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Chemical and functional properties of rose cactus (*Pereskia bleo*) mucilage as affected by different purification mediums

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Abstract

Rose cactus (*Pereskia bleo*) leaves contain a complex polysaccharide called mucilage, which its functionality in foods should be better explored. This study aimed to investigate the effects of different purification mediums on the chemical and functional properties of rose cactus mucilage (RCM). Crude mucilage from leaves of rose cactus was extracted by using 0.14 M NaOH solution at 70°C. Three different purification mediums were employed i.e. isopropanol, saturated barium hydroxide and Fehling solution to obtain mucilage that pure in chemical composition with improved functional properties. Of all mediums, saturated barium hydroxide significantly ($p < 0.05$) gave the highest recovery yield (52.61%) of RCM, with the best properties especially in terms of crude protein content (26.01%), solubility at 60°C (87.19%), water holding capacity (WHC) (393.88%) and also with emulsifying capacity and emulsion stability (at 1% solution) of 14.11% and 10.44%, respectively. The values were also significantly ($p < 0.05$) higher than those recorded for crude mucilage. Fourier transformed infrared spectra revealed that RCM was characterized by a β-(1→4)-D-glucosidic main backbone while galactose, mannose, arabinose and uronic acid were four important monosaccharides identified. The findings signify that purification using saturated barium hydroxide could be used to improve the chemical and functional properties of RCM ensuring its wider application in the food industry.

Keywords: Rose Cactus Mucilage, Purification, Chemical Properties, Functional Properties

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Introduction

Rose cactus (*Pereskia bleo*) is a type of tropical herbs which has traditionally been used as natural remedy for treatments of cancer-related diseases, hypertension, diabetes, diseases associated with rheumatism and inflammation, gastric pain, ulcers, headache and body refreshment (Abdul-wahab et al., 2012). It belongs to Cactaceae family, but it is a type of leafy cactus. In Malaysia, rose cactus is locally known as "Tujuh Duri" among Malay community (Ng and Nor Hayati, 2012) while "Cak Sing Cam" among Chinese community (Abdul-wahab et al., 2012). It has been reported that stem and leaves of rose cactus are known to contain distribution of mucilage bearing

structures, and has been suggested that it is a safe alternative source of food industrial functional ingredient. However, crude mucilage from rose cactus leaves contains high amount of total ash (28.67%) with considerably low emulsifying properties, reflecting high impurities of the mucilage (Ng and Nor Hayati, 2012). Emulsifying properties exhibited by some polysaccharides are highly related to hydrophilic nature of sugar constituents especially galactose. But, insoluble impurities including cellulose and ash will negatively affect other functionalities of the polysaccharides. Thus, further purification of crude polysaccharide is crucial in order to improve its functionality.

Mucilage is a type of hydrocolloid, well known as complex polysaccharides, which form molecular networks due to water absorption capacity contributed by its long chain substance of high molecular weight polysaccharide and also a small fraction of protein which is hydrophilic. Since recent times there are literatures that suggest on the potential use of cactus mucilage as flocculating agent and thickening agents (Gebresamuel and Gebre-Mariam, 2012). Cactus mucilage is largely described by a highly branched structure as arabinogalactan-rich polysaccharides made up of important monosaccharides such as galactose, arabinose, and galacturonic acid in different proportions (Contreras-Padilla et al., 2016). As mentioned by previous study, there was increasing interest of functional food ingredients because of its mucilaginous content with complex sugars. Specifically for rose cactus, it is worth mentioning that mucilage obtained from rose cactus leaves (RCM) has great potential for the use in the food industry as an interesting functional food ingredient. However, due to lack in functionality shown in its crude form, the mucilage needs to be purified by a suitable purification medium subsequently after extraction. Studies on guar gum (Cunha et al., 2007), durian seed gum (Amid and Mirhosseini, 2012a), and locust bean gum (Sébastien et al., 2014) have demonstrated that purification mediums such as saturated barium hydroxide, Fehling solution and isopropanol could successfully reduce impurities in crude extracts including ash, cellulose and natural pigments, resulting in improved gum functionalities. Thus, this study aimed to determine chemical and functional properties of RCM after purification with different mediums in comparison with that of crude mucilage.

Material and Methods

Material

Rose cactus (*Pereskia bleo*) whole leaves were obtained from Department of Agriculture, Kelantan, Malaysia. Chemicals used for extraction, purification and chemical analyses were bought from Sigma-Aldrich, US and of analytical grade. Corn oil was purchased from local supplier in Kuala Terengganu, Malaysia.

Extraction of mucilage

Rose cactus leaves were selected based on a similar green colour of older leaves (L^* = 20.10 ± 1.4, a^* = -32.9 ± 3.4, b^* = 23.7 ± 2.5) and free of visual defects. The leaves were soaked in 0.14 M sodium hydroxide solution at 1:3 ratios for 3 hours and blended for 45 seconds with low speed in order to increase total surface area and homogenized them prior to mucilage extraction. Then the extraction solution was heated by using water bath (Techne 12/TE-10 D, UK) at 70°C for 45 minutes and allowed to cool down at room temperature. The extraction solution was then centrifuged at 8000 rpm for 15 min at 25°C by using centrifuge and crude RCM was precipitated from the supernatant with acetone at 1:3 ratios. The extracted mucilage was dried in a drying oven (Oven Memmert, Germany) overnight at 40°C (modified from Ng and Nor Hayati, 2012).

Crude mucilage purification and yield determination

Three different purification mediums i.e. isopropanol, saturated barium hydroxide and Fehling solution were used as purification mediums. 1% w/v crude RCM was dissolved in deionised water and stirred for 30 minutes at 70°C, 250 rpm with magnetic stirring. The prepared solution (1% w/v) was precipitated in purification medium at 1:3 ratios, and then stirred for 5 minutes at 25°C with 250 rpm. The sample-solvent slurry was allowed to stand for 30 minutes. The precipitate was separated by centrifugation at 10,000 rpm for 15 minutes. The precipitate was collected by filtration and washed twice with the purification medium and acetone before oven drying at 40°C, overnight. Yield of purified RCM was calculated using the following formula (modified from Amid and Mirhosseini, 2012a):

$$Yield\ 1(\%) = \frac{Weight\ of\ dried\ purified\ mucilage\ obtained\ (g)}{Weight\ of\ dried\ crude\ mucilage\ used\ (g)} \times 100$$

$$Yield\ 2\ (\%) = \frac{Weight\ of\ dried\ purified\ mucilage\ obtained\ (g)}{Weight\ of\ leaves\ (g)} \times 100$$

Determination of crude protein and total ash contents

Crude protein and total ash contents in crude and purified RCM were determined by using Kjedhal method (conversion factor of 6.25) and dry ashing method, respectively following the standard AOAC procedures (AOAC, 1990).

Determination of sugar composition by a Fourier Transform Infrared spectroscopy

Infrared spectra of crude and purified RCM were obtained by using a Fourier Transform Infrared spectroscopy (Perkin Elmer, USA, Spectrum Software V 6.0.1) at a scanning range of 4000 cm^{-1} – 400 cm^{-1} at 4cm^{-1} resolution. The samples were firstly prepared in KBr discs (2 mg sample in 200 mg KBr) with a hydrostatic press at a force of 5t cm^{-2} for 5 minutes. The sample holder (KBr crystal) unit was cleaned thoroughly with acetone and background reading or value of the sample was collected. The sample holder was cleaned again with acetone for sample scanning. Absorbance of functional group at a specific infrared band range was used to identify the sugar composition of mucilage samples. Same procedure was done for standard monosaccharides (Prabakaran et al., 2011).

Determination of solubility

Mucilage suspensions (30 mL, 1% w/v) were placed in a water bath (Techne 12/TE-10 D, UK) at each 30, 60, 70 and 90°C for 30 minutes with continuous stirring at 50 rpm. The suspensions were then centrifuged at 800 rpm for 15 minutes. 10 mL of supernatant was poured into crucible and dried in oven at 125°C for 24 hours. The samples were cooled in desiccators before weighted. Final weight of dried samples was then weighted and the percentage of solubility was calculated by the following formula (modified from Sciarini et al., 2009):

$$Solubility\ percentage\ (\%) = \left\{\frac{[(Final\ weight - Initial\ weight) \times 30mL]}{10mL}\right\} \times 100$$

Determination of water holding capacity (WHC)

The mucilage powder was weighted accurately (2.5 g), and then added with 25 ml of deionised water, mixed by magnetic stirrer for 15 minutes at 25°C and centrifuged at 4000 rpm for 30 minutes. Then, the supernatant was removed while the wet sample was weighted and used in calculating the WHC (modified from Chau and Cheung, 1997):

$$Water\ holding\ capacity\ percentage\ (\%) = \frac{Weight\ of\ wet\ sample\ (g) - weight\ of\ dry\ sample\ (g)}{Weight\ of\ dry\ sample\ (g)} \times 100$$

Determination of emulsifying properties

The mucilage suspensions of 0.2, 0.4, 0.6, 0.8 and 1.0% were prepared by mixing with deionised water using magnetic stirrer at 80°C, 250 rpm for 2 hours. The mucilage suspensions were allowed to stand at room temperature for overnight ensuring a complete hydration. Each suspension was then mixed with corn oil at 1: 2 ratios and homogenized for 3 minutes by using a high-speed homogenizer (Ultra Turrax, USA). After that, the emulsion obtained was centrifuged at 800 rpm for 10 minutes. The emulsifying capacity was calculated as:

$$Emulsifying\ capacity\ percentage\ (\%) = \frac{Height\ of\ emulsion\ layer\ (cm)}{Height\ of\ whole\ layer\ (cm)} \times 100$$

To determine the emulsion stability, similar procedure was applied to prepare the emulsion sample. The sample was then heated in water bath (Techne 12/ TE -10 D) at 80°C for 30 minutes. Next, the sample was centrifuged at 800 rpm for 10 minutes. Emulsion stability was calculated as (modified from Sciarini et al., 2009):

$$Emulsion\ stability\ percentage(\%) = \frac{Height\ of\ emulsion\ layer\ (cm)}{Height\ of\ whole\ layer\ (cm)} \times 100$$

Statistical analysis

Each crude and purified RCM were prepared in three independent replications for all analyses except for the yield calculation that involved six replications. All data were reported as mean ± standard deviation. Significant effects of independent variable on the chemical properties and functional properties were analyzed using a One-Way ANOVA with Tukey's Multiple Comparison using a Minitab 17 (Minitab Inc., USA) statistical software at $\alpha = 0.05$.

Results and Discussion

Total yield of purified mucilage

The purification yields ranged from 21.15 to 52.61%, depending on the purification medium (Table 1). The purification mediums provided significant effect ($p <$

0.05) on recovery yield in the following decreasing order: saturated barium hydroxide > Fehling solution > isopropanol. Saturated barium hydroxide and isopropanol seemed to give the highest (52.61%) and the lowest (21.15%) yields, respectively based on a total weight of crude mucilage. Considering the actual yield calculated based on original weight of fresh leaves, generally, the yield (3.03%) of purified RCM by using medium saturated barium hydroxide was higher compared to those reported for *Durio zibethinus* seed gum (0.5% - 1.2%) (Amiza et al., 2007) but lower than *Acanthophyllum bracteatum* roots mucilage (5.8% - 12.4%) (Jahanbin et al., 2012) that similarly used barium as a purification medium.

The extraction efficiency is strongly depended on how different is the medium relative to water in terms of their dielectric constant and polarity. Generally, the closer the medium to water especially in terms of their polarity, the more soluble the medium in water and consequently the better interaction among medium-water-solute (or polysaccharide in the present case). It is known that, of all mediums used, barium hydroxide is much more soluble compared to the other two mediums, because Ba^{2+} interacts more strongly with water than with hydroxide ions upon mixing due to high polarity of both saturated barium hydroxide and also water. This indirectly increased the capability of barium hydroxide to precipitate the polysaccharide from the water matrix and thus resulted in the highest yield recovery (Amid and Mirhosseini, 2012a).

Crude protein and total ash contents

Crude protein and total ash contents of RCM were shown to be significantly ($p < 0.05$) affected by different purification medium (Table 1). All purified mucilage had significant ($p < 0.05$) high protein content compared with the crude mucilage. Basically, purification process may eliminate fat, fiber or ash but sustain the protein that covalently bound to monosaccharide units of mucilage and in turn the protein fractions become increased after purification. As reported by Benhura and Chidewe (2011) during purification process, protein could be covalently bound to polysaccharide or the protein could be a free protein that was co-purified with the polysaccharides. It is suggested that, protein found in RCM was covalently bound to polysaccharide, known as an arabinogalactan-protein. This protein could not be removed by a physical purification process since the covalent bonds are stable to alkali (Xiang and Runge, 2016). This is the case when saturated barium hydroxide was used, which gave the significant ($p < 0.05$) highest crude protein content (26.01%) amongst the purification medium used.

Table 1: Total yield, protein, ash content and absorbance value of monosaccharide composition and protein functional group of crude and purified mucilage from different purification mediums

Type of mucilage	Purification Yield (%)		Crude protein (%)	Total ash (%)	FTIR absorbance value for specific functional group		
	Yield[1]	Yield[2]			Mannose	Uronic acid	Protein
A (Crude mucilage)	-	-	9.11 + 0.04^{d}	28.11 ± 0.16^{a}	0.10	0.12	0.13
B (Purified mucilage: isopropanol)	21.15± 0.91^{c}	1.21	14.41 ± 0.40^{c}	19.22 ± 0.27^{b}	0.18	0.14	0.22
C (Purified mucilage : saturated barium hydroxide)	52.61± 0.72^{a}	3.03	26.01 + 0.18^{a}	10.11± 0.19^{d}	0.14	0.55	0.80
D (Purified mucilage: Fehling solution)	28.09± 0.52^{b}	1.62	16.79 ± 0.10^{b}	16.06 ± 0.17^{c}	0.12	0.19	0.26

[1]Recovery yield calculated based on weight of crude mucilage. Data are reported as mean from six independent replications (n = 6) for each sample.

[2]Average yield converted based on weight of fresh leaves.

Crude protein, ash and FTIR data are reported as mean from three independent replications (n = 3) for each sample.

[a-c] Means with the same letter are not significantly different. Statistical analysis was not applied to FTIR data.

Ash content was higher in crude mucilage (28.11%) than purified mucilage which ranged from 10.11 to 19.22%. The low value of ash content in purified mucilage indicated low level of contamination as well as high purity of mucilage (Jindal et al., 2013). Previous studies have shown that ash content in durian seed gum (Amid and Mirhosseini, 2012a); crude cress seed gum (Razmkhah et al., 2016) and basil seed gum (Razavi et al., 2009) was drastically decreased after purification.

Sugar composition identification

Through the FTIR identification, RCM could be described with a highly branched structured as arabinogalactan-rich polysaccharides. Based on absorbance intensity, four important monosaccharide's of crude and all purified RCM were galactose, mannose (1070-810 cm^{-1}) (Kacurakova et al., 2000; Yang and Zhang, 2009), arabinose (1068-830 cm^{-1}) (Kacurakova et al., 2000; Yang and Zhang, 2009; Szymanska-Chargot et al., 2015) and galacturonic acid (uronic acid) (1740-1210 cm^{-1}) (Yi et al., 2011; Pereira et al., 2013) in different proportions. Generally, the present FTIR results were in accordance with previous findings revealed that mucilage from Cactaceae species (*Opuntia ficus indica* mucilage) are identified as arabinogalactan-rich polysaccharides containing galactose, arabinose and galacturonic acid with different amounts (Contreras-Padilla et al., 2016).

All purified RCM have characteristic as glucomannan and galactoglucomannan with β-(1→4)-mannan backbone and β-(1→4)-D-glucosidic backbone. Glucomannan and galactoglucomannan were detected around 1064 cm^{-1} due to stretching vibration of C-O in C-O-H bonds (e.g. glycosidic bonds) (Pawar and Lalitha, 2014). Glucomannan was also detected at 1092 cm^{-1}. As reported by Kacurakova et al. (2000) glucomannan and galactoglucomannan are formed due to presence of mannose units. Relative to other sugars, the purification process seemed to give most pronounced effect in terms of increasing amount of mannose and uronic acid (Table 1) implying that there were possible changes in the sugar chemical bonds. It is supported by previous research by Jia et al. (2012), stating that purification process by using different chemicals medium does not degrade the polysaccharide chain structure, but only disrupt the sugar chemical bonds in the centre of the chains. Wu et al. (2013) reported that during extraction process of crude mucilage, cell wall prevents break-up of intracellular polysaccharide substance. However, when purified with alkaline solution, the cell wall will damage and thus facilitate intracellular polysaccharide dissolution. This dissolution occurred due to polysaccharides constituents are easily oxidized with chemical medium (Wu et al., 2013) i.e. saturated barium hydroxide and Fehling solution used in the present case.

In terms of uronic acid content in RCM, saturated barium hydroxide (purified mucilage C) recorded the highest absorbance value (0.55) amongst the medium used (Table 1).This is in parallel with the findings for *Pleurotus tuber-regium* sclerotia polysaccharide, as higher amount of glucuronic acid was detected in this polysaccharide when used alkaline medium as compared hot water medium (Wu et al., 2013). Glucuronic acid (uronic acid) were formed when mannose residues linked by α-(1→4) bonds and glucuronic acid residues linked by β-(1→2) bonds, with side β-(1→6)-linked xylose or galactose as well as 1→3-linked arabinose (Chen, 2014).

Besides, all samples exhibited the characteristic IR absorption of protein with strong absorbance peak of ester carbonyl stretching at around 1650 cm^{-1} (Charles et al., 2008). Of all samples, purified mucilage C (saturated barium hydroxide) recorded the highest absorbance value (Table 1), attributable to the highest protein content. Thus, this result is in parallel with the crude protein content result. Previously, it has also been reported that high protein content in crude cassava mucilage was evidenced by a strong absorbance similarly at 1650 cm^{-1} (Charles et al., 2008).

Solubility

Higher solubility improved the functionality of hydrocolloid. A maximum solubility means hydrocolloids can fully well dissolved in water. Fig. 1 demonstrates that solubility of all purified mucilage was drastically increased when the temperature increased from 30 to 60°C, and achieved their maximum solubility at this point (81-87%). Differently, crude mucilage required higher temperatures (70°C and 90°C) for a better solubility. Increases in mucilage solubility at elevated temperatures are due to more breaking of the hydrogen bonds among polysaccharide chains as well as exposing OH-groups to water (Amid and Mirhosseini, 2012a). In addition, high temperature also promoted the hydrolysis process of mucilage to happened (Muhammad Aslam et al., 2011).

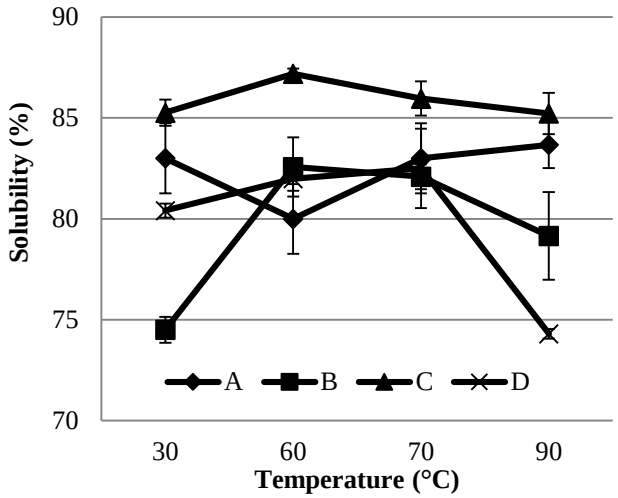


Fig. 1: Solubility of crude and purified mucilage from leaves of rose cactus. Data are reported as mean from three independent replications (n = 3) for each sample. See Table 1 for sample abbreviations.

However, if the mucilage does not have enough of heat to break the bond, the energy required (high temperature) for the net dissolving reaction is endothermic (Amid and Mirhosseini, 2012b). However, as shown in Fig. 1, the solubility of all purified mucilage's started to drop after 60°C, due to polysaccharide already unfolded and showing a lower dependence on high temperature (Sciarini et al., 2009). It is important to note that only purified mucilage C exhibited a constant higher solubility at all tested temperatures, as compared to crude mucilage which could be attributed to its higher uronic acid content (Razavi et al., 2016).

Water holding capacity (WHC)

The purification process significantly ($p < 0.05$) improved the WHC of purified RCM, which varied from 340.08% - 393.88% as compared to the control sample (crude mucilage) (300.20%) (Fig. 2). These values were observed to be pronouncedly higher than other reported new mucilage or commercial gum such as durian seed gum (207 to 228 %) (Amid and Mirhosseini, 2012a); *Citrus grandis* Osbeck mucilage (44.5%) and xanthan gum (274%) (Wang et al., 2016). Difference in WHC among mucilage samples is basically because of difference in ramification proportion. The purification process can cause the unfolding of the constituent polysaccharides, thus resulting in a higher proportion of ramification which affects the WHC. These factors decreased chain-to-chain interaction degree (Sciarini et al., 2009; Amid and Mirhosseini, 2012a). Therefore, purified mucilage had higher WHC due to it was easier for water to interact with polysaccharide chains as opposed to crude mucilage.

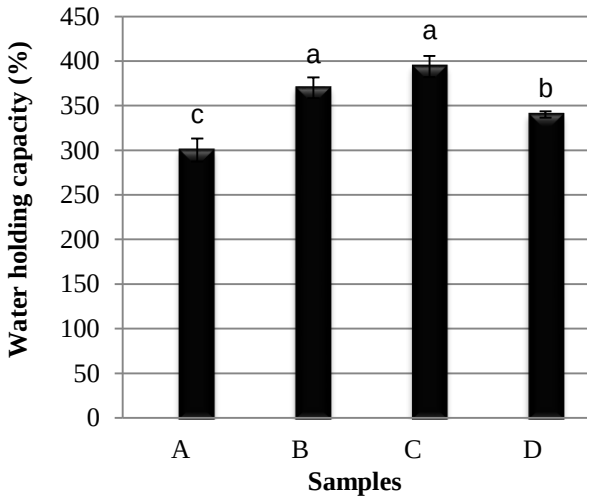


Fig. 2: Water holding capacity of crude and purified mucilage from leaves of rose cactus. Data are reported as mean from three independent replications (n = 3) for each sample. See Table 1 for sample abbreviations.

As referred to Fig. 2, the purified mucilage C exhibited the highest WHC, whilst crude mucilage A showed the lowest as compared to others. This might be due to purification process has eliminated minor components such as minerals which could be considered as impurities. On the contrary, low WHC in crude mucilage A might due to stronger chain-to-chain interaction degree among polysaccharides molecules and impurities, giving rise to lower interaction degree with water molecules (Amid and Mirhosseini, 2012a). The higher protein content in purified mucilage also might resulted higher hydrogen bonding and higher electrostatic repulsion due to strong correlation of protein hydration with polar constituents and the hydrophilic interaction through hydrogen bonding (Muhammad Aslam et al., 2011).

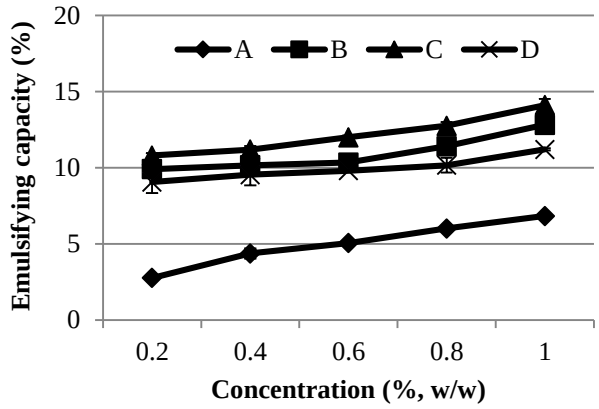
Emulsifying properties

Emulsifying capacity was expressed as molecule's ability to facilitate solubilization where it measures maximum amount of oil that the sample solution can be emulsified by hydrocolloid dispersion without losing its emulsion characteristics. While, emulsion

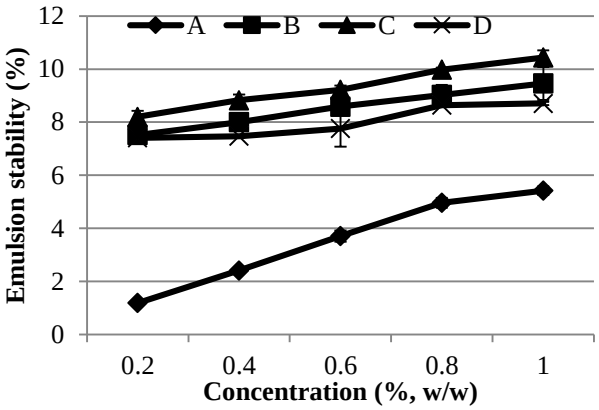
stability was determined as ability of an emulsion with a specific composition to maintain stable and its resistance to phase separation even though under changed condition (Gannasin et al., 2016). According to Fig. 3, emulsifying properties of purified mucilage were found to be better than that of crude mucilage at all emulsion concentrations. The emulsifying capacity range of purified RCM at concentration of 0.2 - 1% was from 9.06 - 14.11% whilst crude mucilage was 2.77 - 6.83% as shown in Fig. 3 (a). On the other hand, based on Fig. 3 (b), the emulsion stabilities of purified RCM at concentration of 0.2 - 1% were ranging from 7.41 to 10.44% while crude mucilage was ranging from 1.19 to 5.42%. Based on Fig. 3 (a), increasing the concentration of crude and purified mucilage was found to increase the emulsifying capacity within the samples significantly ($p < 0.05$). Similar trend was observed for emulsion stability result as depicted in Fig. 3 (b). Higher hydrocolloid concentration generally increases both emulsifying capacity and thus emulsion stability due to decreases in surface tension of oil-water interphase (Ng and Nor Hayati, 2012).

Higher emulsifying properties of purified mucilage could be partially contributed by their higher protein fraction as opposed to crude mucilage (Jahanbin et al., 2012). According to Ritzoulis et al. (2014), the emulsifying capability of polysaccharides is most probably based on a small fraction of surface-active proteins attached to the highly branched polysaccharides and as for this case, in the form of arabinogalactan-protein complex. As referred to polysaccharide-protein complex, both hydrophobic protein chains would adsorb onto the surface of oil droplets because of non polar radical amino acids while hydrophilic polysaccharide fractions limit oil droplet aggregation and coalescence by steric and/or repulsive electrostatic forces (Andrade et al., 2015).

Moreover, Naji-Tabasi and Razavi (2016) found that protein eliminated from basil seed gum resulted in low emulsion stability. It is also worth to mention that purified RCM have higher total content uronic acid as compared to crude mucilage. This led to higher emulsifying properties of purified mucilage due to purified mucilage have surface activity that induce a strong electrostatic repulsion between oil droplets (Naji-Tabasi and Razavi, 2016).



(a)



(b)

Fig. 3: Emulsifying capacity (a) and Emulsion stability (b) of crude and purified mucilage from leaves of rose cactus. Data are reported as mean from three independent replications (n = 3) for each sample. See Table 1 for sample abbreviations.

Conclusion

The present study successfully demonstrated that RCM could potentially be used as a promising food ingredient for emulsion-based food products. The purified RCM was found to be significantly ($p < 0.05$) different from crude mucilage in terms of their chemical and functional properties. Four important sugar constituents in RCM were galactose, mannose, arabinose and uronic acid. As compared to others, purified mucilage C (saturated barium hydroxide) contained higher crude protein fraction and uronic acid, resulting in a higher emulsifying properties which is desirable for emulsion-based food products.

This mucilage also exhibited higher WHC compared to crude mucilage and also other mucilage purified by Fehling solution and isopropanol, that would contribute to better thickening and gelling property. In short, saturated barium hydroxide could be a medium of choice for purification in order to produce higher mucilage yield with good functional properties especially solubility, WHC (reflects a gelling ability) and emulsifying properties.

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Contribution of Authors

Aluwi NFM: Conducted the study and prepared the manuscript draft
Ibrahim NH: Designed the study, supervised the study and prepared the manuscript draft
Hamzah Y: Supervised the study and prepared the manuscript draft
Rozaini MZH: Supervised the study and prepared the manuscript draft

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