharide, is considered as a food encapsulating agent because it exhibits low viscosity at high solid content and good solubility. To increase the interfacial property required for higher encapsulation efficiency, carbohydrate is associated with other encapsulating materials, such as protein [9]. Protein can improve the emulsifying and film-forming properties during encapsulation [10]. As an emulsifier, soy lecithin is necessary to avoid aggregation and coalescence in feed emulsion [11].

To the best of authors's information, no writing work has been accounted for on the investigation of freeze-dried roselle extract characterization encapsulated with maltodextrin, sodium caseinate, and soy lecithin altogether. This research aimed on the effect of total solids content (TSC) in feed emulsion on the physico-chemical properties as well as thermal stability of freeze-dried roselle extract (FDRE). To analyse the thermal stability of FDRE, anthocyanin concentration of FDRE and roselle extract (as a control) was determined by the first order of degradation kinetics, so the half-life of FDRE could also be predicted.

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II. METHODOLOGY

Material and Chemicals

Dried roselle calyces was obtained from ANR Sdn. Bhd. (Selangor, Malaysia). Wall materials for this study weremaltodextrin DE10 , sodium caseinate, and soy lecithin. All stock solutions were prepared by purified deionised water (MilliQ purification system, Millipore, France).

Roselle Calyces Extraction

The method of anthocyanin roselle extraction was adapted from Nafiunisa et al. [12] with some modifications. The ratio between solid and water solvent was 1:5 (w/v). Roselle calyces were ground with a household miller (Panasonic MX-GM1011 H, Malaysia). Anthocyanin from the roselle calyces was extracted by homogenizing 200 g of ground roselle with 1000 mL of deionised water by using ultrasonic-assisted extraction method at a frequency of 40% amplitude for 30 minutes (Sartorius Stedim, Labsonic P, Canada). Then, residue of ground roselle was filtered from roselle extract at vacuum condition (Merck Millipore WP6122050, USA) through a filter paper. Finally, the roselle extract was kept overnight under refrigeration at 4°C prior to the analyses.

Total Anthocyanin Content (TAC)

The determination of TAC was conducted with pH differential method which has been performed by Lee et al. [13]. The ratio of sample and buffer was must be 1:4 and the appropriate dilution factor was determined by diluting 0.5 mL of sample with 2 mL of pH 1.0 buffer until absorbance at 520 nm was within the linear range of the spectrophotometer (UvLine 9400, Schott Instruments, Germany). Using this dilution factor, prepare another test portion with 2 mL of pH 4.5 buffer. The absorbance of test portions was read at both 520 and 700 nm in which 700 nm could correct for haze. The diluted test portions were read versus a blank cell filled with deionised water.

pH, Antioxidant Activity and Color

The pH electrode was put in the sample and the measurement was noted after it was stable for few minutes. The pH meter was standardized using standard pH 4, 7, and 10 buffers prior to usage.The antioxidant activity of roselle extract (RE) was determined with DPPH free radical scavenging method based on previous studies of Thoo et al. [14]. Each 0.l mL of sample and 95% (v/v) ethanol (as the control), was added to 3.9 mL of ethanolic DPPH. Soon after vortexing the test portion for 1 minute, the tubes were placed in dark for 30 minutes and absorbance was measured at 517 nm (UvLine 9400, Schott Instruments, Germany). The 95% (v/v) ethanol was also used as the blank.The Hunter color solid system was used to characterize the: (i) lightness (L), (ii) redness/greenness ($\pm a^*$), and (iii) yellowness/blueness ($\pm b^*$) objectively by using colorimeter (ColorFlex EZ, Hunterlab, USA).

Preparation of Rosells Pre –Mix Solution (RPMS)

Based on researcher's previous trials, it was determined 3 formulations of roselle pre-mix solutions: 17, 23, and 28%. The ratio of wall materials in solid phase was carried out according the method of Ng et al. [15]. Wall materials of sodium caseinate and maltodextrin DE10, encapsulated roselle extract at a fixed protein/carbohydrate ratio of 1:9 and an emulsifier of soy lecithin was added at the ratio of 0.1:1 with respect to the protein as shown in Table 1. All of the wall materials were mixed in roselle extract by using a homogeniser (IKA T25 Digital Ultra-Turrax, ThermoFisher Scientific, New Zealand) at an ambient temperature (25^{o}C).

Moisture Content, Water Activity, Hygroscopicity

The moisture contents of FDRE (1 g) was determined gravimetrically by oven drying at 105^{o}C for 3 hours and was calculated. A water activity meter (Aqua Lab Water Activity Meter, Series 3, Decagon Devices Inc., USA) was used to examine the aw of FDRE at 25°C.Approximately 1 g of FDRE was placed in 25°C desiccator containing saturated NaCl solution (75.29% RH). After one week, FDRE was weighed and hygroscopicity was expressed as g of adsorbed moisture per 100 g dry solids [28].

Water Absorption Index (WAI) and Water Solubility Index (WSI)

The determination of WAI and WSI of FDRE were performed according to the method of Grabowski et al., [35]. FDRE (0.83 g; m_s) was mixed with 10 mL of deionised water in a 15 mL preweighed centrifuge tube. The mixture was incubated in a 30^{o}C water bath for 30 min and centrifuged at 3,000×g for 15 min.

Thermal Degradation Kinetics of FDRE

Based on method conducted by [36] with some simplifications, the thermal degradation of FDRE could be measured by heating the samples and roselle extract (as a control) with conditions at 80°C and 126°C for 20, 40, 60 and 80 minutes. Each FDRE formulation (8 g) was separated in 4 dishes and 80 mL of roselle extract were also separated in 4 dishes. They were heated, one dish of FDRE and one dish of roselle extract would be taken out from the drying oven every 20 minutes.The determined anthocyanin concentrations in FDRE followed the first order of degradation kinetics.

III. RESULT AND DISCUSSION

Roselle Extract Characterization

Table 1 shows that the total anthocyanin content of roselle extract and different TSC of FDRE was significantly different ($p < 0.05$). The total anthocyanin content in the freeze-dried roselle extract decreased compared to the roselle extract. Results obtained was supported by the previous studies done by Arueya & Akomolafe [37] where further processes of

FDRE production decreased total anthocyanin content of FDRE. During both processing and storage, anthocyanin is quite unstable from pH, temperature, oxygen and light, which relates to enzymatic systems in the roselle [16].The extracting solution of anthocyanin should be slightly acidic to maintain the stability of flavylium cation form, which was red and stable in highly acidic medium [17]. During the preparation of RPMS, deionized water as roselle anthocyanin solvent, sodium caseinate, maltodextrin and soy lecithin were used, which had pH of 7.0; 6.5–6.9; 4.0–7.0; and 7.0, respectively. Therefore, the pH of RPMS reached neutral and might cause the degradation of the anthocyanin. The pHs for different formulations of FDRE were significantly different ($p < 0.05$) and the higher TSC contributed to the higher neutral condition in FDREFDRE showed a great impact on the antioxidant activity rather than roselle extract, whereby the antioxidant activity for the FDRE was significant higher ($p < 0.05$) than roselle extract. However, there was no significant difference ($p > 0.05$) for the antioxidant activity of different TSC. There was a relationship between moisture content and antioxidant activity in which the lower moisture content caused the higher antioxidant activity of FDRE (Table 1). Anthocyanin is classified as relatively good electron donor [18]. DPPH unstable radical (due to an unpaired electron) becomes more stable after accepting an electron from anthocyanin [19]. Water also has O^{2-} electron so the presence of high moisture content, especially in roselle extract, may contribute to the decrease of antioxidant activity. Other components present in both roselle extract and FDRE, like polyvalent organic acids (citric, tartaric, malic, and ascorbic acids from roselle extract), phospholipids (soy lecithin from FDRE), and protein (sodium caseinate from FDRE) react with antioxidant as the synergists so they increase the antioxidant activity [20].On the redness (+*a**) and yellowness (+*b**) level of color determination, they were clearly showed significant differences ($p < 0.05$) while the lightness between roselle extract and FDRE of 17% TSC were no significant difference ($p > 0.05$). FDRE of 28% TSC had lightest, reddest, and yellowest color among roselle extract and other FDRE. Maltodextrin and sodium caseinate as the highest portion of wall materials used in all of RPMS, especially in 28% TSC. It had white solid physical appearance, therefore they imparted to the lightest color of 28% TSC. The redness and yellowness level of both roselle extract and FDRE were affected by initial condition of roselle calyces.Metal ions such as magnesium, manganese, tin and copper, accumulate in the roselle vacuoles and form stable complexes with anthocyanin, thereby affecting the hueof roselle [21].Table 1 also shows that total anthocyanin content was negatively correlated with reddess level (a*) and this result was supported by previous studies of Kasim et al. [22]. Their studies found that total anthocyanins level was negatively correlated with chromatic parameters (a*, b*, hue angle (h) and chroma (C*). These foundings may be caused by the effect of other components presented in roselle extract, like amino acids, proteins, phenols which contributed to the forming of polymeric brown compounds or decrease of redness level (a*) in FDRE.

Table. 1 Physico-chemical properties of roselle extract and freeze-dried roselle extract (FDRE)

Properties		Roselle Extract	17% TSC	23% TSC	28% TSC
Total Anthocyanin Content (mg/L)		1181.45 ± 76.55[a]	993.58 ± 98.37[b]	822.60 ± 42.60[c]	760.01 ± 25.90[c]
pH		2.01 ± 0.01[d]	2.04 ± 0.02[c]	2.11 ± 0.00[b]	2.17 ± 0.00[a]
Antioxidant Activity (%)		79.67 ± 4.11[b]	90.68 ± 1.09[a]	93.79 ± 0.52[a]	94.00 ± 2.68[a]
Color	*L**	2.51 ± 0.19[c]	2.66 ± 0.04[c]	5.66 ± 0.13[b]	7.77 ± 0.05[a]
	*a**	3.26 ± 0.07[d]	7.38 ± 0,14[c]	13.56 ± 1.28[b]	17.22 ± 1.30[a]
	*b**	± 0.04[d]	2.26 ± 0.06[c]	5.06 ± 0.32[b]	7.58 ± 0.46[a]

Rpms Characterization

Table 2 displays that the increased of TSC impacts to the higher viscosity of FDRE. Results obtained supported by the previous studies by Ahmed et al. [23] that viscosity increased with increasing MD concentration. There was significant difference of viscosity between all of RPMS. Maltodextrin $(C_6H_{10}O_5)n \cdot H_2O$ as a polysaccharide produced from starch, would undergo granule gelatinization and pasting when they met water molecules. As a plasticizer, water molecules enter between granules chains until much of the water is absorbed by them. They break interchain bonds, establish hydration layers around the separated molecules, and force them to swell. As starch granules swell, hydrated amylose molecules diffuse through the mass to the external phase (water), a phenomenon responsible for some aspects of paste behavior and visosity increase [24].

Table. 2 Roselle pre-mix solution viscosity in different total solids content (TSC)

TSC	Roselle Pre-Mix Solution Viscosity (cP)
17%	8.94 ± 0.75[c]
23%	10.59 ± 0.40[b]
28%	12.08 ± 1.10[a]

Analyses of FDRE Physio-chemical properties

Moisture content is how much water there is in a sample while water activity (a_w) is how difficult it is to remove the water from a sample [25]. Based on the definition, there would be a correlation between both of them which was the higher moisture content causes the higher a_w in a sample and it was proven by the results shown in Table 3 The moisture content and a_w of all the three FDRE were significantly different ($p < 0.05$) in which the highest was 17% TSC while the lowest was 28% TSC. According to Anselmo et al. [26], maltodextrin with low DE (less than 20) is more efficient, possess better encapsulant properties and low moisture diffusivity. The term of 'diffusivity' is characterized to depict the rate of dampness development in an example so 17% TSC (with 83% of fluid stage) would hold more dampness content than 28% TSC (with 72% of watery stage). The benefits of lower dampness content are to restrict the capacity of water as a plasticizer and to lessen the glass change temperature [27]. Then again, there was no critical distinction ($p > 0.05$) in all FDRE hygroscopicity and past explores done by Tonon et al. [28] likewise got similar outcomes in which the diminished of dampness content showed the higher hygroscopicity. A lower dampness content had a more noteworthy ability to ingest the encompassing dampness, which was identified with the higher water focus angle between the item and the encompassing air so the lower dampness content was, the higher hygroscopicity of an example.

Water assimilation file (WAI) measures the volume involved by the granule or starch polymer in the wake of swelling in abundance of water while water dissolvability record (WSI) decides the measure of polysaccharide discharge from the granule on the expansion of overabundance of water [29] Table 3 demonstrates that WAI between the most minimal (17%) and most elevated (28%) TSC was fundamentally extraordinary ($p < 0.05$). In view of the meaning of WAI, WAI had a relationship with thickness in which the higher the TSC was, the higher the consistency and WAI of FDRE were. Their WAI variated because of distinction in the level of commitment of hydroxyl gatherings to frame hydrogen and covalent bonds between starch chains [24]. Then, WSI of FDRE increased with increasing of TSC concentration but WSI of all FDRE was same ($p > 0.05$). Maltodextrin as the dominant wall material used in FDRE, is soluble and readily dispersible in water [30]. All FDRE had high WSI percentage of 84.13% - 89.53% so the encapsulated anthocyanin inside the FDRE could be released well for food and pharmaceutical uses.

Table. 3 Physico-chemical characterics of freeze-dried roselle extract (FDRE)

TSC	Moisture content (%)	Water activity (aw)	Hygroscopicity (g of adsorbed moisture/ 100 g dry solids)	WAI (g of gel obtained/ g of dry solids)	WSI (%)
17%	8.30 ± 0.82a	0.335±0.00a	0.1569 ± 0.00a	0.56 ± 0.06b	84.13±5.83a
23%	5.33 ± 0.25b	0.245±0.01b	0.1626 ± 0.01a	0.62 ± 0.06a, b	85.85±6.85a
28%	4.48 ± 0.30c	0.211±0.00c	0.1725 ± 0.01a	0.67 ± 0.06a	89.53±6.19a

Table 4 displays that both TAC and SAC of all FDRE were significantly different ($p < 0.05$). SAC is an amount of anthocyanin that is not entrapped in the capsule wall and remained on the surface of the capsule [31]. The variation of TAC and SAC between three FDRE was due to the degradation of anthocyanin by enzymatic systems during both processing and storage of FDRE After preparing the roselle pre-mix solution (RPMS), one by one of RPMS were proceeded to viscosity analysis from 17% TSC, 23% TSC, and 28% TSC, in sequence. When viscosity analysis of 17% TSC was conducted, RPMS of 23% TSC and 28% TSC were put in room condition with the presence of light. Then, RPMS of 17% TSC was put in cold and dark condition, the viscosity analysis of next RPMS was continued. Room condition with the presence of light might degrade some anthocyanin content in 23% TSC and 28% TSC so the TAC and SAC of 23% TSC and 28% TSC decreased.

Encapsulation efficiency refer to the potential of the wall materials to encapsulate or hold the core material inside the microcapsule. The value of TAC and SAC was also proportional, the higher the TAC was, the SAC also increased. Encapsulation efficiency of all FDRE was significantly different ($p < 0.05$), except for 17% TSC and 23% TSC. Determination of FDRE encapsulation efficiency had been conducted by Idham et al. [32] and Nafiunisa et al. [12]. Idham et al. [32] prepared 4 variations of 20% TSC wall materials: maltodextrin+gum Arabic (M+GA), maltodextrin (M), gum Arabic (GA), soluble starch (SS) which EE were 99.87±0.04%; 99.69±0.06%; 98.4±0.11%; 96.7±0.35%, respectively. Nafiunisa et al. (2017) prepared 10% TSC of maltodextrin and the obtained EE was 33.3%. Compared with the previous studies, wall materials of maltodextrin, sodium caseinate, and soy lecithin used in this study's FDRE, worked synergically to reach the EE as high as Idham et al [32].

Table. 4 Physico-chemical characterics of freeze-dried roselle extract

TSC	TAC (mg/L)	SAC (mg/L)	EE (%)
17%	993.58±98.37a	51.49±3.41a	94.76±0.82b
23%	822.60±42.60b	37.11±0.00b	95.48±0.24b

28%	760.01±25.90b	20.87±0.00c	97.80±0.10a

Thermal Degradation Kinetics of FDRE

Fig. 1 and Fig. 2 described that the data showed a good fit to a linear equation (R^2> 0.90) between [ln Ct/C0] and time, so the reaction was considered to be the first order. This study was in agreement with some stability studies to the anthocyanin extracts such as roselle [32] urmu mulberry [33], sour cherry [34], commercial anthocyanin sample [35]. Either 80°C or 126°C shows that the lower the TSC is, the higher the rate constant (*k*) is. Without any wall materials, anthocyanin in both control samples degraded drastically, especially in higher heating temperature (126°C).

Then, the increased of *k* was associated with the decreased of half-life period as shown in Table 5. The half-life of both control samples were shorter than the half-life of all FDRE, they were 30.01 min (126°C) and 41.76 min (80°C). At 80°C, the half life of 28% TSC was 4 times longer than 17% TSC while at 126°C, the half life of 28% TSC was 2 times longer than 17% TSC. The shelf-life of TAC in the powder was also related to the encapsulation efficiencies (Idham et al., 2010). Based on Table 4, 28% TSC which had the highest EE (97.25±0.10%), the shelf-life was also the highest among other TSC (277.26 min at 80°C and 154.03 min at 126°C). The results proved that the concentration of wall materials played an important role to prevent roselle anthocyanin degradation from heat.

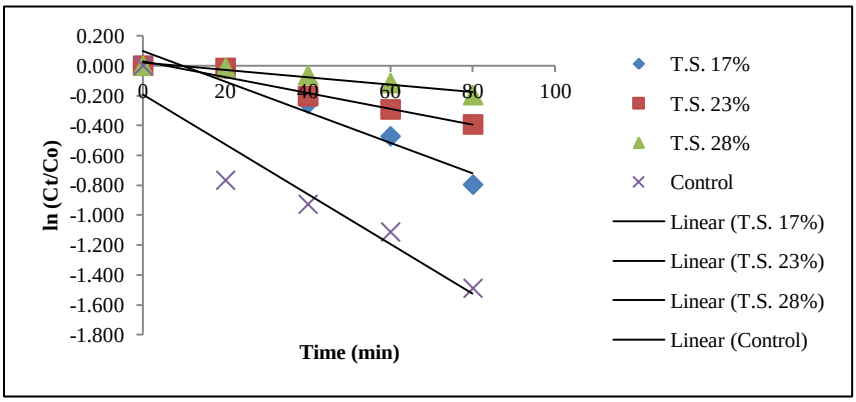

Fig. 1 First-order kinetic plot for retention of TAC in different total solids content (TSC) and control at 80°C

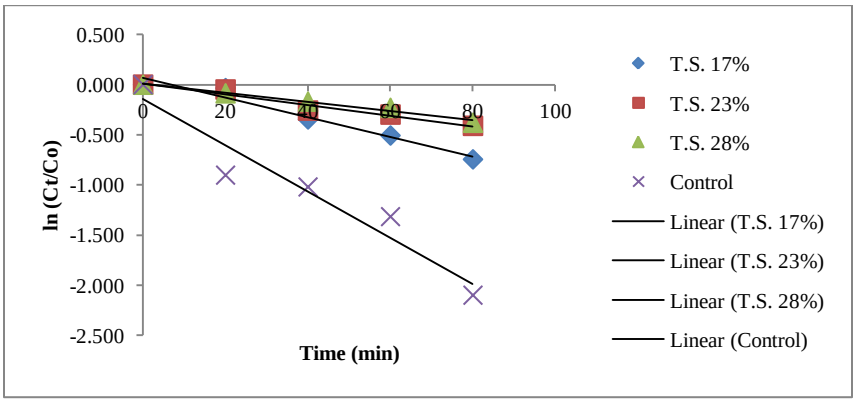

Fig. 2 First-order kinetic plot for retention of TAC in different total solids content (TSC) and control at 126°C

Table. 5 Kinetic parameters of anthocyanins in different total solids content (TSC) and control at 80°C and 126°C

Sample		Kinetic parameters	
TSC	T (°C)	k (× 10-2 min-1)	Half-life (min)
17%	80	1.02 (0.94)	67.96
23%		0.53 (0.96)	130.78
28%		0.25 (0.95)	277.26
Control		1.66 (0.91)	41.76
17%	126	0.98 (0.96)	70.73
23%		0.53 (0.95)	130.78
28%		0.45 (0.98)	154.03

Control	2.31 (0.93)	30.01

IV. CONCLUSION

In this study, freeze drying technique was effective to encapsulate roselle anthocyanin by improving the physico-chemical properties and thermal stability of freeze-dried roselle extract (FDRE). Encapsulation efficiency represented that total solids content of 28% was the best formulation of FDRE among others. Encapsulation efficiency also affected the shelf-life of FDRE in which the higher the encapsulation efficiency was, the longer the shelf-life of FDRE.

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