Alginate Encapsulation as a Preservation Method of Pitaya Fruit Juice (*Stenocereus* spp.)

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Abstract: Alginate is a widely used polymer matrix in food industry since it allows formation of spherical, soft, and strong membranes adequate for encapsulation of a large amount of products, including food. The flow rate of alginate solutions and the permeability of the capsules were evaluated within an acidic-low acidic pH range and different alginate concentrations. In solutions adjusted at different pH (3.0 to 7.0) with concentrations of alginate of 0.8, 1.0, and 1.2% w/v, flow rates at 20 °C were 6.95 to 10.00, 4.54-5.35, and 2.60-2.80 mL s^{-1}, respectively. Permeability of the capsules was evaluated in terms of the diffusion of H$^+$ions (expressed as pH) and soluble solids (°Brix). Meanwhile both diffusions were minor at $4.0 < pH < 7.0$ and were significantly superior at more acidic pH ($P < 0.05$), alginate concentration did not present significant effect. Yellow, purple, and red juices from *Stenocereus* spp. fruits (pitayas) were encapsulated using 1.0% of alginate and stored with isotonic solution (3 mL g^{-1}) at 4 °C in the dark. The capsules were spherical with diameter between 4.59 and 470 mm, weight from 82.60 to 97.50 mg, and volume of 0.075-0.098 mL. Pigment (total betalains content) diffusion reached equilibrium at 24 h of storage, at which point retentions of total betalains in the yellow, purple, and red capsules were 87.79, 96.13, and 85.13%, respectively. Also, changes in the color of the capsules were observed during storage.

Key words: *Stenocereus*, pitaya, betalains, alginate encapsulation, color stability.

1. Introduction

The *Stenocereus* genus (pitaya) is a plant native to America with some species endemic to Mexico [1]. The fruits from pitaya may have yellow, red or purple pulp [2], whose color is due to the presence and concentration of betalains, water-soluble pigments with antioxidant properties and positive effects on human health [3]. According to their chemical structure, betalains are divided into two groups: yellowish betaxanthins, and reddish betacyanins [4].

The production of pitaya in Mexico has increased more than 200% in the last 10 years [5]. The fruits are locally consumed fresh and their juices have great sensorial acceptance due to their pulp color and high acidity, which confers a pleasant sweet-sour taste [2]. Nevertheless, the fruit shelf-life is no longer than 6 days [6] and new methods of preservation of pitaya fruit, pulp or juice are required in order to extend product shelf-life, for instance encapsulation [7].

Encapsulation is a technology of packaging solid or liquid materials, which are covered with semipermeable, spherical, and strong membranes [8] that provide enhanced stability under unfavorable environmental conditions [9]. This technology can be used for many applications in food industry: to stabilize the core material, control the oxidative reaction, provide controlled or sustained release, mask flavors, colors or odors, extend shelf life or preserve active compounds against loss, such as fatty acids [10], pigments [11], phenolic compounds [12], and

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microorganisms [13] among others.

The use of natural polymers in techniques of food encapsulation is advantageous over the use of synthetic ones due to their biocompatibility. Equally important is that natural polysaccharides are available in their raw form from natural sources. Furthermore, they are biodegradable, inexpensive, and friendly with the environment [14]. Several matrixes, such as maltodextrin, starch, gelatin, whey protein, caseinate, Arabic gum, chitosan, or alginate [15] are mainly used, applying different encapsulation technologies. Alginate encapsulation is an ionic gelation method and is one of the most widely used in food conservation due to its biocompatibility and low toxicity [16]; it also allows preserving ready-to-eat food for long storage periods in adequate conditions [17].

Alginic acid is extracted from seaweeds and is composed of units of mannuronic (M) and guloronic (G) acids in different proportions, depending on the source and growth conditions. These units are present in block copolymers (MM, GG, and MG). The binding of divalent cations and subsequent gel formation depend on the arrangement and relation of the blocks [18]. The GG blocks exhibit preferential binding sites for divalent counter-ions (for instance Ca^{2+}, Ba^{2+}, Fe^{2+}, or Sr^{2+}), and the bound ions interact with other blocks to form links that subsequently form gel structures; on the other hand, MM and MG blocks provide flexibility to the structure. Interfacial polymerization is instantaneous when sodium alginate solution is added to a calcium solution, with calcium alginate precipitation followed by a gradual gelation of the interior of the capsule as Ca^{2+} permeate trough the alginate system [17]. The characteristics of the gel can be manipulated by modifying manufacturing conditions: temperature, pH, ion concentration or alginate concentration.

The flow rate of aqueous alginate solutions and permeability of alginate capsules were analyzed as a function of the alginate concentration and pH of the aqueous solution. In addition, encapsulation of pitaya

juices was performed to evaluate its preservation during storage.

2. Materials

2.1 Chemicals

Food-grade sodium alginate, calcium carbonate, citric acid, sodium chloride, and sucrose (Mexico).

2.2. Plant Material

Yellow fruits (pitayas) from *Stenocereus pruinosus*, purple, and red pitayas from *Stenocereus stellatus* were collected from Santiago Tonahuiztla, Puebla, Mexico, during harvesting season in 2013.

3. Methods

3.1 Alginate Solutions Encapsulation

Sodium alginate was dissolved in water (adjusted at pH 3, 3.5, 4, 5, 6, and 7 with citric acid) at 3 different concentrations (0.8, 1.0, and 1.2% w/v) at 60 °C. Using a separation funnel, alginate solutions were dropped at 20 °C into a 1.0% w/v calcium chloride solution without agitation, where capsules were kept for one minute. After that, capsules were washed with water and then storaged in aqueous medium. Flow rate of alginate solutions and permeability of alginate capsules were measured.

3.2. Flow Rate of Alginate Solutions

Flow rate was determined using samples of alginate solutions at different concentrations and pH; 100 mL samples were placed into a separation funnel opened to a quarter of the total aperture and total flow time was measured at 20 °C. The results were expressed as mL of solution per second (mL s-1).

3.3. Permeability of Alginate Capsules

Capsules were elaborated according to 3.1. section and then storaged with distilled water as packing liquid with a solution/capsule ratio of 2 mL g-1 during 0, 1, 2, 3, 4, 5, 6, 7, 10, 17, 21, and 24 d at 4 °C in the

dark.

Capsules were drained and packing liquid was measured to H^+, expressed as pH (Denver Instrument, UB-10, USA) and total soluble solids (Boeco, VBR32, Germany), expressed as °Brix.

3.4 Fruit Selection and Juice Preparation

Undamaged yellow, purple, and red pitayas without spines were packed in Food Saver ® plastic bags of 500 g to 800 g whole fruit under vacuum, and stored at -20 °C until encapsulation. Prior to encapsulation, samples were placed at 20 °C for 1 day, and peel. Using a juice extractor (Nutri-Max, Mexico), seeds were removed from the pulp, which was sieved through 710 μm mesh, and then centrifuged (Velocity 14R, Dynamica Scientific, UK) at 10,576 × g for 20 min at 20 °C. Juices were analyzed for pH, total betalains, and color parameters.

3.5. Alginate Encapsulation of Pitaya Fruit Juices

Sodium alginate was mixed with pitaya juice (1.0% w/v) until homogenization at 60 °C. Using a separation funnel, homogenized pulp with sodium alginate was dropped at 20 °C into a 1.0% w/v calcium chloride solution without agitation, where capsules were kept for one minute. After that, capsules were washed with water and then storage with isotonic solution (0.1 M sodium chloride, 0.3 M sucrose) as packing liquid with a solution/capsule ratio of 3 mL g-1 during 0, 1, 2, 4, 6, 24, 48, 72, 96, and 120 h at 4 °C in the dark.

For analysis, capsules were drained and their color was determined, the packing liquid was used to measure betalain content.

3.6 Quantification of Betalains

Total betalains (the sum of betacyanin and betaxanthin) content were quantified using a UV-Vis spectrophotometer [19]. Pigment concentrations were calculated using Eq. (1).

$$B = \frac{(A \times DF \times W)}{(\varepsilon \times L)} \times 1,000 \qquad (1)$$

Where B is betaxanthin and betacyanin content (μg mL-1 or μg g-1) using indicaxanthin and betanin respectively as reference. A is absorbance (483 nm for betaxanthin and 538 nm for betacyanin), DF is dilution factor, W is molecular weight: 550 g mol-1 for betanin, and 308 g mol-1 for indicaxanthin; ε represents the molar extinction coefficient (60,000 L mol-1 cm-1 for betanin and 48,000 for indicaxanthin), and L is path length (1 cm).

A mass balance was performed to determinate betalain retention in capsules. At each time, betalain content in capsules was calculated by the difference between initial content in capsules and pigment content in the isotonic solution.

3.7 Color

Chromatic parameters (L*, a*, and b*) were measured at 0, 24, 48, 92, 96, and 120 h using a colorimeter (Hunter Lab, Color Flex EZ, USA). Samples of drained capsules were placed in a quartz cell with a measurement area of 5 cm (2 in) in diameter. The illuminant used was D65, and the standard observer angle was 10° against a white background. With a* and b* values, Hue angle (H°) and Chroma (C*) were calculated using Eqs. (2) and (3), respectively.

$$H° = \tan^{-1}\left(\frac{b^*}{a^*}\right) \qquad (2)$$

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \qquad (3)$$

3.8 Statistical Analysis

Results are reported as mean ± standard deviations (n = 3). Means were compared by analysis of variance and Tukey's comparison test (P < 0.05). Data were analyzed using Minitab 16 (Minitab Inc., USA).

4. Results and Discussion

4.1. Flow Rate of Alginate Solutions

The mechanical and physicochemical properties of alginate structures are affected by the composition and the formation process [20]. Regardless pH values,

changes in the consistence and in the flow rate of solutions were observed with different alginate concentrations (Table 1): the higher alginate concentration in the solution, the greater viscosity or flow resistance [21].

Aqueous alginate solutions present shear-thinning behavior and are non-Newtonian fluids [16]. When the alginate is in solution it behaves as pseudoplastic and as Newtonian fluids at concentrations of 2.5% and 0.5%, respectively [22], but there is not a well-defined characterization of the fluids at concentrations in between.

On the other hand, flow rate (or thickness) for the 0.8% alginate solution peaked at pH of 6. Moreover, the maximum resistance to flow was reached at pH 3.5 for solutions formulated with 1.0 and 1.2% of alginate. It has been reported that greater thickness of alginate solutions is reached at $3 < pH < 4$ [23]; in contrast, some authors reported that the resistance to flow increases at pH 5 and it is unstable above 11 [22].

4.2. Permeability of the Capsules

The permeability and other properties of alginate gel structures (such as viscosity, strength, molecular weight, among others) are directly influenced by several process conditions, in particular alginate concentration, pH, temperature, alginate composition, and availability of calcium ions [24, 21].

Regardless alginate concentration, with capsules of $pH < 3.5$ solutions diffusion of H^+ ions through membranes was increased ($P < 0.05$) after 24 d of storage among different encapsulated solutions. Thus,

pH of liquid packing (water) was diminished over time. Indeed, pH decreased from 7.5 to 4.5, 4.3, and 4.1 after 24 d in liquid packing of capsules manufactured with solution at pH = 3.0 and 0.8 (Fig. 1A), 1.0 (Fig. 1B) and 1.2% (Fig. 1C) of alginate, respectively. Solutions with less H^+ diffusion were those encapsulated at $4.0 < pH < 7.0$ for all alginate concentrations assayed ($P < 0.05$). Alginate films in aqueous medium are fully permeable due to their high porosity and large amount of water in the gel structure [14].

Similarly to diffusion of H^+ ions, acidic pH values (< 4.0) increased soluble solids diffusion from capsules to the medium and $4.0 < pH < 7.0$ showed less °Brix in liquid packing.

Therefore, low acidic pH grants superior capsule integrality over time. Indeed, in 0.8% alginate capsules the minimal diffusion of solids was observed with pH = 6.0; however, statistical differences were no significant ($P < 0.05$) after 24 d of storage among pH range tested (Fig. 2A).

Furthermore, pH 4.0 and 6.0 showed fewer soluble solids permeability than other pH values assayed ($P < 0.05$) for 1.0% alginate capsules (Fig. 2B). Moreover, in those manufactured with 1.2% of alginate, pH = 7.0 guaranteed physical stability of capsules over time, even though there were no significant statistical differences ($P < 0.05$) among conditions evaluated (Fig. 2C).

4.3. Alginate Encapsulation of Pitaya Juices

Pitaya juice ($4.05 < pH < 4.87$) adequately formed

Table 1   Flow rates of solutions at several pH values (3-7) and different alginate concentrations (0.8%, 1.0%, and 1.2%).

| pH | Flow rates of alginate solutions (mL s^{-1}) | | |
	0.8%	1.0%	1.2%
3	6.95 ± 0.08^d	4.54 ± 0.40^b	2.68 ± 0.03^b
3.5	9.01 ± 0.28^{bc}	5.35 ± 0.08^a	2.80 ± 0.02^a
4	9.01 ± 0.16^{bc}	5.29 ± 0.13^a	2.79 ± 0.04^a
5	8.77 ± 0.10^c	4.76 ± 0.08^b	2.67 ± 0.05^b
6	10.00 ± 0.11^a	5.00 ± 0.06^{ab}	2.70 ± 0.02^b
7	9.29 ± 0.10^b	4.59 ± 0.06^b	2.79 ± 0.02^a

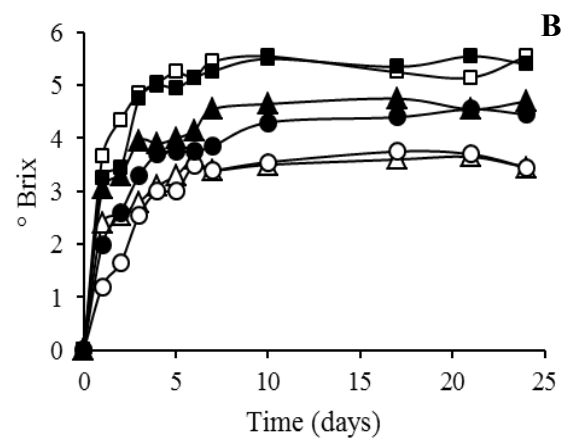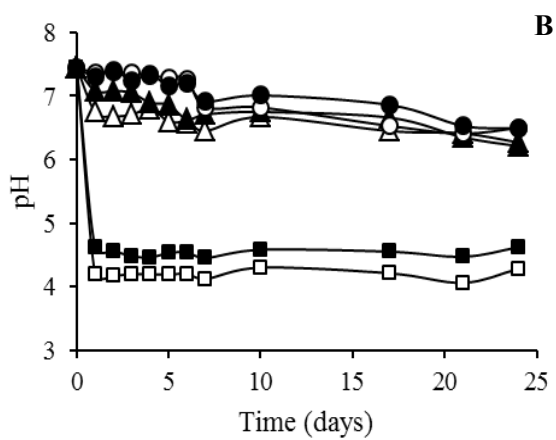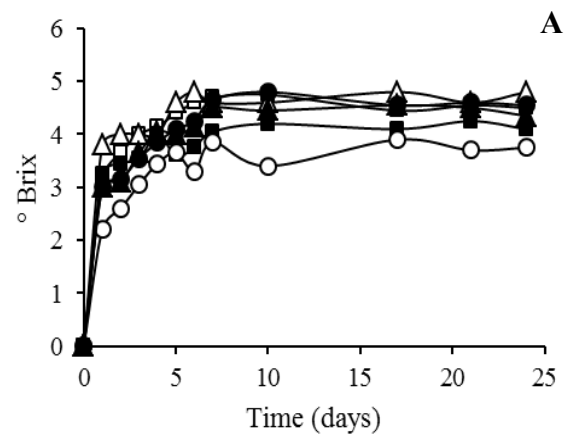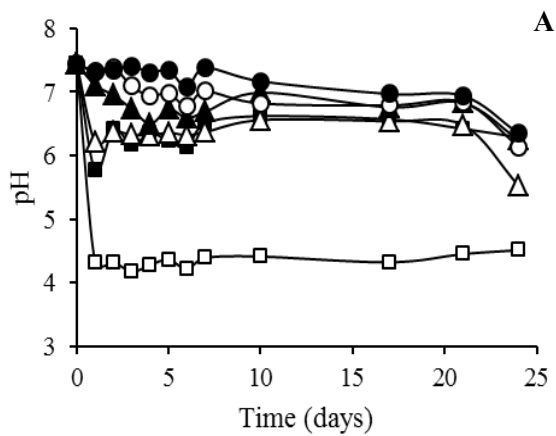Different letters represent significant differences between pH values ($P < 0.05$).
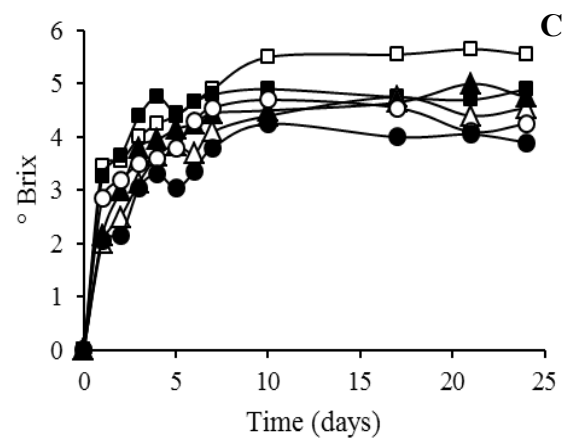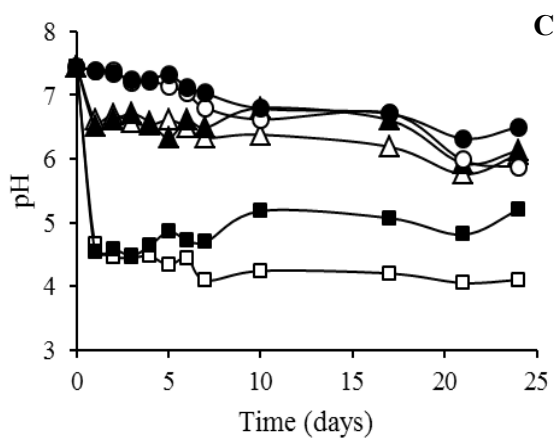
Fig. 1   Diffusion of H⁺ions from capsules to medium expressed as pH. Capsules were prior adjusted at pH 3 (□), 3.5 (■), 4 (△), 5 (▲), 6 (○), and 7 (●). Capsules were manufactured with 0.8% (A), 1.0% (B), 1.2% (C) of alginate.

Fig. 2   Diffusion of soluble solids from capsules to medium expressed as °Brix. Capsules were prior adjusted at pH 3 (□), 3.5 (■), 4 (△), 5 (▲), 6 (○), and 7 (●). Capsules were manufactured with 0.8% (A), 1.0% (B), and 1.2% (C) of alginate.

capsules (Fig. 3) using 1.0% of sodium alginate as polymer matrix. The capsules presented a spherical shape with similar diameter, weight, and volume among fruits (Table 2).

Equilibrium of pigment diffusion from the capsules to the media was reached within 24 h with retention of total betalains in the capsules of 87.79 ± 0.06, 96.13 ± 0.05, and $85.13 \pm 0.12\%$ in the yellow, purple, and red juice, respectively; mentioned retentions were maintained for 120 h without significant differences (Fig. 4).

Pigment diffusion affected the chromatic parameters of the capsules during storage (Table 3). Changes in L*values suggested that capsules presented brighter colors at the end of the experiment.

On the other hand, H° indicated changes in the tone of the product along with more saturated colors (C* values) with storage.
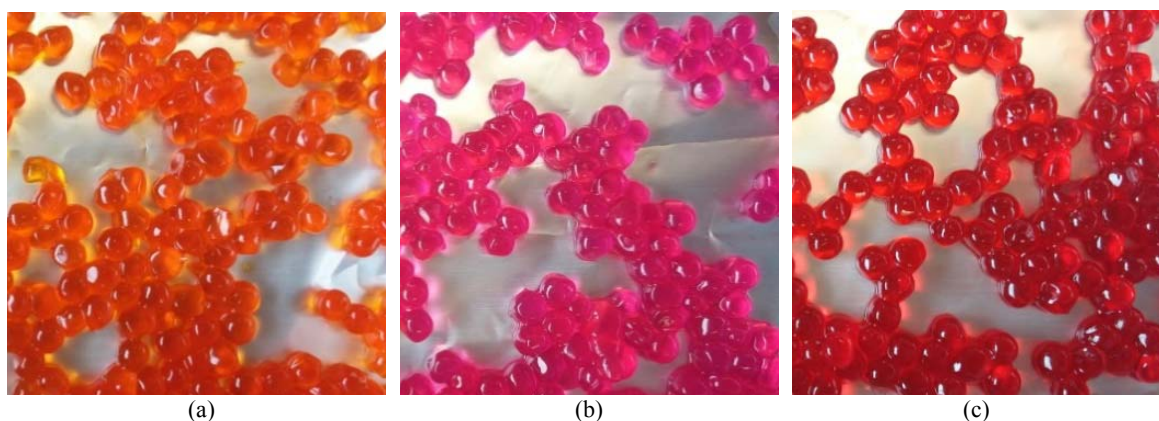

| (a) | (b) | (c) |

Fig. 3 Alginate capsules of yellow (A), purple (B), and red (C) pitaya juice.

Table 2 Physical characterization of juice capsules.

Pitaya capsules	Diameter (mm)	Weight (mg)	Volume (mL)
Yellow	4.61 ± 0.19	82.60 ± 0.75	0.075 ± 0.01
Purple	4.59 ± 0.14	83.10 ± 7.56	0.080 ± 0.00
Red	4.70 ± 0.16	97.50 ± 0.35	0.098 ± 0.01

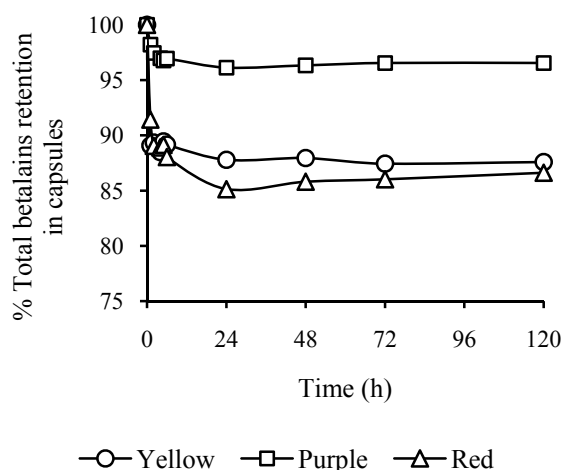Presented results are the mean $\pm$ S.D. of 3 replicates.

Fig. 4 Retention of total betalains in capsules of pitaya juices preserved using isotonic solution during 120 h of storage period at 4 °C.

Table 3   Chromatic parameters of alginate capsules at 0, 24 and 120 h of storage at 4 °C.

Capsules	Time (h)	L*	H°	C*
Yellow	0	23.96 ± 0.42	37.52 ± 0.17	54.49 ± 1.07
	24	28.44 ± 0.56	45.42 ± 0.37	65.48 ± 0.85
	120	29.04 ± 0.35	45.17 ± 1.46	66.57 ± 1.01
Purple	0	22.05 ± 0.09	21.93 ± 0.39	49.98 ± 0.42
	24	25.39 ± 0.44	17.73 ± 0.13	54.83 ± 0.35
	120	25.82 ± 0.84	17.24 ± 0.44	56.20 ± 1.65
Red	0	17.89 ± 0.03	27.90 ± 0.01	45.82 ± 0.08
	24	20.22 ± 0.82	33.39 ± 0.58	58.24 ± 1.45
	120	20.27 ± 1.10	33.11 ± 1.40	58.63 ± 2.90

Presented results are the mean ± S.D. of 3 replicates.

In products pigmented with betalains, similar color changes have been reported [25, 26] during storage or stability tests, and they are directly related with the pigment stability, which is influenced by its concentration, temperature, presence of light, water activity, among others [4], conditions that should be controlled if a good color stability are desired.

5. Conclusions

In this paper it was demonstrated that alginate encapsulation is an adequate method to preserve food under controlled conditions.

Physical properties of the capsules are determined by the alginate concentration and pH used during process. Importantly, it was found that pH plays an important role in capsules stability during storage. Particularly, pH between 4.0 and 7.0 allows obtaining uniform and strong membranes with minimal diffusion of H^+ and soluble solids through the capsules.

Indeed, encapsulation of pitaya juices preserves betalain content in the capsules with good color stability during storage. Further sterilization after food encapsulation is recommendable in order to extend shelf life and to guarantee appropriate microbiological quality. This information may help to develop new products, and also increase commercialization and value of pitayas.

Acknowledgements

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