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RESEARCH

Effect of Sucrose on Some Physical Properties of Different Philippine Agars

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The effect of sucrose on some gelling properties of agar extracts from six Philippine agarophytes: *Gracilaria eucheumoides*, *G. firma*, *G. salicornia*, *Gelidiella acerosa*, *Gracilariopsis heteroclada* and *Laurencia flexilis* were investigated with Bacto-agar, (Difco) as reference. Sucrose-agar gels consisted of 50% sucrose in 1.5% (w/w) agar solution. Control gels contained 1.5% aqueous agar solution. Addition of sucrose resulted in significant increase ($p<0.05$) in the gel strength and the gelling and melting temperatures of gels prepared from *G. eucheumoides*, *G. firma*, *G. salicornia*, *L. flexilis*, *G. heteroclada* and Bacto-agar. On the other hand, syneresis index decreased. Similar effects were observed with *Gelidiella acerosa* agar except for its gel strength which decreased in the presence of sucrose. Chemical analysis indicated high 3,6-anhydrogalactose and low sulfate contents of agar samples. The FT-IR spectra indicated sulfation at C-4 on the galactose residues of *G. eucheumoides*, *G. firma*, *G. salicornia* and *L. flexilis* agars. These agars were classified "sucrose-reactive" based on their gel strengths which increased more than twice that of the control upon sucrose addition.

Key words: agarophytes, gel strength, syneresis index, sucrose-agar gel, sucrose-reactive agar, FT-IR spectroscopy

Agar has a wide variety of uses as human and animal foods in addition to having numerous industrial applications. The multitude of uses from this polysaccharide is based on its behaviour in aqueous solution (Armisén 1991).

In the food industry, agar is employed predominantly for its stabilizing and gelling characteristics. The thickening effect of agar when dispersed in water medium is the basis for its use as bulking, stabilizing and emulsifying agent in foods. Its gels are used as texture modifier. Furthermore, it has the unique ability of holding large amounts of moisture (Meer 1980) whereby preventing quick dehydration of confectionery products (Armisén & Galatas 1987).

Soft and elastic gels used in the food industry are obtained from *Gracilaria* species (Yaphe & Duckworth 1972). Armisén & Galatas (1987) indicated that addition of high sugar concentration (above 50%) to *Gracilaria* agar increases its gel strength much more than *Gelidium* agar does. Murano (1995) called this type of agar "sugar-reactive" which is considered the most expensive phycocolloid today (Abbot 1996).

Few studies have been done on the effect of sucrose addition on the gelling properties of phycocolloids. Addition of increasing amounts of sucrose (up to at least 60%) increased melting temperature (Nishinari *et al.* 1990) and improved resistance to rupture and firmness (Fiszman & Durán

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1992) of k-carrageenan and alginate gels. Gel strengths of agar solutions increased upon addition of sucrose (Que et al.1995) while Matsuhasi (1990) observed doubling of the gel strength when 50% sucrose was added.

The objective of the study was to assess the effect of sucrose addition on the gel strength, syneresis index and other properties of agar from different agarophytes. Chemical and spectral analyses of the samples were done to support some observations. Moreover, the "sucrose-reactivity" potential of agar from different *Gracilaria* species and other agarophytes were also evaluated. The results obtained in this study may be used as a basis for the optimum utilization of agar in the food industry especially in bakery and confectionery.

Materials and Methods

Agarophytes

Agarophytes were collected from different places in the Philippines. *Gracilaria eucheumoides* Harvey and *Gelidiella acerosa* (Forsskål) Feldmann *et* Hamel were collected from Bolinao, Pangasinan; *Gracilaria salicornia* (C. Agardh) Dawson from Tawi-Tawi; *Gracilaria firma* Zhang *et* Xia and *Laurencia flexilis* (Setchell) from Currimao, Ilocos Norte and *Gracilariopsis heteroclada* (Zhang *et* Xia) Zhang *et* Xia from Maimbong, Sulu. Powdered Bacto-agar, obtained from Difco Laboratories, USA, was used as reference material for all determinations and analyses.

Agar Extraction

Algae were thoroughly cleaned of epiphytes and washed with running water to remove excess salts then oven-dried at 60°C. Alkali modification was done prior to extraction. *Gracilaria eucheumoides* was pretreated with 10% NaOH using the optimized method of Villanueva et al.(1997). *Gracilaria firma*, *G. salicornia*, *Gracilariopsis heteroclada* and *L. flexilis* were pretreated with 5% NaOH at 90°C for 1 h. After pretreatment, samples were washed with water and soaked with 0.5% acetic acid solution for 1 h to further neutralize whatever alkali was left. Extraction of agar from seaweed samples was done by boiling or by autoclaving (44 kg cm^{-2}, 121°C for *Gelidiella acerosa*) the thalli with water for 1 h. Algal mixtures were finally blended and pressure-filtered with the aid of diatomaceous earth. The agar extracts were frozen, thawed, dehydrated with 2-propanol and oven-dried at 60°C. Extraction was done in three replicates.

Gel Preparation

Two types of gel were prepared. The control gel was made of 1.5% aqueous agar solution. The sucrose-agar gel, on the other hand, was prepared by incorporating sucrose to constitute 50% sucrose in 1.5% agar solution.

Gel strength

Gel strengths of the control and the sucrose-agar solutions were measured using a Marine Colloids Gel Tester (Model GT-1). The plunger had a cross-head area of 1 cm^2 and a descent rate of 2.5 mm/s.

Gelling and Melting Temperatures

Dynamic gelling temperatures of the control and sucrose-agar solutions were determined. Each solution was poured into a test tube fitted with a thermometer with its bulb situated just below the surface of the solution. The solution was allowed to cool and glass beads (2.85 mm dia., wt.: 30 mg) were dropped at intervals of 0.5°C. The temperature at which a bead failed to sink was taken as the dynamic gelling temperature. The gel was allowed to stay overnight at room temperature after which a lead shot (4.30 dia mm., wt.: 500 mg) was placed on the surface. The gel was placed in a water bath and gradually heated. The temperature at which the lead shot dropped to the bottom was recorded as the melting temperature.

Syneresis Index

The amount of water exuded from the gel samples after standing for a certain period of time was determined and quantified using a modified method of Fiszman and Durán (1992).

Approximately 10 grams of hot 1.5 % (w/w) agar extracts were poured into test tubes (21mm dia) and allowed to gel at room temperature (28-31°C) for 24 h. The initial weights of these gels were measured before placing them on dry Whatman (No. 1) filter papers. Loss of exudate from the gels was monitored by weighing the gels after 2 h. The sucrose-agar gels were treated similarly.

The syneresis index values of the gel samples were taken as the difference between the initial weight of the gel and its final weight after 2 h. This value indicates the water holding capacity of the gel.

Chemical Analysis

The amount of 3,6-anhydrogalactose (3,6-AG) present in agar extracts of the different agarophytes were determined by the resorcinol-acetal method of Yaphe and Arsenault (1965) while the method of Jackson and McCandless (1978) was adapted in the

determination of the sulfate content after hydrolysis with 1N HCl at 110°C for 4 h.

Spectral Analysis

Fourier-Transform Infrared (FT-IR) spectra of agar samples were recorded on films using Shimadzu 8201 PC FT-IR spectrometer. Films were prepared by drying 5 mL of 0.5% agar solutions in a teflon-coated pan at 60°C. Relative amounts of 3,6-AG to sulfate contents of the agar samples were determined by taking the ratio of the absorbances of bands at 930 cm^{-1} (3,6-AG) and 1250 cm^{-1} (total sulfate ester).

Statistical Analysis

Analysis of Variance (ANOVA, p=0.05) using a Statistical Analysis Software v. 6. 10 (SAS Institute Inc., NC, USA) program was used to analyze results while a Duncan's Multiple Range Test (DMRT, p=0.05) was used to compare treatment means.

Results

The gel strengths of agar extracts from different agarophytes in the presence and absence of sucrose are presented in Fig 1. Agar solutions (1.5% w/w) prepared from *Gracilariopsis heteroclada* and *Gelidiella acerosa* exhibited the highest gel strength followed by *Laurencia flexilis and Gracilaria firma* then *Gracilaria salicornia. Gracilaria eucheumoides* agar possessed the softest gel among the samples studied. The gel strength of Bacto-agar, the reference material, was lower than that of *Gracilariopsis heteroclada and*

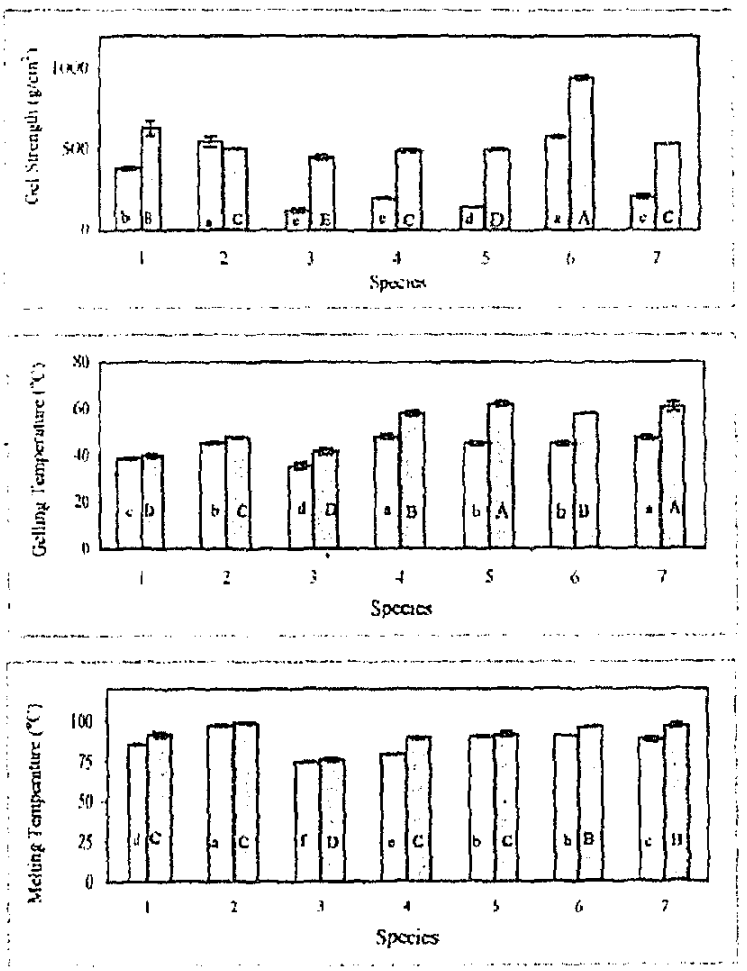

Figure 1. Gel strength, gelling temperature and melting temperature of agar gels from different agarophytes with (shaded) and without (unshaded) sucrose. Means with similar letter do not differ significantly (small letters for gels without sucrose; capital letters with sucrose. ($p<0.05$; $n=3 \pm SE$). 1. Bacto-agar; 2. *Gelidiella acerosa*; 3. *Gracilaria eucheumoides*; 4. *Gracilaria firma*; 5. *Gracilaria salicornia*; 6. *Gracilariopsis heteroclada*; 7. *Laurencia flexilis*

Gelidiella acerosa but higher than the other agar samples studied.

Sucrose-agar gels prepared from all agarophytes, except *Gelidiella acerosa*, exhibited significantly higher ($p<0.05$) gel strengths than the control. The gel strength of Bacto-agar also increased when sucrose was added but the increase was not as much as those of *Gracilaria eucheumoides*, *G. firma*, *G. salicornia* and *Laurencia flexilis*. Although agar extracts of *Gracilaria eucheumoides*, *G. firma*, *G. salicornia* and *Laurencia flexilis* were softer than the other agars studied, they possessed gel strengths which increased more than twice that of the control when sucrose was added. These results corroborate the observations made by Matsuhashi (1990).

The sucrose-agar solutions were prepared by adding 50% (w/w) sucrose in 1.5% (w/w) aqueous agar solution. Assuming that sucrose acted purely as co-solute, it would constitute half of the total weight of the solution thereby reducing the amount of solvent by half. On the other hand, the control gel was made of 1.5% (w/w) agar solution which was twice diluted than the sucrose-agar solution. Therefore, if the concentration of agar in the two solutions were compared, agar would be two times less concentrated in the control than in the sucrose-agar solution.

The strength of agar gels vary with concentration. In fact, agar gels can be formed from very dilute solutions containing a fraction of 1% agar (Glicksman 1983). Hence, the more concentrated the agar solution, the harder the gel becomes. It is therefore expected that the sucrose-agar gels would be at least two times stronger than the control.

Agars which exhibit gel strengths more than twice the control when 50% sucrose is added may then be classified as "sucrose-reactive". At this juncture, *Gracilaria eucheumoides*, *G. firma*, *G. salicornia* and *Laurencia flexilis* could be considered potential sources of "sucrose-reactive" agar.

The gelling and melting temperatures of the two gel preparations are shown in Fig. 1. The 1.5% agar solution prepared from *Gracilaria firma* and *Laurencia flexilis* had the highest gelling temperature, while that of *G. eucheumoides*, the lowest. On the other hand, *G. salicornia* agar solution exhibited the highest gelling temperature upon sucrose addition. Although the gelling temperature of *Gelidiella acerosa* agar increased upon addition of sucrose, the increase was too minimal. Similarly, Bacto-agar exhibited minimal increase in gelling temperature upon sucrose addition.

Generally, gels containing 50% sucrose exhibited significantly higher ($p<0.05$) gelling and melting temperatures than those without sucrose. Of the agars studied, *G. firma* and *L. flexilis* agars showed the highest gelation and melting temperatures. Bacto-agar also increased its gelling and melting temperatures upon addition of sucrose.

The extent of syneresis as measured by the syneresis index values of the two gel preparations from different agar extracts are shown in Table 1. The amount of water exuded from the control gels were significantly higher ($p<0.05$) than those of the sucrose-agar gels. Among the control gels, *Gelidiella acerosa* agar showed the highest syneresis index while those of *Gracilaria salicornia* and *Laurencia flexilis*, the lowest. The sucrose-agar gel of *Gelidiella acerosa* also had the most amount of synerized water while *Gracilaria firma* had the least.

The difference in the syneresis index values (DSI) of both the control and sucrose-agar gels (Table 1) were measured to determine the amount of water retained when sucrose was added to the gel. *Gracilaria eucheumoides* and *Gracilariopsis heteroclada* retained the most amount of water while *Gelidiella acerosa* the least. The seaweed source of Bacto-agar was unknown, however, it showed similarity with *Gracilaria firma* extract

Table 1. Syneresis index values of agar gels from different agarophytes with (B) and without (A) sucrose. Difco Bacto-Agar was used as reference. Means with similar letter do not differ significantly. ($n = 3 \pm SE$).

Source	A(g)	B(g)	ΔSI(g)
Bacto-Agar	$1.53^{b} \pm 0.05$	$0.66^{b} \pm 0.10$	0.87^{b}
Gelidiella acerosa	$2.06^{a} \pm 0.07$	$1.60^{a} \pm 0.25$	0.46^{e}
Gracilaria eucheumoides	$1.66^{b} \pm 0.17$	$0.61^{b} \pm 0.16$	0.95^{b}
G. firma	$1.38^{bc} \pm 0.20$	$0.49^{b} \pm 0.03$	0.89^{b}
G. salicornia	$1.21^{c} \pm 0.10$	$0.56^{b} \pm 0.03$	0.65^{c}
Gracilariopsis heteroclada	$1.55^{b} \pm 0.15$	$0.58^{b} \pm 0.09$	0.97^{a}
Laurencia flexilis	$1.22^{c} \pm 0.16$	$0.67^{b} \pm 0.20$	0.55^{d}

ΔSI is the difference between the amount of water exuded by the control and sucrose-agar gels which denotes the water holding capacity of sucrose-agar gels.

Table 2. Chemical composition of agar extracts from different agarophytes. Difco Bacto-Agar was used as reference. Means with similar letter do not differ significantly. ($n = 3 \pm SE$).

Source	% 3, 6-anhydrogalactose	% Sulfate
Bacto-Agar	$33.64^{cd} \pm 0.79$	$2.96^{cd} \pm 0.18$
Gelidiella acerosa	$34.83^{c} \pm 0.74$	$2.62^{e} \pm 0.37$
Gracilaria eucheumoides	$40.44^{b} \pm 1.90$	$3.63^{a} \pm 0.12$
G. firma	$28.30^{e} \pm 0.78$	$2.70^{de} \pm 0.10$
G. salicornia	$32.20^{d} \pm 0.95$	$3.09^{bc} \pm 0.01$
Gracilariopsis heteroclada	$42.59^{a} \pm 1.35$	$3.39^{ab} \pm 0.09$
Laurencia flexilis	$29.30^{e} \pm 0.63$	$2.22^{f} \pm 0.16$

ΔSI is the difference between the amount of water exuded by the control and sucrose-agar gels which denotes the water holding capacity of sucrose-agar gels. $\Delta SI = A - B$

in terms of its water holding capacity.

The 3,6-anhydrogalactose (3,6-AG) and sulfate contents of agar extracts and Bacto-agar are shown in Table 2. Chemical analysis indicated high 3,6-AG content ranging from 28.30 to 42.59%. *Gracilariopsis heteroclada* agar contained the highest 3,6-AG while *Gracilaria firma* the lowest. The sulfate content of the samples ranged from 2.22 to 3.63%, the highest of which was *Gracilaria eucheumoides* and *Laurencia flexilis* the least. The 3,6-AG and sulfate contents of Bacto-agar were comparable with those obtained for *Gracilaria salicornia*.

Results of the chemical analysis of agar extracts are supplemented by the FT-IR spectra shown in Figure 2. Prominent bands appear at 930 cm^{-1} (3,6-AG) and weak bands at 1250 cm^{-1} (total sulfate). All agar samples showed sulfation at *O*-4 on their galactose residues as revealed by bands at 845 cm^{-1} (Armisen & Galatas 1987, Caceres *et al.* 1997). The sulfate contents of these agars obtained through chemical analysis may be attributed to these alkali-stable sulfate group.

Alkali treatment efficiently eliminated sulfation at *O*-6 of the 4-linked galactose residues of agar. This was evident from the spectra of alkali-treated extracts of *G. eucheumoides*, *G. firma*, *G. salicornia*, *Gracilariopsis heteroclada* and *L. flexilis* since no band was observed at 820 cm^{-1} (assigned to equatorial hemi-ester sulfate at *O*-6 of the galactose residues). Bacto-agar preparation was also free of this sulfation. On the contrary, FT-IR spectra of *Gelidiella acerosa* extract revealed another band at 820 cm^{-1} which was expected since the seaweed was not pretreated prior to extraction. The amount of sulfate present in *G. acerosa* could still be reduced through alkali treatment.

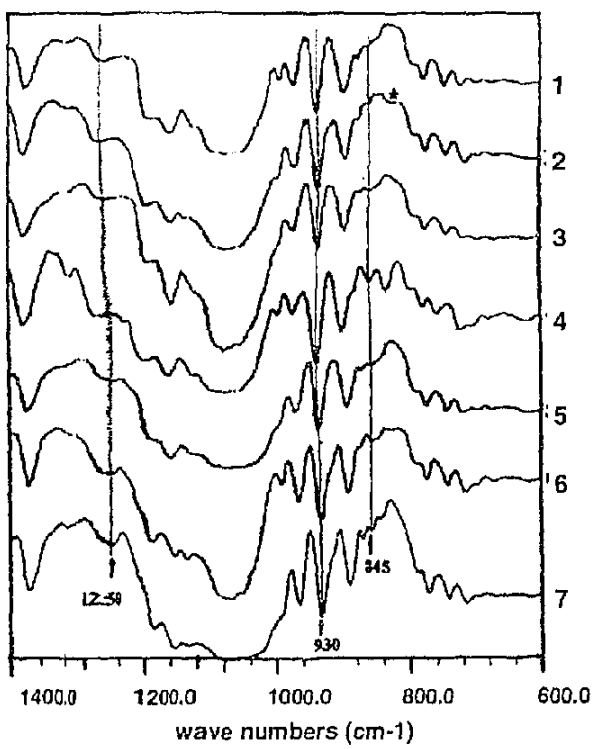

Figure 2. FT-IR spectra of agar extracted from different agarophytes. 1.Bacto-Agar; 2. *Gelidiella acerosa*; 3. *Gracilaria eucheumoides*; 4. *Gracilaria firma*; 5. *Gracilaria salicornia*; 6. *Gracilariopsis heteroclada*; 7. *Laurencia flexilis*.
* 820 cm^{-1} (peak assigned to equatorial hemi-ester sulfate at *O*-6 of the galactose residues)

Discussion

The results corroborate previous observations. (Glicksman 1983, Nishinari *et al. 1990,* Fiszman & Durán 1992, Que et al. 1995) that sucrose markedly increased the gel strength, gelling and melting temperature of agar solutions.

The differences in the gelation temperatures of the different agar gels are attributed to the variation in their individual methoxy contents (Guiseley 1970) although the methyl contents of agar samples used in this experiment were not determined. However, agar gels added with sucrose showed an increase in the gelling temperature. Similarly, the melting temperature of these gels increased. This may be due to the interaction of sucrose with the agar polymer.

Gel formation involves association of chain segments resulting in a three-dimensional framework that contains solvent in the interstices. The associated regions are known as junction zones, and may be formed from two or more chains. It is interesting to note that polymer chains usually form interconnected network that gives rise to characteristic texture and properties, in the interstices of which are molecules of solvent and other species (Rees 1966). Sucrose which is present in the interstices reinforces the strength of the junction zone as noted by Nishinari *et al.* (1990).

It was also observed that agar gels undergo syneresis in the absence or presence of sucrose. However, syneresis was reduced in the presence of sucrose. Syneresis indicates gel network stability (Fiszman and Durán 1992). It is a phenomenon by which water is spontaneously released with the contraction of the gel matrix that may occur upon standing. The process is spontaneous and constitutes a shift to a more stable state (Rees 1966) hence, the agar framework is said to continually break and re-form. This is due to the rotational and restricted translational motion by the polymer segments thereby breaking the junction zones. In the presence of sucrose, breaking of the junction zones is speculated to be minimized thereby creating a more stable agar network.

Hemi-ester sulfates were present in the agar samples studied as revealed by the FT-IR spectra. Sulfate contents of agar extracts were relatively low compared to the 3,6-AG (Table 2) indicating that it occurs occasionally along the agar backbone. It is speculated that sucrose may align itself with the sulfate groups minimizing repulsion while at the same time interacting with the sulfate as well as the agar backbone through hydrogen bonding. It may be the manner by which the framework is stabilized. This is possible since sulfate groups may be situated far from each other offering the right fit for sucrose.

Sucrose becoming part of the gel network is likewise suspected to trap water (Fiszman & Duran 1990) thereby reducing syneresis. This has been observed in the agar samples studied. Although the liberation of water from the gels was minimized with the presence of sucrose, the degree by which syneresis occurs vary. Among the agars studied, *Gracilariopsis heteroclada* and *G. eucheumoides* agar retained the most amount of water after standing for 2 h.

Gelidiella acerosa agar, on the other hand, retained the least amount of water. It should be noted that the agar was not treated prior to extraction and the amount of sulfate indicated in Table 2 could be partly due to alkali-labile sulfate on O-6 of the 4-linked L-galactose residues. Sulfation at C-6 of the galactose moities creates kinks which hinders gelation (Moirano 1977, Rees 1969). Furthermore, these sulfate groups may push agar polymers apart creating distances between them which may not offer the right fit for sucrose. It is suspected that sucrose is found around the kinks creating instability hence lowering its gel strength.

The results of the study indicate that sucrose improves some of the physical properties of agar which are quite useful in the food industry. *Gracilaria eucheumoides, G. firma, G. salicornia* and *Laurencia flexilis* are potential sources of "sucrose-reactive" agars based on their gel strengths which can increase more than two-fold upon sucrose addition. However, other properties of the agar need to be investigated. Likewise, the mode of interaction between sucrose and agar need further studies and must be supported by experimental and spectral data.

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