คุณสมบัติของอิเทอริไฟด์โพลีแซคคาร์ไรด์จากกากมะพร้าวในอิมัลชันน้ำมันในน้ำ

Characteristics of Etherified Polysaccharide from Coconut Pulp in O/W Emulsion

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บทคัดย่อ

ในด้านอาหาร อิมัลซิไฟเออร์เป็นสารรักษาความคงตัว สารเพิ่มความเข้มข้น ฟิล์มเคลือบ สารแขวนลอย และสารเพิ่มปริมาณ กากมะพร้าวเป็นเนื้อมะพร้าวที่คั้นกะทิออกแล้ว กากมะพร้าวไม่มีกลูเตนและเมล็ดและไม่มีสารต่อต้านอาหาร วัตถุประสงค์ของการศึกษานี้ เพื่อสังเคราะห์อิเทอร์โพลีแซคคาร์ไรด์จากกากมะพร้าวและศึกษาคุณสมบัติทางเคมีกายภาพในด้านอุตสาหกรรมอาหาร โดยนำกากมะพร้าวบดมาทำปฏิกิริยาในสารละลายด่าง จากนั้นนำมาเติมลงในขวดก้นกลมและรีฟลักซ์เป็นเวลา 2 ชั่วโมง กรองส่วนที่เป็นตะกอน โพลีแซคคาไรด์จากกากมะพร้าวได้ถูกทำปฏิกิริยาเป็นคาร์บอกซิลเมทิลโพลีแซคคาร์ไรด์โดยปฏิกิริยาอิเทอริฟิเคชั่น ทำการศึกษาเมทิลโพลีแซคคาร์ไรด์ในคุณสมบัติอิมัลซิไฟเออร์ที่พีเอชต่างๆ ผลการศึกษาแสดงว่าเมทิลโพลีแซคคาร์ไรด์มีความสามารถอิมัลซิไฟด์สูงทุกพีเอชที่ทำการศึกษา มีค่าดัชนีของกิจกรรมการอิมัลซิไฟด์สูง และขนาดหยดของอิมัลชันเล็กที่พีเอชต่ำ มีค่าความคงตัวของอิมัลชันใกล้เคียงกับคาร์บอกซิลเมทิลเซลลูโลสเชิงพาณิชย์ในทุกพีเอช (ยกเว้น อิมัลชันที่พีเอชต่ำ) โดยไม่มีการเปลี่ยนแปลงขนาดหยดอิมัลชันระหว่างการเก็บรักษาที่อุณหภูมิห้อง ผลการศึกษาแสดงว่าอิเทอริไฟด์โพลีแซคคาร์ไรด์จากกากมะพร้าวเป็นอิมัลซิไฟเออร์ที่ดีสำหรับการนำไปประยุกต์ใช้ในอุตสาหกรรมอาหารต่อไป

คำสำคัญ: อิมัลซิไฟเออร์ กากมะพร้าว อิเทอริไฟด์โพลีแซคคาร์ไรด์

Abstract

In foods, polysaccharide emulsifier is used as a stabiliser, thickener, film former, suspending agent and bulking agent. Coconut pulp is coconut meal that is leftover from making the milk out. Coconut pulp is naturally gluten and grain free and free of antinutrients. The objectives of this study were to synthesize etherified polysaccharide from coconut pulp and to evaluate their physiochemical properties as a potential material for food industry. The ground coconut pulp was treated with an alkali solution. The mixture was transferred into a round bottom flask and treatment was performed at reflux for 2 h. The solid was then filtered. Polysaccharide from coconut pulp was modified to carboxyl methyl polysaccharide by reaction of etherification. Carboxyl methyl polysaccharide was investigated in terms of its emulsifying properties at various pH values. The results showed that it had higher emulsifying capacity, higher emulsifying activity

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index. Carboxyl methyl polysaccharide also had high emulsifying capacity at all pH values tested, high emulsifying activity index at acidic pH, and small emulsion droplet size. The stability of based emulsions were similar to those of commercial carboxyl methyl cellulose (except the emulsion stabilized at acidic pH), with no significant changes in droplet size during storage at room temperature. The results show that the etherified polysaccharide from coconut pulp is effective emulsifier in food industry.

Keywords: emulsifier, coconut pulp, etherified polysaccharide

Introduction

Food emulsions are compositionally complex. Their droplets are stabilized to differing extents by proteins, small-molecule surfactants (emulsifiers), and, in certain cases, polysaccharides (hydrocolloids). In terms of the underlying mechanisms of (de)stabilization, there are some similarities and differences between particles and the other types of emulsifying agents.[1-3] The most widely used polysaccharide emulsifiers in food applications are gum arabic (*Acacia senegal*), modified starches, modified celluloses, some kinds of pectin, and some galactomannans.[4,5] The surface activity of these hydrocolloids has its molecular origin in either (i) the non-polar character of chemical groups attached to the hydrophilic polysaccharide backbone (in hydrophobically modified starch/cellulose) or (ii) the presence of a protein component linked covalently or physically to the polysaccharide (some gums, pectins, etc.).

Natural biopolymer structural assemblies are obviously attractive as particle building blocks. Polysaccharides such as starch and cellulose represent a readily accessible and inexpensive source of particulate material for potential food use. After appropriate hydrophobic modification, these materials can be highly effective in the stabilization of emulsions and foams.[6, 7]

Coconut pulp is coconut meal that is leftover from making the milk out. Coconut pulp represents high amount of polysaccharide of 61%.[8] The objectives of this study were to prepare carboxyl methyl polysaccharide from coconut pulp in comparison with the characteristics of commercial carboxyl methyl cellulose (CMC) and to evaluate their physiochemical properties as a potential material for food industry.

Materials and Methods

Source of material

Coconut pulp used as a raw material was a by-product from a local coconut milk factory (Nakhon Pathom province, Thailand).

Preparation of coconut pulp

The coconut pulp was dried at 60 ºC for 12 h in a hot-air oven and sieved to obtain particle size of coconut pulp in the range of 0.4–3 mm. The dried coconut pulp was kept in polyethylene bag and kept at room temperature until used.

Extraction of polysaccharide

The coconut pulp was soaked in distilled water for 72 hours, the water was changed every 8 hours. The coconut pulp was subsequently boiled for about 15 min and then washed several times in fresh water until supernatant was clear. The coconut pulp was finally dried in the sun and

ground using a ultracentrifugal mill. The ground coconut pulp was treated with 4% NaOH at 125 ºC for 2 h. The ratio of the coconut pulp to liquor was 5:100 (g/mL). Each step was repeated several times, and the coconut pulp was washed with distilled water after each treatment.

Carboxyl methyl polysaccharide Synthesis

CMCs were synthesized following the procedure describe by Browning.[9] Polysaccharide was suspended in isopropyl alcohol under mechanical stirring at room temperature and 40 % NaOH was added to the mixture. Five gram of monochloroacetic acid (MCA) was added to the mixture and its temperature was maintained at 55 ºC for 3.5 hour. Then, methanol (70% v/v) was added and the mixture was neutralized with acetic acid (90% v/v). CMC was then recovered by filtration with Whatman filter paper No. 1 and washed with ethanol/water (70/30 v/v). The product was washed with methanol and dried at 60 ºC. After the first etherification reaction, CMCs were submitted to a second reaction. The second etherification treatment was performed with the same chemicals, but the concentration of the MCA was slightly increased to 7 g per 5 g of each polysaccharide sample.

Emulsion formulation and particle size determination

Oil in water emulsions (o/w emulsions) were prepared according to the method described by Agboola et al.[10] using 1% (w/v) emulsifier and 20% (v/v) oil in 10 mM Sodium Phosphate buffer (pH 7.0) containing 0.5% w/v sodium azide.

First, coarse emulsions containing oil and the emulsifier were obtained with a rotor-stator system (Ultra-Turrax) at 4°C. Then, the coarse emulsion was prepared at 60°C with high shear mixing using a Fluko homogenizer FA25 model by rapidly adding the oil phase to the aqueous phase at 10,000 rpm. The high shear mixing process was carried out for 8 minutes at 19,000 rpm and the final emulsion was obtained by high-pressure homogenization using homogenization equipment (APV-2000, Germany) at 1000 bar for seven cycles. The temperature of the whole homogenization process was maintained below 40°C in a cycle ice-water bath.

The emulsions were evenly distributed into screw cap tubes and stored at 25 ºC for 7 days. The mean particles size was measured with a Mastersizer E (Malvern Instruments, UK) using Milli-Q water as dispersant, from day 0 to day 7 and the emulsions were observed physically for stability, creaming and oiling-off.

Emulsifying capacity (EC)

EC was determined according to the method described by Webb et al.[11] with modifications. The carboxyl methyl polysaccharide sample (50 mg) was dispersed in 40 mL of 10 mmol/L phosphate buffer at pH 4, 7 and 9 and then stirred for 1 min using a Dumax Laboratory Stirrer (Milperra, Australia). The sample solution was added to 40 mL oil and the mixture was stirred for 1 min to form an emulsion. A volt-ohm meter with 2 electrodes immersed in the mixture was used to determine the electrical resistance of the emulsions. The mixture was continuously stirred while additional oil was added to the mixture using a burette until a sudden increase in electrical resistance of the dispersion occurred upon the collapse of the emulsion. EC was expressed as mL oil emulsified per g sample.

Emulsifying activity index (EAI)

EAI of carboxyl methyl polysaccharide samples at pH 4-9 was determined according to the spectroturbidimetric method of Pearce and Kinsella[12] with modifications. Immediately after the emulsions were prepared as described above, the emulsions (10 ml) were diluted appropriately with 1 mg/mL sodium dodecyl sulfate (SDS) solution to give an absorbance between 0.1 and 0.6 at 500 nm measured by a Helios Gamma UV-vis spectrophotometer (Thermo Electron Corporation, UK) using the SDS solution as a blank. The EAI was then calculated as EAI = 2T/øC, where ø is the volume fraction of the dispersed phase, C is the weight of protein per unit volume of aqueous phase before the emulsion is formed, and T is turbidity (defined by 2.303A/l, where A is the observed absorbance and l is the path length of the cuvette).

Experimental design

All experimental parameter measurements were done in duplicate. The experiment was used to generate the experimental model for a factorial design.

Statistical analysis

All analyses were carried out in triplicate and the means with standard deviation were reported. Data collected were subjected to analysis of variance (ANOVA), and Fisher's least significant difference test was used to determine if the means were significantly different ($p < 0.05$) by using SPSS™ statistical software version 17.

Results and Discussions

Emulsifying capacity (EC)

The ECs of CMC and carboxyl methyl polysaccharide at pH 4, 7 and 9 are shown in Figure 1. CMC and carboxyl methyl polysaccharide showed significantly different EC at each of the pH tested. The capacity of CMC to form emulsion was 426 mL/g at pH 4, increased to 829 mL/g at pH 7, then further increased to 1014 mL/g at pH 9.

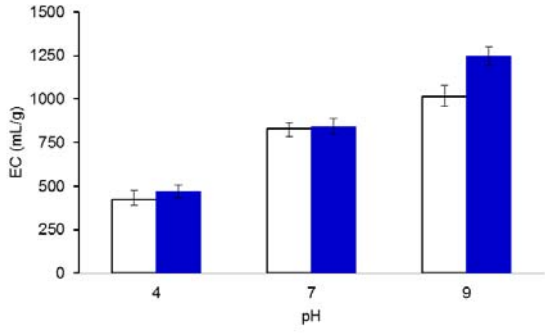

Figure 1 Emulsifying capacity (EC) of CMC (□) and carboxyl methyl polysaccharide (■) at pH 4, 7, and 9. Data are means of triplicate ± standard deviation.

It is noteworthy that the relatively constant, significantly and consistently higher EC of CMC in comparison to carboxyl methyl polysaccharide (at pH 4, 7 and 9) indicates that carboxyl methyl polysaccharide may be a useful emulsifier with wider application in food.

Emulsifying activity index (EAI)

The EAI of CMC and carboxyl methyl polysaccharide is shown in Figure 2. Carboxyl methyl polysaccharide had a higher EAI than CMC at all pH tested. The EAI of carboxyl methyl polysaccharide and CMC increased with increasing pH. The low EAI of CMC than that of carboxyl methyl polysaccharide could probably be due to the high molecular weight.[13] In addition, the high molecular weight,[14] could have further reduced the overall flexibility of the structures, thus decreased their ability to unfold and associate with the oil droplets (27-30 μm).

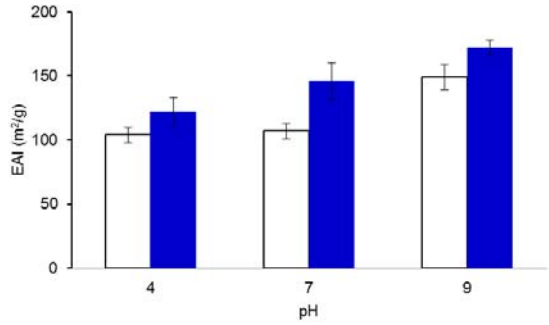

Figure 2 Emulsifying capacity (EAI) of CMC (□) and carboxyl methyl polysaccharide (■) at pH 4, 7, and 9. Data are means of triplicate ± standard deviation.

Changes in droplet size

Figure 3 (A), (B) and (C) shows the changes in average droplet size over 24 hours of storage at 25 ºC for emulsions stabilized by carboxyl methyl polysaccharide and CMC at pH 4, 7, and 9, respectively. Most of the emulsions showed good stability with no significant changes in the droplets size over 24 hours of storage. This result demonstrates that, in terms of changes in droplet size, the stability of emulsions formed with carboxyl methyl polysaccharide was comparable to that of CMC. Emulsions stabilized carboxyl methyl polysaccharide at pH 9 decreased in droplet size during storage (Figure 3 (C), probably as a result from disruption and viscosity modification of emulsion droplets. Viscosity increase the viscosity of the medium, which helps create and maintain the suspension of globules of dispersed phase.

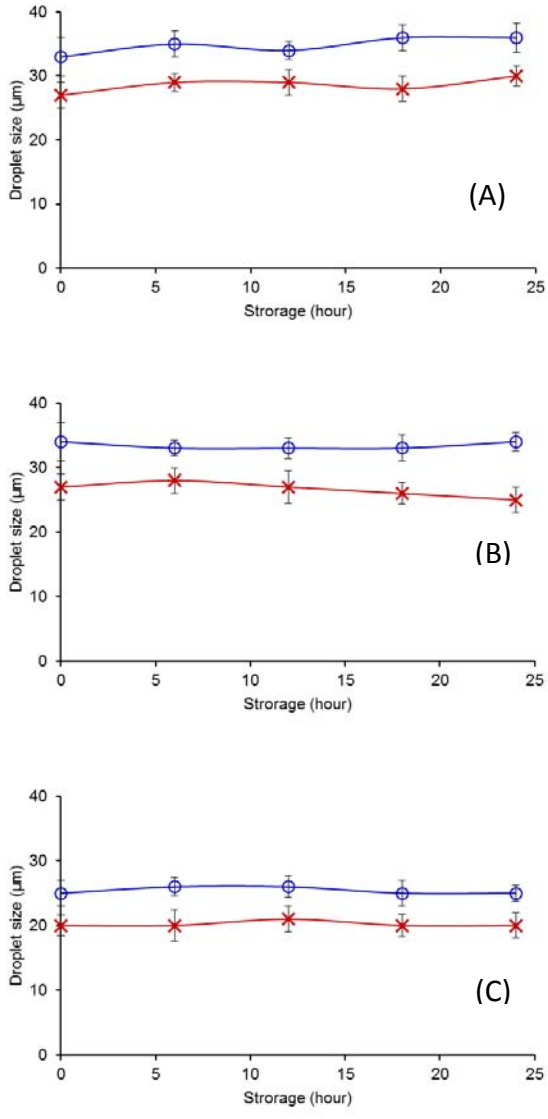

Figure 3 Droplet size of emulsions stabilised by CMC (○) and carboxyl methyl polysaccharide (×) at pH 4 (A), pH 7 (B), and pH 9 (C) over a storage period of 24 hours at 25ºC. Data are means of triplicate ± standard deviation.

Disaggregation is also possible especially if the carboxyl methyl polysaccharide unfold significantly and inadequately cover the oil surfaces. Furthermore, according to Stoke's law, large droplets become to larger size droplets faster than that of small droplets.[15] The significant decrease in droplet size of carboxyl methyl polysaccharide stabilized emulsion at pH 9 during

storage thus indicates its significantly more stable in comparison to emulsions stabilized by CMC.

As shown in Figure 3, the average particle size measurement of the emulsions shows that after 6 hour of storage formed smaller emulsion droplets than the control (CMC). While there was a clear difference between the particle size of the emulsions formed using the CMC and carboxyl methyl polysaccharide, there was significant difference in particle size between the CMC emulsions and carboxyl methyl polysaccharide

Carboxyl methyl polysaccharide stabilized emulsions even when the carboxyl methyl polysaccharide had largely different EAI at different pH values indicating the high tolerance of carboxyl methyl polysaccharide to pH and thus wider application. These results demonstrate that emulsions are complex systems, the changes in EAI of emulsifier as the environmental pH changes may (positively or negatively) or may not have a direct effect to the average droplet size. EAI-average droplet size relationship of a emulsifier is very much emulsifier dependent.

O/w emulsions are widely used in the food industry and are produced by homogenization of oil and aqueous phases together. In order to stabilize emulsions emulsifiers are generally required. The presence of polysaccharide at the interface resulted in an increased stability of the emulsions subjected to environmental stresses. An effective emulsifier should adsorb at the interface of the oil droplets, it should increase the surface pressure and should protect the droplets towards coalescence when the emulsion is exposed to environmental stresses.[16]

Carboxyl methyl polysaccharide is a anionic polysaccharide whose charge characteristics are determined by its carboxyl groups. At relative low pH, the carboxyl methyl polysaccharides carry low charges and the macromolecules relatively less soluble. As the pH of the solution increases, the carboxyl methyl polysaccharides have higher negative charges and higher solubility. They can create thicker interfaces by using complexes of surface active components of carboxyl methyl polysaccharide with non-surface active component. These complexes are usually created by electrostatic interactions. Therefore, at high pH, the emulsions provide the small droplets and more stable droplets compared with pH 7 and pH 4.

A large number of studies have been investigated on the use of charged biopolymers to form the second and higher level layers around lipid droplets by electrostatic deposition.[17] However, little is known about the preformed polysaccharide emulsifier complexes to stabilize o/w emulsions.[18,19] This study represented the bulk phase and the effect of pH on complex formation. Additionally, the ability of the complexes to stabilize o/w emulsions was shown the stability of the emulsions under different pH.

Conclusions

Carboxyl methyl polysaccharide had better emulsion forming ability (higher EAI and EC), and the average droplet size of emulsions it stabilized was consistently smaller irrespective of pH compared to CMC. In comparison to CMC and carboxyl methyl polysaccharide also had higher EC at all pH tested, higher EAI at acidic pH, and smaller or comparable average emulsion droplet size at both pH 4 and 7. Emulsions stabilized by carboxyl methyl polysaccharide showed good stability at all pH range and were comparable to CMC stabilized emulsions with no significant

changes in droplet size during storage for up to 24 hours at room temperature.

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