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Stabilization of Aloe Vera Gel by Interaction with Sulfated Polysaccharides from Red Microalgae and with Xanthan Gum

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Aloe vera gel—the fiber-free mucous exudate of the succulent leaves of aloe vera (*Aloe barbadensis* Miller)—was mixed with sulfated polysaccharides isolated from the red microalgae *Porphyridium* sp., *Porphyridium aerugineum*, and *Rhodella reticulata*, with the natural anionic polysaccharide xanthan gum or with nonionic guar gum to determine possible stabilizing effects. Rheological studies indicated interaction between aloe vera gel and the algal polysaccharides and xanthan gum as shown by increased apparent viscosities, apparent yield points, and, in some cases, hysteresis but not with guar gum. These properties did not deteriorate during storage. It was therefore proposed that the algal polysaccharides or xanthan gum could serve to stabilize the network structure of fresh aloe vera polysaccharides.

INTRODUCTION

Aloe vera gel is the commercial name given to the fiber-free mucilaginous exudate extracted from the hydroparenchyma of the succulent leaves of aloe vera (*Aloe barbadensis* Miller). This is the term used throughout the literature, though the exudate is, properly speaking, a fluid rather than a gel. The therapeutic effect of fresh aloe gel in the topical treatment of injured skin and in diseases of the digestive tract is well documented in folk medicine and is supported by clinical studies, as reviewed by Grindlay and Reynolds (1986). Today, the gel is available commercially in cosmetic formulations and in health foods.

The viscous, pseudoplastic nature of aloe vera gel, due mainly to the presence of polysaccharides composed of a mixture of acetylated glucomannans (Gowda et al., 1979), is lost shortly after extraction, apparently due to enzymatic degradation. This paper reports an attempt to stabilize aloe vera gel and delay its degradation by admixture with natural polysaccharides. Carrageenans have been similarly used to inhibit browning of fresh apple juice (Tong and Hicks, 1991) and cyclodextrins to inhibit aggregation of β-casein (Lee and Fennema, 1991).

The polysaccharides of three red unicellular algae, *Porphyridium* sp., *Porphyridium aerugineum*, and *Rhodella reticulata*, have high molecular weights, $2–8 \times 10^6$ (Arad, 1988). Their solutions, studied only at low concentrations, have been found to exhibit pseudoplastic behavior (Geresh and Arad, 1991) (the apparent viscosities of 0.25% polysaccharide solutions at a shear rate of 40 s^{-1} are 25–75 cP); this behavior was only slightly affected by changes of temperature (20–70 °C), pH (2–9), and salinity. The polysaccharides contain acid groups—sulfate content 2.5–8.0%, glucuronic acid 5–11%—and the main monosaccharides are xylose, galactose, glucose, or rhamnose (in *R. reticulata*) (Dubinsky et al., 1988; Geresh and Arad, 1991; Percival and Foyle, 1979; Ramus, 1972). The possibility of adding these polysaccharides to aloe vera exudate to obtain a homogeneous product with a long shelf life was studied, and the results were compared with those of an admixture with the ionic polysaccharide xanthan gum and the nonionic guar gum. The rheological properties of the aloe vera gel/polysaccharide solutions were characterized before and after storage.

MATERIALS AND METHODS

Preparation of Aloe Vera Gel. Succulent leaves were picked during January from a commercial plantation at 'En Yahav (in the Negev, the southern part of Israel), and kept in a cold room at 10 °C. The fluid was produced within a week postharvest as follows: leaves were peeled, and the colorless hydroparenchyma was ground in a blender. The resulting mixture was centrifuged at 10000*g* for 30 min at 4 °C to remove the fibers. The liquid obtained constitutes fresh aloe vera gel. Each leaf produced about 300 mL of fluid. Six leaves each from mature (3 years old) plants were processed and their gels mixed.

Chemicals. Xanthan gum and guar gum were purchased from Sigma Chemical Co.

Cultivation of the Microalgae. *Porphyridium* sp. (UTEX 637), *R. reticulata* (UTEX LB 2320), and *P. aerugineum* (111.79) were grown outdoors with a continuous flow of air containing 3–4% CO_2, according to the method of Cohen and Arad (1989). The culture (about 3 weeks old) was harvested at the stationary phase of growth.

Production of Algal Polysaccharides. The polysaccharides were obtained from the medium after centrifugal separation of the algae, by precipitation with ca. 4 volumes of ethyl alcohol, filtration, dissolution in water, reprecipitation with ethanol, filtration, drying at room temperature, and grinding. The powders of all algal polysaccharides and of xanthan gum contained 12–19% moisture and 10–15% ash.

Preparation of Polysaccharide Solutions. Stock solutions of 2 and 4% w/v algal polysaccharides, guar gum, and xanthan gum were prepared by addition of polysaccharide powder to water with constant stirring at 70 °C. The resulting concentrated solutions are very thick, and it is sometimes difficult to differentiate between dispersion and dissolution. At dissolution the solutions look more homogeneous and clear, although remaining somewhat turbid.

Preparation of Mixtures of Aloe Vera Gel and the Polysaccharides. Aqueous polysaccharide/aloe vera mixtures containing 50% fresh aloe vera gel and 0.5, 0.7, 1.3, or 2.0% w/v polysaccharide powder were prepared on the same day as the aloe gel. Microbial degradation was prevented by the addition of preservatives, either 0.05% sodium azide or 0.1% Givgard (Givaudan). The solutions were kept at 10 °C for 1 day to allow for the disappearance of air bubbles, and rheological measurements were then performed. In addition, the solutions were stored for 1 month at room temperature and characterized again.

Chemical Analyses. The fresh aloe gel obtained by mixture of the exudates of several leaves was analyzed for dry matter content. It was dialyzed for removal of low molecular weight sugars and quinones, and the dialysate was analyzed for polysaccharide content by the phenol–sulfuric acid method (DuBois et al., 1956). (Quinones were removed since they interfere with the

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Table I. Monosaccharide Profile of Polysaccharides of Aloe Vera Gel[a]

monosaccharide	monosaccharide content,[b] %	monosaccharide	monosaccharide content,[b] %
mannose	60.2	methylhexose[c]	5.3
glucose	22.2	dimethylhexose[c]	2.0
galactose	1.6		

[a] Polysaccharide content in the gel, 0.2% (w/v); total dry matter content, 0.85%; pH 4.4. [b] Expressed as percentage of total sugars in the polysaccharides. [c] Methylated hexoses, identified by comparison with reference standards for methylated hexoses (the nature of the hexose and the place of the methyl were not established).

reaction.) The monosaccharide profile of the polysaccharides in the dialysate was determined by gas chromatography of the alditol acetate sugar derivatives (York et al., 1980). Identification of monosaccharides was done according to retention times of standards, including mono- and dimethylhexoses. The methylhexoses were not, however, identified, nor was the precise nature of the methylated hexose or the methylated hydroxyl identified at this stage.

The IR spectrum of the aloe vera polysaccharides was studied on a film obtained by drying a dialyzed sample to determine the presence of acetyl and methyl groups. Uronic acid content was determined according to the *m*-hydroxybiphenyl method (Blumenkrantz and Asboe-Hansen, 1973).

Rheological Studies. Rheological properties were determined at 25 °C with a Haake RV2 Rotovisco viscometer equipped with coaxial cylinders, MV1 or SV1 sensors, and a MK50 measuring head. Shear stress was measured at seven shear rates (1.25–57 s^{-1}), first in increasing order and then in decreasing order. The duration of the shear at each rate did not affect the shear stress at the short times studied (up to 1 min). The duration of shear at each rate was 30 s. Results were plotted as shear rate vs shear stress. Hysteresis was calculated from the area bounded by the up and down shear stress vs shear rate curves, obtained by first increasing and then decreasing the shear rate. This area was measured in mm^2 and converted to units of dyn cm^{-2} s^{-1}. The apparent yield point was calculated according to Casson's equation. Logarithmic plots of shear stress minus apparent yield stress vs shear rate served to calculate *n*, the slope of the resulting curve. The rheological behavior of the aloe vera gel/polysaccharide solutions was compared with that of solutions of aloe vera gel and of each polysaccharide alone.

RESULTS

The fresh aloe vera gel used in this study contained 0.2% polysaccharides, composed mainly of mannose and glucose in a ratio of about 3:1 (Table I). Their IR spectrum showed peaks at 1240 and 1740 cm^{-1}, which are characteristic of ester groups and point to the occurrence of acetyl groups on the polysaccharide (Paulsen et al., 1978). A peak seen at 2920 cm^{-1} is characteristic of *O*-methyl groups of the methylated sugars (Christiaen and Bogard, 1983). The presence of methylated sugars was also detected by gas chromatography of the sugars (Table I). The flow curve, shown in Figure 1, shows shear thinning behavior with no apparent yield point. This curve represents the particular aloe sample used for the rheological studies of aloe gel/polysaccharides mixtures. Samples produced from other shrubs also displayed shear thinning behavior but differed in their apparent viscosities. The IR spectra of the various samples were very similar. Various treatments such as heat and freeze–thaw reduced the apparent viscosities of the gels.

Solutions of the algal polysaccharides alone (without aloe vera) showed pseudoplastic–shear thinning behavior similar to that of xanthan gum (Table II). *P. aerugineum* polysaccharide (1% w/v solution) was closest to xanthan gum with respect to apparent viscosity and apparent yield point but showed a lower hysteresis; the other algal polysaccharide exhibited lower apparent viscosities and apparent

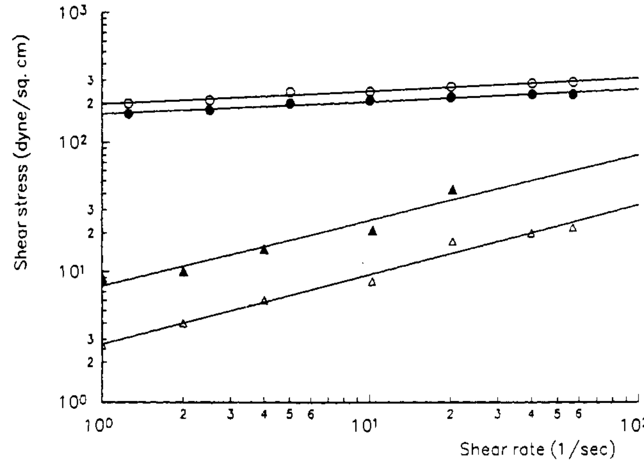

Figure 1. Effect of addition of *P. aerugineum* polysaccharide on flow curve of aloe vera gel. (▲) Aloe vera gel, undiluted; (△) aloe vera gel 50% v/v; (●) *P. aerugineum* 1% w/v; (○) *P. aerugineum* 1% w/v/aloe vera gel 50% v/v.

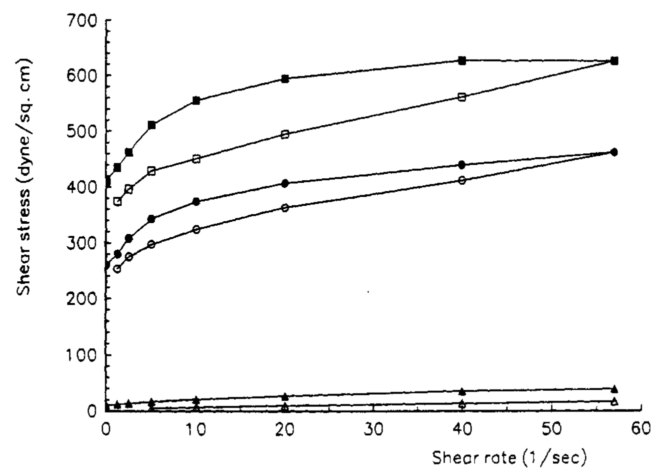

Figure 2. Up and down flow curves of mixture of xanthan gum/aloe vera compared with those of xanthan gum and aloe vera alone. Concentrations: mixture, xanthan gum 1.3% w/v, aloe vera 50% v/v; solutions, xanthan 1.3% w/v, aloe vera 50 and 100% v/v in water. Xanthan gum/aloe vera mixture: (■) up curve; (□) down curve. Xanthan gum: (●) up curve; (○) down curve. Aloe vera gel: (▲) 100%; (△) 50% v/v in water.

Table II. Effect of Admixture with Various Natural Polysaccharides on the Rheological Properties of Aloe Vera Gel[a]

aloe vera gel	source of polysaccharide	app yield point,[b] dyn cm^{-2}	hysteresis,[b] dyn cm^{-2} s^{-1}	n^b	app viscosity at 40 s^{-1}, cP
+100%		none	none	0.43	125
+50%		none	none	0.45	50
–	*R. reticulata*	64	none	0.10	205
+	*R. reticulata*	85	355	0.11	302
–	*Porphyridium* sp.	60	none	0.10	220
+	*Porphyridium* sp.	70	none	0.14	335
–	*P. aerugineum*	168	none	0.14	577
+	*P. aerugineum*	199	370	0.16	700
–	xanthan gum	155	1310	0.11	687
+	xanthan gum	163	1510	0.16	797
–	guar gum	none	none	0.33	1000
+	guar gum	none	none	0.33	1000

[a] In all mixtures the concentration of the aloe vera gel was 50% v/v and that of the additive polysaccharides 1% w/v. In solutions not containing aloe vera the concentration of polysaccharide was also 1% w/v. [b] For method of calculation see Materials and Methods.

yield points (Table II) and showed no hysteresis at this concentration. Figure 2 illustrates the time-dependent behavior of xanthan gum (1.3% w/v) on a linear scale,

Table III. Effect of Concentration of *P. aerugineum* Polysaccharide and of Storage on the Rheological Properties of Its Mixtures with Aloe Vera Gel[a]

aloe vera gel	*P. aerugineum* polysaccharide, % w/v	app yield point,[b] dyn cm^{-2}	hysteresis,[b] dyn cm^{-2} s^{-1}	n^b	app viscosity at 40 s^{-1}, cP
		Fresh Solutions			
−	0.5	40	none	0.14	165
+	0.5	55	none	0.14	247
−	0.7	83	none	0.14	302
+	0.7	112	342	0.15	407
−	1.0	168	none	0.10	577
+	1.0	199	370	0.10	700
−	2.0	417	855	0.06	1182
+	2.0	537	1252	0.07	1347
		1-Month-Old Solutions[c]			
−	0.5	none	none	0.37	142
+	0.5	none	none	0.41	192
−	0.7	58	none	0.23	287
+	0.7	56	none	0.27	325
−	1.0	135	none	0.12	550
+	1.0	146	342	0.15	564
−	2.0	460	455	0.08	1393
+	2.0	280	682	0.12	1122

[a] The aloe vera gel concentration was 50% v/v in all mixtures. [b] See note *b* Table II. [c] The solutions were stored at room temperature.

Table IV. Effect of Concentration of Xanthan Gum and of Storage on the Rheological Properties of Its Mixtures with Aloe Vera Gel[a]

aloe vera gel	xanthan gum, %	app yield point, dyn cm^{-2}	hysteresis,[b] dyn cm^{-2} s^{-1}	n^b	app viscosity at 40 s^{-1}, cP
		Fresh Solutions			
−	0.7	94	740	0.16	495
+	0.7	101	1052	0.20	687
−	1	155	1310	0.13	687
+	1	163	1510	0.17	795
−	1.3	224	1652	0.15	1100
+	1.3	332	3562	0.13	1567
		1-Month-Old Solutions[c]			
−	0.7	97	555	0.15	380
+	0.7	101	1102	0.17	451
−	1	147	665	0.12	500
+	1	147	1102	0.14	606
−	1.3	246	1367	0.12	875
+	1.3	332	1955	0.08	1123

[a] The aloe vera gel concentration was 50% v/v in all mixtures. [b] For methods of calculation see Materials and Methods. [c] The solutions were stored at room temperature.

Table V. Rheological Behavior of Guar Gum and Its Mixture with Aloe Vera Gel before and after 1 Month of Storage

aloe vera gel	guar gum, %	shear stress at 1 s^{-1}, dyn cm^{-2}	app viscosity[b] at 10 s^{-1}, cP	n^b
		Fresh Solutions		
−	2	470	10890	0.36
+	2	400	9790	0.38
		1-Month-Old Solutions[c]		
−	2	108	2100	3.2
+	2	176	2750	5.0

[a] The aloe vera gel concentration was 50% in all mixtures. [b] Shear stress and apparent viscosity were measured at the indicated shear rates. For calculation of *n* see Materials and Methods. [c] The solutions were stored at room temperature.

showing the hysteresis at the low shear rate range 1–57 s^{-1}.

Compared with the polysaccharides taken singly, the anionic polysaccharide/aloe vera mixtures all showed significantly higher apparent viscosities and apparent yield points. In addition, three of the mixtures—those with xanthan, *P. aerugineum*, and *R. reticulata* (Table II)—showed an increase or appearance of hysteresis areas. The increase in apparent yield point and apparent viscosity is seen in the shear stress vs shear rate curves for *P. aerugineum*/aloe vera (logarithmic scale, Figure 1) and for xanthan/aloe vera (linear scale, Figure 2). The effect on hysteresis is clearly seen in Figure 2. Guar gum nonionic galactomannans (1% w/v; Table II) showed a different behavior; the flow curve reflected shear thinning behavior with no apparent yield point, no hysteresis was seen, and the curves of guar gum solutions with and without aloe gel coincided.

The effect of increasing the concentration of the polysaccharide additive in mixtures containing aloe vera gel is shown for *P. aerugineum* in Table III (for fresh and stored mixtures) and for xanthan gum in Table IV. These two polysaccharides were chosen as they gave higher viscosities and exhibited hysteresis. With increasing concentrations of *P. aerugineum* and xanthan gum polysaccharides, the mixtures show a clear increase in apparent yield points and apparent viscosities as well as an increase or appearance of hysteresis areas. After 1 month of storage, the aloe vera gel had lost most of its apparent viscosity and approached a Newtonian viscosity of 20–27 cP at 60–68 s^{-1} (Figure 3). A precipitate had formed which consisted of a small part (~0.003% dw) of the aloe gel polysaccharides.

Storage also changed the rheological behavior of the *P. aerugineum* solutions (Table III): at the lowest concentrations (0.5%) the apparent yield point decreased and *n* increased with storage, while at the highest concentration (2%) the apparent yield point remained about the same. The properties of aloe vera/*P. aerugineum* polysaccharide mixtures also changed with storage. However, at 1 and 2% concentrations of *P. aerugineum* (Table III), differences in hysteresis were retained. Differences in apparent viscosity and apparent yield point between the mixtures with aloe vera and the algal polysaccharides alone

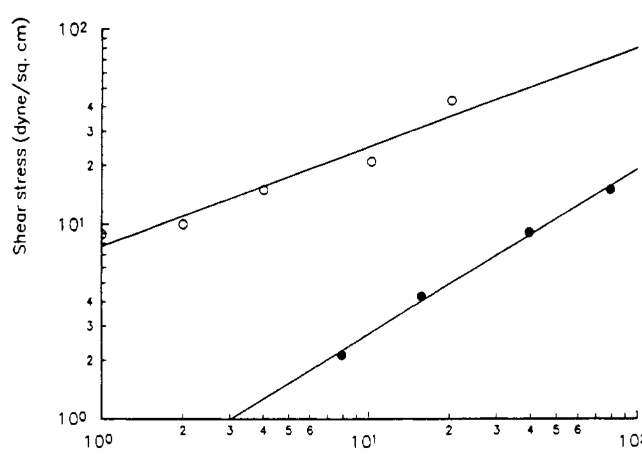

Figure 3. Flow curves of fresh (○) and 1-month-old (●) (chemically preserved) aloe vera gel (undiluted).

were retained only at 1% concentration. Xanthan gum (0.7, 1, 1.3%)/aloe vera solutions retained their differences in hysteresis on storage; the difference in apparent yield points was retained only at 1.3% xanthan/aloe vera (50%) (Table IV). The pseudoplastic properties of guar gum solution alone and with aloe vera deteriorated toward Newtonian behavior in storage at room temperature; with a high decrease in apparent viscosities (Table V), stored guar gum/aloe vera solutions had higher viscosities than the guar gum without aloe vera (Table V).

Aloe vera gel mixtures with the different concentrations of *P. aerugineum* polysaccharide or xanthan gum also remained homogeneous after prolonged storage at room temperature (more than 1 year).

DISCUSSION

The composition of aloe vera gel polysaccharides varies depending on production conditions, and the fact that no pentosans and only low uronic acid content were found in the gel could indicate that cell wall polysaccharides were not extracted or were not incorporated in the gel. The gel polysaccharides consisted of methylated and acetylated glucomannans. Fluctuations in the ratio of mannose to glucose have been reported for plants of different species and for different growth conditions. Gowda et al. (1979) recorded mannose/glucose ratios of 19:1 to 0.6:1, in four fractions isolated from aloe vera associated with acetyl group contents varying between 1.1 and 17.2%.

Rheological characterization revealed that fresh aloe vera gel displays shear thinning behavior. This behavior is largely lost during storage (Figure 3), possibly due to enzymatic degradation.

Since at medium to high concentrations xanthan gum is strongly shear thinning at very low shear rates, the extrapolated yield point should be interpreted as an apparent yield point. This accords with the findings of Lim et al. (1984), who report a strong drop in viscosity at low shear rates, indicating the existence of an apparent yield point at 1% w/w. Whitcomb and Macosko (1978) also report an apparent yield point in xanthan gum 0.5–1% w/w solutions. The shear stresses recorded by Lim et al. (1984) for 1% xanthan gum at low shear rates are similar to the values recorded by us. The time-dependent nature of 1% w/v xanthan gum at the low shear rates tested is recorded as hysteresis. Rochefort and Middleman (1987) found thixotropic behavior for the lower (0.5%) concentration, measured in terms of the time required to regain structure post shear (up to 24 h).

Shear thinning and thixotropy seem to indicate intermolecular associations (such as hydrogen bonds, hydrophobic bonds, and electrolytic interactions) which break and re-form upon shear; Lim et al. (1984) showed that for 1% w/w xanthan gum solutions liquid crystalline regions also exist which break and re-form under shear.

In this study *P. aerugineum* showed the highest apparent yield points and viscosities of the three algal polysaccharides, and its flow curve was very similar to that of xanthan gum. Both *P. aerugineum* and 1% xanthan gum showed interactions with aloe vera gel, as indicated by the large increase in apparent yield points and the increase or appearance of hysteresis.

In Figure 4 we tried to relate the apparent yield point to the total polysaccharide content in solution in a linear curve, for solutions of aloe vera, *P. aerugineum*, and xanthan gum, alone or mixed. At low concentrations (up to 1% total polysaccharides in the solution) the apparent yield point increased linearly with total polysaccharide content. At higher concentrations the increase in apparent yield point continued to be linear for xanthan and *P. aerugineum* polysaccharides; however, the aloe vera/polysaccharide mixtures deviated from this behavior and showed a sharper increase in apparent yield point (Figure 3), possibly indicating the appearance of new interactions and the formation of a network in solution. The hysteresis recorded also seems to indicate the formation of shear-dependent structure. The flow curve of the nonionic guar gum (1%)/aloe vera solutions coincided with the flow curve of guar gum alone, suggesting an absence of interaction

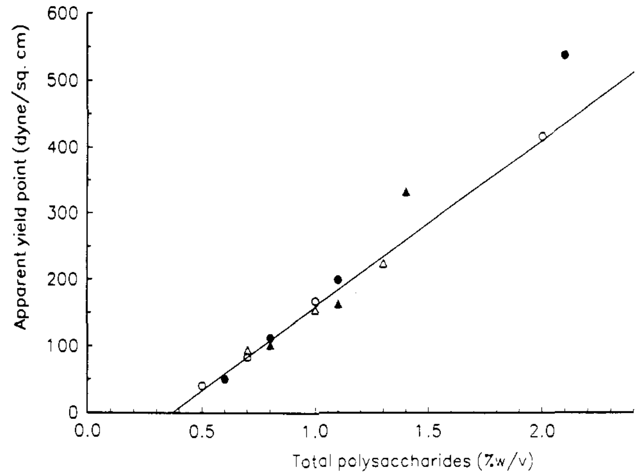

Figure 4. Correlation between apparent yield points and total polysaccharide content in solutions of xanthan gum alone (△), xanthan gum with aloe vera (▲), *P. aerugineum* polysaccharides alone (○), and *P. aerugineum* with aloe vera (●). The contribution of the aloe vera gel to the total polysaccharide content of the mixtures was 0.1% w/v throughout.

(Table II) or negative interaction as shown for 2% guar gum solution (Table V). Such behavior is probably due to aggregation of molecules.

The lower apparent viscosities and smaller hysteresis areas recorded after 1 month of storage in most mixtures of aloe vera with the algal polysaccharides or xanthan gum, as well as in the polysaccharide solutions without aloe vera, may indicate that the molecular association changed with time, probably due to aggregation of molecules. However, the fact that the interaction of aloe vera and the polysaccharides was retained after 1 month of storage (mainly at concentrations of 1% for *P. aerugineum* and 1.3% for xanthan) seems to indicate that the aloe vera polysaccharides retained their original network structure. It seems that addition of the algal polysaccharides or xanthan gum stabilizes the aloe vera gel, preserving both the structure of the fresh aloe vera polysaccharides and the homogeneity of the gel on storage. The anionic polysaccharides could serve for preservation of aloe vera gel in a homogeneous long-shelf-life product.

The chemical structure of the aloe vera polysaccharides in the stored product was not evaluated; however, unlike untreated aloe vera gel, the product was homogeneous, suggesting that gel degradation may have been delayed.

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Registry No. Xanthan gum, 11138-66-2; guar gum, 9000-30-0.