



Effect of crude sea grape hydrocolloids extract on the rice starch physicochemical and digestibility properties

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Abstract

The combination of starch and hydrocolloids is widely used in food manufacturing as a thickening agent for texture modification, but a comprehensive understanding of the influence of hydrocolloids on starch properties is lacking. Sea grape (*Caulerpa lentillifera*) is an underutilized edible green macroalga, which is mainly cultivated in closed systems in Thailand. Here, the effects of a crude aqueous extract of sea grape hydrocolloids on thermal properties, pasting properties, gel morphology, and starch digestibility of rice starch were compared with the addition of carrageenan and sodium alginate, which are the seaweed hydrocolloids widely used in the food industry. Results showed that the addition of crude sea grape hydrocolloid extract increased retrogradation enthalpy similar to carrageenan. The seaweed hydrocolloids also impacted peak viscosity and breakdown of pasting properties. Swollen granules identified in gel morphology studies suggested that the addition of seaweed hydrocolloids restricted starch granule disruption and altered starch digestibility by increasing the rapidly digested starch content. The addition of sea grape hydrocolloids to starch produced a similar outcome to carrageenan and sodium alginate as a possible future substitute.

Keywords: crude sea grape hydrocolloids extract; rice starch; physicochemical properties; starch digestibility.

Practical Application: A comprehensive understanding of the influence of seaweed hydrocolloids on starch properties remains unclear, especially for aspects of starch digestion. Results indicated that three different varieties of seaweed hydrocolloids had similar effects on rice starch properties, with retrogradation enhanced as retrogradation enthalpy increased. Peak viscosity and breakdown from pasting properties and gel morphology suggested that the hydrocolloids maintained a granular structure under heat and shear force, while hydrocolloid addition improved starch digestion.

1 INTRODUCTION

The combination of starch and hydrocolloids is widely used in food manufacturing to modify starch properties or create unique characteristics by improving texture and mouthfeel. Previous studies on the influence of hydrocolloids on starch properties mostly investigated gelatinization properties, especially pasting properties and starch swelling but rarely on starch retrogradation and digestibility.

Zhang et al. (2018) reported that xanthan gum reduced starch granule swelling, resulting in thicker slurry due to phase separation and intermolecular interaction between leached starch components and gum (Varela et al., 2016). Intermolecular interaction caused either weaker or stronger gel strength depending on the type of hydrocolloids, while Liu et al. (2021) reported that carboxymethylcellulose (CMC) addition yielded weaker chestnut starch gel by obstructing network ability, but a harder gel was achieved with sodium alginate addition. Adding highly branch hydrocolloids, such as gum Arabic, retarded the amylose junction zone and reduced the strength of corn starch gel (He et al., 2015). However, the influence of

hydrocolloid addition on thermal properties remains unclear, hydrocolloid addition may change the temperature profile or the enthalpy or no effect on the thermal properties (Leite et al., 2012; Zhang et al., 2018). Bárcenas et al. (2009) commented that different hydrocolloids showed diverse interactions with other components in the system.

Seaweed hydrocolloids are widely used in the food industry as a thickening agent, gelling agent, and stabilizer. The sulfate subunit is a unique characteristic of seaweed hydrocolloids that have biological activities such as antimicrobial and immunostimulating activities (Carina et al., 2021; Pereira et al., 2013). Sea grape (*Caulerpa lentillifera*) is an edible green macroalga commonly found in the Gulf of Thailand and along the Andaman Coast (Sompong et al., 2020). It is used for water treatment in shrimp farms and has been cultivated in closed culture systems since 1993 (Osotprasit et al., 2021), but over 60% of the production is underutilized (Honwichit et al., 2022). The bioactivity of the aqueous extract of *Caulerpa* hydrocolloids has been identified as sulfated xylogalactomanan with a molecular weight of more than 3,000 kDa (Honwichit et al., 2022; Maeda et al., 2012; Sun et al., 2018) with potential as an antimicrobial

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prebiotic, but limited studies have investigated the interaction of this hydrocolloids with other food components.

Gluten-free food products are now attracting high market demand with gluten-free materials such as rice flour and rice starch used instead of wheat flour. As rice is the main crop of Thailand, the gluten-free market draws a lot of attraction to food manufacturers. Unfortunately, the rapid staling or retrogradation of gluten-free material is an unwanted characteristic, and blending with the retrogradation delay agent is one of the methods used to solve this problem. In addition, hydrocolloids are used as food texture-modifying agents, and seaweed hydrocolloids that do not cause food allergies can also serve the vegan market. Alginate and carrageenan are commonly used seaweed hydrocolloids in food industries. This study investigated how an aqueous extract of sea grape hydrocolloids impacted the gelatinization and retrogradation properties of rice starch including thermal properties, pasting properties, gel morphology, and also starch digestibility compared with alginate and carrageenan. Findings will improve current knowledge on the use of sea grape hydrocolloids in the food industry and add value to sea grape culture.

2 MATERIALS AND METHODS

2.1 Materials

Cultivated sea grape (*C. lentillifera*) was purchased from a local farm in coastal Phetchaburi, Thailand. Non-waxy rice starch was obtained from Cho Heng Rice Vermicelli Factory Co., Ltd., Thailand, and food-grade carrageenan and sodium alginate were bought from a local food ingredient supplier.

2.2 Sea grape hydrocolloid extraction and starch-hydrocolloid mixture

The sea grape was dried at 60°C for 12 h before hydrocolloid extraction, following the method of Chaiklahan et al. (2020). Briefly, sea grape powder was soaked in 30-fold water for 30 min before blending using an electronic blender and boiling for 3 h. Hydrocolloids were precipitated with 95% ethanol, dried at 45°C, and ground to a powder. The crude sea grape hydrocolloid powder was kept in a zip lock bag until use.

Rice starch was mixed with crude sea grape hydrocolloids using a food processor to obtain 0.5 and 1% hydrocolloid addition. The starch mixture was kept in a zip lock bag until further analysis. Starch properties were altered by sea grape hydrocolloid addition, and the results were compared with carrageenan and alginate commercial algal hydrocolloids at the same concentration.

2.3 Thermal properties

The thermal properties of rice starch and starch mixtures were studied by differential scanning calorimetry (DSC) (Diamond, Perkin-Elmer). Investigated temperatures ranged from 25 to 180°C at a heating rate of 10°C/min. The pan was then cooled to 25°C at 10°C/min. The samples were kept for 7 days at 5–7°C in a refrigerator before reanalyzing for retrogradation

under the same condition. An empty stainless steel pan was used as the reference.

2.4 Pasting properties

The pasting properties of rice starch and the starch-hydrocolloid mixtures were determined by a rapid visco analyzer (RVA) (Newport Scientific, Australia) using rice profiles. A 3.0 g sample (12% moisture basis) was mixed with 25 mL of distilled water. The mixture was held at 50°C for 1 min, then heated to 95°C within 3.45 min, and held there for 2.30 min. It was then cooled to 50°C over 4.51 min and held there until the end of the test. The total profile time was 12.30 min.

2.5 Gel morphology

The gel from RVA analysis was diced into small pieces and dehydrated in 50, 70, and 90% and absolute ethanol to prevent the structure from collapsing before freeze drying. Gel morphology was studied by a scanning electron microscope (SEM) (SU8020, Hitachi, Japan). The dried samples were attached to a stub by carbon tape and platinum-coated using a sputter coater (Q150R ES, Quorum, UK) before examination at 5,000× magnification.

2.6 In vitro digestibility

The samples were treated with mixed porcine amylase, invertase, and glucoamylase as described by Englyst et al. (1992) for starch digestibility. Rapidly digestible starch (RDS) and slowly digestible starch (SDS) were assayed at 20 and 120 min after adding the enzyme, respectively. Resistant starch (RS) content was calculated after total starch was analyzed.

2.7 Statistical analysis

All experiments were carried out in duplicate, except morphology analysis. Mean values and standard deviations were reported. Analysis of variance (ANOVA) was performed with mean separations conducted by Duncan's test ($p \leq 0.05$) using the SPSS software (SPSS Inc., Chicago, USA).

3 RESULTS

3.1 Thermal properties

The gelatinization onset temperature of rice starch increased, while the conclusion temperature was slightly altered by hydrocolloid addition, as shown in Table 1. The gelatinization temperature range of hydrocolloid-added rice starch narrowed, except for alginate addition at 1.0%. Moreover, the gelatinization enthalpy of crude sea grape hydrocolloid added rice starch was notably lower than rice starch and other seaweed hydrocolloid addition.

For retrogradation properties, the onset temperature of seaweed hydrocolloids decreased while the conclusion temperature significantly increased. This resulted in a broadened retrogradation peak due to a wider temperature range, as shown in Table 2. The retrogradation enthalpy of rice starch was remarkably lower than seaweed hydrocolloid-added rice starch. The amount of

hydrocolloid addition also had pronounced effects on the decreasing of the retrogradation enthalpy. The addition of 0.5% hydrocolloid gave higher enthalpy than the 1% addition. Furthermore, alginate seems to have less effect on the starch retrogradation enhancement due to the lower enthalpy compared with the other seaweed hydrocolloids. Hydrocolloid sources also had an impact on enthalpy alteration. Crude sea grape hydrocolloid and carrageenan had higher enthalpy values than alginate.

3.2 Pasting properties

Peak and final viscosities of crude sea grape hydrocolloid- and carrageenan-added samples were increased from rice starch. The increases in peak and final viscosities were more noticeable with the lower amount of addition. On the contrary, alginate had an adverse effect on the peak and final viscosities. Breakdown viscosity shared the same trend as peak and final viscosities, while setback viscosity had the opposite

result. The amount of hydrocolloid addition had an effect on the breakdown and setback viscosity alteration, but the amount of carrageenan addition had less effect on the setback viscosity. Among seaweed hydrocolloids, alginate addition had the lowest breakdown viscosity (Table 3).

3.3 Gel morphology

The morphology of rice starch gel prepared by RVA is shown in Figure 1. At 5,000× magnification, swollen starch granules appeared embedded in the flossy matrix of leached components in all gel samples (marked in a circle), while a sheet-like structure (marked in a square box) appeared in all samples except for 1% carrageenan addition (Figures 1D and 1E). Among the seaweed hydrocolloids, crude sea grape hydrocolloid addition made them the most similar to rice starch gel morphology (Figures 1B and 1C), while alginate addition limited flossy structure formation (Figures 1F and 1G).

Table 1. Gelatinization of rice starch and seaweed hydrocolloid-added rice starch.

Sample		Enthalpy, (DH, j/g db)	Temperature (°C)		
			Onset	Conclusion	Temperature range
Rice starch		9.35 ± 1.87a	60.24 ± 1.38a	79.54 ± 0.20ab	19.31 ± 1.58a
Crude sea grape hydrocolloids	0.5%	8.64 ± 0.26a	61.59 ± 0.18b	79.57 ± 0.13ab	17.98 ± 0.05a
	1.0%	8.45 ± 0.28a	61.89 ± 0.13b	79.52 ± 0.30ab	17.63 ± 0.44a
Carrageenan	0.5%	9.57 ± 0.04a	61.69 ± 0.04b	79.55 ± 0.03ab	17.86 ± 0.27a
	1.0%	9.34 ± 0.34a	62.16 ± 0.02b	79.98 ± 0.03ab	17.83 ± 0.05a
Alginate	0.5%	9.10 ± 0.29a	61.51 ± 0.08ab	79.29 ± 0.10a	17.78 ± 0.18a
	1.0%	9.21 ± 0.56a	61.55 ± 0.23ab	80.77 ± 1.36b	19.23 ± 1.59a

Different letters in the same column indicate statistical differences ($p \leq 0.05$).

Table 2. Retrogradation of rice starch and seaweed hydrocolloid-added rice starch.

Sample		Enthalpy (DH, j/g db)	Temperature (°C)		
			Onset	Conclusion	Temperature range
Rice starch		2.09 ± 0.15a	45.13 ± 1.12ab	57.88 ± 1.05a	12.75 ± 2.17a
Crude sea grape hydrocolloids	0.5%	4.27 ± 0.12d	43.98 ± 1.12a	60.36 ± 0.22bc	16.38 ± 1.34c
	1.0%	3.51 ± 0.12c	44.31 ± 0.05ab	59.01 ± 0.13ab	14.70 ± 0.08abc
Carrageenan	0.5%	4.45 ± 0.27d	44.91 ± 0.11 ab	60.19 ± 0.40bc	15.28 ± 0.30abc
	1.0%	3.50 ± 0.13c	44.95 ± 0.52 ab	60.46 ± 0.06c	15.51 ± 0.58bc
Alginate	0.5%	3.09 ± 0.01b	44.60 ± 0.09ab	59.37 ± 0.57bc	14.78 ± 0.47abc
	1.0%	2.86 ± 0.19b	45.74 ± 0.30b	59.14 ± 0.58abc	13.40 ± 0.88ab

Different letters in the same column indicate statistical differences ($p \leq 0.05$).

Table 3. Pasting properties of rice starch and seaweed hydrocolloid-added rice starch.

Sample		Viscosity (RVU)			
		Peak	Breakdown	Final	Setback
Rice starch		224.54 ± 1.36b	42.92 ± 0.94e	285.88 ± 0.06ab	61.34 ± 1.29b
Crude sea grape hydrocolloids	0.5%	253.17 ± 1.06d	48.42 ± 1.89f	298.38 ± 7.01b	45.21 ± 8.08a
	1.0%	234.67 ± 2.12c	39.13 ± 1.12d	284.67 ± 0.00ab	50.00 ± 2.12ab
Carrageenan	0.5%	232.92 ± 2.12c	34.63 ± 0.77c	292.00 ± 4.60b	59.08 ± 2.47b
	1.0%	227.79 ± 0.76b	22.25 ± 0.82ab	287.21 ± 3.95ab	59.42 ± 0.14b
Alginate	0.5%	215.54 ± 2.53a	25.46 ± 0.41b	273.50 ± 2.72a	57.96 ± 0.18b
	1.0%	232.75 ± 2.94c	20.17 ± 2.95a	294.71 ± 12.08b	61.96 ± 9.14b

Different letters in the same column indicate statistical differences ($p \leq 0.05$).

3.4 *In vitro* digestibility

Seaweed hydrocolloids altered starch digestibility differently (Table 4). RDS was digested within 20 min after the enzyme was added, while SDS was digested within 120 min after the enzyme was added. Starch not digested within 120 min is called RS (Englyst et al., 1992). All samples with added hydrocolloids

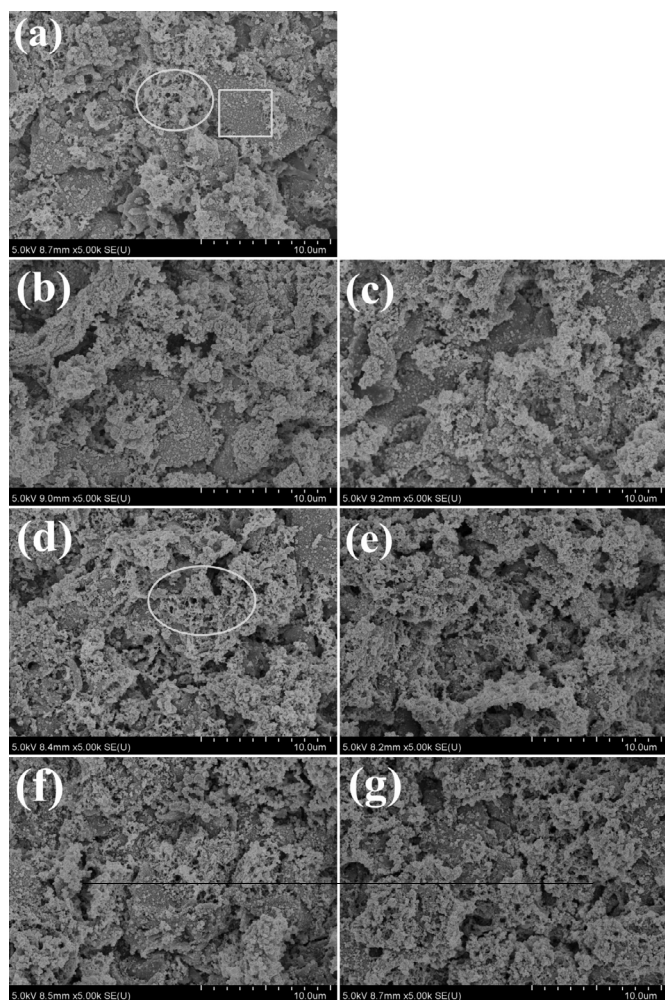


Figure 1. Morphology of rice starch and seaweed hydrocolloid-added rice starch gel, while (A) represents rice starch gel, (B) and (C) represent crude sea grape added at 0.5 and 1%, respectively, (D) and (E) represent carrageenan added at 0.5% and 1%, respectively, and (F) and (G) represent alginate added at 0.5 and 1%, respectively.

Table 4. *In vitro* digestibility of rice starch and seaweed hydrocolloid-added rice starch.

Sample		RDS (%)	SDS (%)	RS (%)
Rice Starch		74.99 ± 0.45a	3.27 ± 0.46ab	21.74 ± 0.01d
Crude sea grape hydrocolloids	0.5%	93.67 ± 1.30d	3.22 ± 0.79ab	3.11 ± 0.50a
	1.0%	91.37 ± 0.43c	3.65 ± 0.41ab	4.98 ± 0.02b
Carrageenan	0.5%	93.98 ± 0.11d	3.04 ± 0.06a	2.97 ± 0.05a
	1.0%	93.49 ± 0.30d	3.49 ± 0.14ab	3.02 ± 0.44a
Alginate	0.5%	84.68 ± 0.49b	3.99 ± 0.31ab	11.33 ± 0.18c
	1.0%	84.51 ± 0.25b	4.09 ± 0.14b	11.41 ± 0.11c

Different letters in the same column indicate statistical differences ($p \leq 0.05$).

had higher RDS contents than rice starch, while RS contents decreased. Among seaweed hydrocolloids, carrageenan-added samples had the highest RDS content and lowest RS content. Crude sea grape hydrocolloid and alginate addition resulted in decreased RS content depending on the amount of hydrocolloids added. The effect of adding hydrocolloids on SDS content was inconclusive, with different hydrocolloids giving diverse results. Carrageenan addition increased SDS content, while alginate addition had the reverse effect. This effect was more pronounced when adding increasing amounts of hydrocolloids, while for crude sea grape hydrocolloids, the effect was inclusive.

4 DISCUSSION

Thermal properties studied by DSC indicated that seaweed hydrocolloids were located in the continuous phase surrounding the starch granules (Zhang et al., 2018). The decrease in gelatinization enthalpy resulted from incomplete melting of the crystalline structure of starch granules because the water molecules were immobilized by the hydrocolloids (Varela et al., 2016) or the hydrocolloids were preventing the water penetration into the starch granules (Zhang et al., 2018). An increase in gelatinization onset temperature was also induced by restricted water adsorption to the amorphous region in the starch granules (Zhang et al., 2018). In contrast, starch retrogradation was promoted by seaweed hydrocolloid addition, with increased retrogradation enthalpy. This result concurred with He et al. (2015) who reported that adding xanthan, guar, and gum Arabic induced short-term retrogradation, while Zou et al. (2023) reported that adding Konjac glucomannan reduced retrogradation enthalpy via the water-holding capacity of polysaccharide. The increase in short-term retrogradation was caused by the aggregated acceleration of the leached starch component (Liu et al., 2021).

The addition of hydrocolloids impacted pasting viscosity, as previously reported. Cengiz and Dogan (2021) mentioned that hydrocolloids induced higher viscosity of the coating batter and the impact was more pronounced when more hydrocolloids were added due to lower water diffusivities (Qazi et al., 2011) as hydrocolloids have high water-holding capacity (Saleh et al., 2016). Viscosity increase during heating was induced by the interaction between hydrocolloids and the starch component and also the phase separation (Funami et al., 2005; Liu et al., 2021; Zhang et al., 2018). The influence of hydrocolloids on the pasting properties differed depending on each parameter. Peak viscosity refers to the water-holding capacity (Balet et al., 2019), and the increase in peak viscosity after adding hydrocolloids demonstrated that starch granule swelling was impacted by hydrocolloids, apart from the viscosity of the hydrocolloids itself (Leite et al., 2012). After passing the viscous peak, the viscosity of the slurry dropped due to the melting of the crystalline region, thereby allowing water molecule movement (Balet et al., 2019). The difference between the peak and trough is the breakdown, representing swollen granule disruption, which relates to starch granule stability to heat and shear force (Zou et al., 2023). Lower breakdown for seaweed hydrocolloid addition indicated that hydrocolloids acted as a barrier cover for the granule surface, which lowered the shear force effect and inhibited the leaching of starch components (Varela et al., 2016). The final viscosity

is the viscosity at the end of the pasting analysis and the difference between the final and trough is the setback viscosity (Balet et al., 2019). The final and setback viscosities represent the aggregation of leached amylose during the cooling period on the RVA profile (Varela et al., 2016). The aggregation of leached amylose commonly reflects retrogradation (Sun et al., 2017), which is not always reflected by the setback value (Zou et al., 2023). The addition of seaweed hydrocolloids decreased setback viscosity, especially for crude sea grape hydrocolloids, indicating that the hydrocolloids interfered with the starch gelling system but did not delay retrogradation as the thermal properties indicated that retrogradation was accelerated.

The morphology showed a flossy structure representing the leached starch components, while a sheet-like structure appeared in all samples, except for a 1% carrageenan addition. Swollen granules were also noticed in all the samples but were more visible when hydrocolloids were added. Results suggested that hydrocolloids hampered the swelling and disruption of starch granules, with more noticeable swollen granules. Starch digestion was enhanced by hydrocolloid addition as high RDS content with very low RS content. Gularte and Rosell (2011) reported that hydrocolloids favor starch digestion by increasing the RDS content. The starch digestible increment varies due to the type of starch and hydrocolloids in the system. However, information about the impact of hydrocolloids on starch digestibility remains inadequate.

Crude sea grape hydrocolloids had a similar effect on rice starch properties compared with the commercial seaweed hydrocolloids carrageenan and sodium alginate and showed potential for use in the food industry. Increased utilization of undergrade sea grape from farms can add value to this product.

5 CONCLUSION

Pasting properties, thermal properties, gel morphology, and starch digestibility of rice starch and rice starch mixed with seaweed hydrocolloids were studied. Each seaweed hydrocolloid altered the starch properties differently due to their disparate structures. Hydrocolloid addition impacted starch gelatinization and retrogradation by coating the starch granules and limiting swelling and disruption, resulting in high peak viscosity and low breakdown. Retrogradation was enhanced, while starch digestibility increased with hydrocolloid addition.

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