DEVELOPMENT OF VITAMIN C BUTTERFLY PEA JUICE BEADS BY REVERSE SPHERIFICATION TECHNIQUE

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Abstract

The edible beads of calcium lactate for encapsulating vitamin C butterfly pea juice were transformed by frozen reverse spherification technique. This juice consisted of 1 L blue butterfly pea tea, 20 g erythritol, 50 ml lime juice and 1.2 g ascorbic acid. The influence of sodium alginate concentration solutions at 0.5% w/v, 1.0% w/v, and 1.5% w/v on the physicochemical characteristics, for example, length, width, sphericity ratio, color (*L*, *a*, and *b* values), pH, and brix were investigated. The 9-point hedonic scale, just about right, and acceptance tests of three samples were also examined. The Duncan's multiple range test of data was performed on the experiment.

The findings revealed that the application of higher concentration of sodium alginate was significantly ($P<0.05$) leading to higher *L* and *a* values of the bead, nevertheless, lower *b* value, brix, preference score of appearance, sweetness, bead membrane texture, overall liking and acceptance of the bead. The most of the respondents was male (67%) aged ranging between 21 to 35 years. The sensory results indicated that hedonic score in term of appearance, sweetness, bead membrane texture and overall liking of the vitamin C juice bead immersed at the lowest concentration (0.5% w/v) was the highest. Additionally, the respondents 41 from 50 panelists or 82% accepted this product.

Keywords: Beads, Reverse spherification, Sodium alginate, Physicochemical properties, Sensory Attributes and Just about right scale

Introduction

Spherification is a modern cuisine technique that involves creating semi-solid spheres with thin membranes out of liquids. This product provides a burst-in-the-mouth effect with the liquid inside, when chewing a sphere membrane. There are two commonly types of spherifications namely basic and reverse spherification. Basic spherification is where the liquid/beverage containing sodium alginate is submerged in a water bath containing calcium, whereas reverse spherification is where the liquid/beverage containing calcium is dropped into a water bath containing sodium alginate (Sullivan, 2019). When these two solutions come into contact, the beads or spheres are created and formed. Calcium lactate and sodium alginate are the two basic components used for this technique in this research. Sodium alginate is naturally taken from seaweed, while calcium lactate is a type of salt that widely uses in food industry. Sodium alginate is used as a binder, stabilizer, thickening agent and viscosity inducing agent. The mixing of alginate with the calcium salt, it intensifies the viscosity of liquid and transform into strong gel due to alginate polymer cross-linking (Patomchaiviwat et al., 2022).

Vitamin C, also known as L-ascorbic acid, is a water-soluble vitamin that is an essential nutrient for human health, which frequently applies in foods and beverages. It is interesting to combine vitamin C in the beverage, such as butterfly pea juice, in order to enhance health benefits,

including improve immune function, extend product shelf life, improve skin health and promote wound healing as shown in this research. Mukherjee et al. (2008) reported that the butterfly pea popularly used in traditional medicine, particularly as a supplement to enhance cognitive functions and alleviate symptoms of numerous ailments including fever, inflammation, pain, and diabetes. Erythritol is a sugar alcohol that is commonly used as a sugar substitute. This sweetener naturally presents in low amounts in calorie, approximately 0.24 calories per gram, while sugar contains 4 calories per gram.

It is essential to use high-quality ingredients and employ the innovative technology, such as frozen reverse spherification technique, to ensure that the final product is safe, nutritious, and appealing for consumers. Therefore, this research aims to apply all these mentioned materials, namely, sodium alginate, calcium lactate, vitamin C, butterfly pea flower, and erythritol to study and develop the healthy edible beads. The physicochemical and sensory properties of the edible beads in different sodium alginate concentrations were also investigated.

Research Objectives

1. To study the effect of sodium alginate concentrations at 0.5% w/v, 1.0% w/v, and 1.5% w/v of vitamin C butterfly pea juice beads
2. To study the physicochemical properties of vitamin C butterfly pea juice beads
3. To study the sensory evaluation of vitamin C butterfly pea juice beads

Literature Review

1. Reverse spherification technique

The spheres can be made of different sizes and have been given names like caviar when they are small, eggs, gnocchi and ravioli when they have larger size. The resulting spheres have a thin membrane and are filled with the flavored liquid, which is flexible and can be carefully manipulated (Dhrubo Jyoti Sen, 2017). The Reverse spherification technique consists of submerging a liquid with calcium content in a bath of sodium alginate. When the liquid drops into the bath, a thin coat of gel forms around the droplet as the calcium reacts with the sodium alginate. Previous study revealed that the reverse spherification technique was effective in protecting the probiotics in the bead during storage and simulated gastric conditions (Siripatrawan and Kaewklin, 2016).

2. Vitamin C

Vitamin C, or ascorbic acid, is an essential micronutrient for human, which must be taken daily through food or supplements. It is required for human body like the biosynthesis of collagen, L-carnitine, certain neurotransmitters, and protein metabolism (Li, 2007). Furthermore, vitamin C is also an important physiological antioxidant within the body (Carr and Frei, 19 99) . Thus, the lack of vitamin C results in impaired immunity and higher susceptibility to infections. The recommended dietary allowance for vitamin C is shown in Table 1.

Table 1: Recommended dietary allowances (RDAs) for vitamin C adequate intake (Institute of Medicine, 2000)

Age	Male (mg/day)	Female (mg/day)	Pregnancy (mg/day)	Lactation (mg/day)
0–6 months	40	40		
7–12 months	50	50		
1–3 years	15	15		

Age	Male (mg/day)	Female (mg/day)	Pregnancy (mg/day)	Lactation (mg/day)
4–8 years	25	25		
9–13 years	45	45		
14–18 years	75	65	80	115
19+ years	90	75	85	120
Smokers	Individuals who smoke require 35 mg/day more vitamin C than nonsmokers.			

The RDAs for vitamin C is based on its known physiological and antioxidant functions in white blood cells and are much higher than the amount required for protection from deficiency. Previously, it was reported that daily supplementation with vitamin C improved skin elasticity and reduced the appearance of wrinkles in middle-aged women (Pullar, 2017).

3. Butterfly pea juice

Butterfly pea juice, also known as *Clitoria ternatea* juice, is a beverage made from the flowers of the butterfly pea plant. The bright blue color of this juice is unique. It is commonly consumed in Southeast Asian countries due to several health benefits. Moreover, it is widely used in traditional medicine in order to reduce fever, headache, and constipation symptom. Several studies reported that the butterfly pea extract had anti-inflammatory and antioxidant properties (flavonoids and anthocyanin), which could potentially protect against chronic diseases such as cancer and cardiovascular disease (Singh et al., 2022). It also could improve cognitive function and memory in rats as described in the research of Damodaran et al. (2018).

4. Erythritol

Erythritol, an ingredient category called sugar alcohol, is a common artificial sweetener. Low amounts occur naturally in fruits, vegetables, fermented foods and beverages. It is used as a zero-calorie sweetener to help replace calories from carbohydrates. In addition to providing sweetness, erythritol also helps foods retain moisture. When used as a sweetener, erythritol levels are typically more than 1,000-fold greater than levels found naturally in foods, which are not required to be listed individually on nutrition facts labels (Witkowski et al., 2023).

Methodology

1. Materials

Distilled water, sodium alginate, calcium lactate, erythritol and ascorbic acid (vitamin C) were obtained from Krungthepchemi Co., Ltd. (Bangkok, Thailand), which were food grade. Fresh lime and dried butterfly pea flower were purchased from a local market in Nonthaburi, Thailand.

2. Preparation of vitamin C butterfly pea juice

The dried butterfly pea flower (8 g) was added in the boiling water (1.2 L) and then stirred and pressed the flowers against the pot for 3 minutes. After that, the blue tea was strained with a mesh strainer in order to discard the flowers. The twenty grams of erythritol was put in the one litre of warmed blue tea. Next, the fresh limes were cut and then squeezed the water out by hands. The lime juice about 50 ml and ascorbic acid 1.2 g was mixed in the butterfly pea juice.

3. Preparation of juice beads by reverse spherification technique

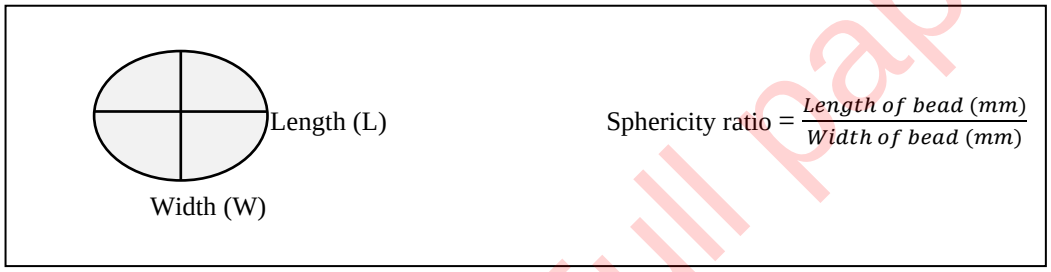
Calcium lactate 0.06 g was dissolved and mixed with 1 L vitamin C butterfly pea juice and they were poured into sphere silicone mould. After that, they were frozen at -15°C for 24 hr. The frozen liquids were immersed into the bath containing various concentrations (0.5-1.5% w/v) of sodium alginate solution for 5 min to form the bead membrane. The obtained beads were then

removed from the bath and transferred to three distilled water baths to rinse off any excess sodium alginate. This reverse spherification method was slightly modified in accordance to the research of Kamprawet and Vatthanakul (2018). The vitamin C juice beads, soaked in different sodium alginate solutions including 0.5% w/v, 1.0% w/v, and 1.5% w/v, were investigated and compared the physicochemical and sensory properties in this study.

4. Physicochemical properties determination

4.1 Sphericity ratio

The sides of beads were measured by using a digital Vernier. The length (L) and width (W) of the bead were determined. The findings were represented as the mean of ten measurements. Sphericity ratio was calculated by using the equation in the same way as described in the research of Kamprawet and Vatthanakul (2018). If the ratio was close to 1, it meant that the bead was round due to the little difference between their length and width.



4.2 Weight

The bead was weighed with a Mettler analytical balance. The findings were reported as the mean of six measurements.

4.3 Color

The color of samples was measured with a portable colorimeter as lightness (L), redness (a), and yellowness (b). The colorimeter was calibrated against a standard white tile (L = 94.81, a = −0.53, and b = 5.672. Measurements were conducted in triplicate. The L, a, and b values represent as described by Samard and Ryu (2018).

4.4 Acidity and sweetness (brix)

The pH and sweetness measurements of the vitamin C juice inside the beads (i.e., before and after burst of bead) were examined by using a calibrated digital pH meter and a calibrated digital refractometer, at 25°C, respectively. The results were averaged of six treatments.

5. Sensory Evaluation

A 9-point hedonic scale was commonly used to assess the acceptability of food product. The criteria used were: blue color, appearance, sweetness, sourness, flavor, bead membrane texture and overall liking on a scale of 1 to 9, where 9 represented “like extremely” and 1 represented “dislike extremely.” The 3-scale just about right test was used for measuring attribute intensity and acceptability simultaneously (Carvalho et al., 2017). The attributes were designed as continuous line scale with three descriptive principles, low intensity (much too weak) on the left end, just-about-right or acceptance at the center, and high intensity (much too strong) on the right end, score ranging from 1 to 3, respectively. If the net score is less than -20, the attribute intensity should be increased. On the contrary, if it is higher than 20, the attribute intensity should be decreased. The net score between -20 and 20 means that the attribute intensity is just about right. Moreover, the acceptance of vitamin C juice beads was inquired.

A panel of 50 untrained students and staff aged ranging from 21 to 35 years, Food Business Management Department, Panyapiwat Institute of Management, attended in the sensory evaluation. Every panelist was served three beads from three different sodium alginate concentration solution s (0.5% w/v, 1.0% w/v, and 1.5% w/v). Samples were blind coded with random three-digit numbers and the order of serving samples was randomized so that each sample occurred equally. One cup of water was served to rinse.

Statistical Analysis

Vitamin C juice bead data: percentage, mean and standard deviation were analyzed by using IBM SPSS software version 24.0 (IBM, Armonk, NY, USA). Significant differences among treatments were determined at $p<0.05$ using Duncan's multiple range test.

Results and Discussion

The physicochemical properties of vitamin C butterfly pea juice beads at three different sodium alginate concentration solutions, including 0.5% w/v, 1.0% w/v, and 1.5% w/v, presented in **Table 2**. There was no significant difference in the length, width and sphericity ratio of all three samples, which could be due to using the same size of sphere silicone mold. In addition, the weight of the bead, soaked in the lowest concentration solution at 0.5% w/v (15.06 g), was significantly ($p<0.05$) lighter than those of the rest concentration solutions (16.67 g to 16.77 g), which might be attributed to the lower forming of the bead membrane texture.

The color parameters of all samples were significantly different ($p<0.05$) by the immersion of sodium alginate concentration solution as shown in **Table 2**. The application of higher sodium alginate concentrations brought to the higher *L* and *a* values of the beads, however, the lower in *b* value. The pH of the vitamin C juice before and after bead bursting, ranging from 5.23 to 5.32, did not differ significantly ($p<0.05$), which was in accordance to the pH of energy drink in the previousresearch of Patomchaiviwat et al. (2022). As regards to sweetness (brix), the increasing of sodium alginate concentrations from 0.5 % w/v to 1.5 % w/v was significantly different leading to the lower sweetness of the vitamin C juice.

Table 2: Physicochemical properties of vitamin C butterfly pea juice beads

Properties	Sodium alginate concentration (% w/v)		
	0.5	1.0	1.5
Length (mm)	34.20 ± 1.00^{ns}	33.77 ± 1.57^{ns}	34.60 ± 1.00^{ns}
Width (mm)	26.51 ± 1.00^{ns}	26.03 ± 1.00^{ns}	26.81 ± 1.00^{ns}
Sphericity ratio	1.29 ± 0.97^{ns}	1.30 ± 0.98^{ns}	1.29 ± 1.00^{ns}
Weight (g)	15.06 ± 0.97^{b}	16.67 ± 0.98^{a}	16.77 ± 1.00^{a}
L	34.48 ± 1.52^{b}	35.12 ± 1.52^{a}	35.37 ± 1.62^{a}
a	-0.42 ± 1.52^{b}	-0.07 ± 0.97^{a}	0.05 ± 0.87^{a}
b	16.02 ± 1.00^{a}	15.00 ± 1.00^{b}	14.74 ± 1.00^{c}
pH	5.32 ± 0.97^{ns}	5.23 ± 1.12^{ns}	5.27 ± 1.15^{ns}
Brix (%)	7.85 ± 1.15^{a}	6.30 ± 1.23^{b}	6.44 ± 1.50^{b}

Values are means ± standard deviation
Different letters (a–c) in the same row are significantly different at $p<0.05$

The three samples of vitamin C butterfly pea juice beads were immersed into different sodium alginate concentration solutions at 0.5 % w/v, 1.0 % w/v, and 1.5 % w/v, respectively. The results indicated that the fifty respondents who participated in the sensory evaluation of this research were female 33% and male 67%, aged ranging from 21 to 35 years. The sensory attributes of three juice beads were significantly different ($p<0.05$) dependent on sodium alginate concentration solutions (0.5% w/v to 1.5% w/v) as indicated in Table 3. The highest overall liking score (6.58) was the sample at 0.5% w/v sodium alginate concentration, compared to those two concentrations (1.0% w/v and 1.5% w/v). The increasing of sodium alginate concentrations from 0.5% w/v to 1.5 % w/v was leading to the lower score of sweetness, bead membrane texture and overall liking ($p<0.05$). Moreover, the preference score of blue color, sourness, and flavor scores showed no statistically significant differences at 0.05 level among three samples.

Table 3: Sensory attributes of vitamin C butterfly pea juice beads

Attributes	Sodium alginate concentration (% w/v)		
	0.5	1.0	1.5
Blue color	4.20 ± 0.30^{ns}	4.07 ± 0.57^{ns}	4.00 ± 1.00^{ns}
Appearance	7.06 ± 0.75^{a}	6.67 ± 0.83^{b}	6.77 ± 1.60^{b}
Sweetness	6.32 ± 0.01^{a}	5.53 ± 1.74^{b}	4.63 ± 1.32^{c}
Sourness	5.20 ± 1.02^{ns}	5.43 ± 1.23^{ns}	5.37 ± 1.24^{ns}
Flavor	5.02 ± 0.52^{ns}	5.27 ± 0.22^{ns}	5.37 ± 0.72^{ns}
Bead membrane texture	6.02 ± 0.00^{a}	5.00 ± 0.10^{b}	4.74 ± 0.50^{c}
Overall liking	6.58 ± 1.45^{a}	5.30 ± 1.23^{b}	5.44 ± 1.80^{b}

Values are means ± standard deviation
Different letters (a–c) in the same row are significantly different at $p<0.05$

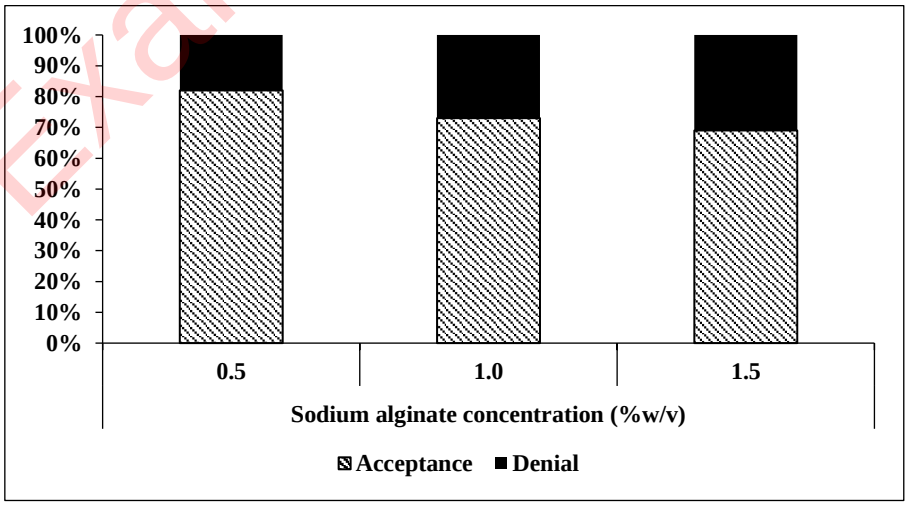
The just about right (JAR) score of the sample at 0.5% w/v sodium alginate concentration provided the different consideration amongst three samples as demonstrated in **Table 4**. The results reveled that all three vitamin C butterfly pea juice beads were too dark, and the blue color intensity of the beads needed to be decreased, while the sourness and flavor of three samples were too low, and considered to be increased. Besides, the appearance intensity of three beads was just about right. For the sweetness, the samples at sodium alginate concentrations of both 1.0% w/v and 1.5% w/v were not enough and needed to be increased, while the bead membrane texture of these samples were too tough to chew and needed to be softer. This might be due to these membrane beads were quite thick and sticky, especially the sample at the highest concentration (1.5% w/v). It might be explained that the higher sodium alginate concentration lead to the stronger membrane bead, which the respondent felt difficult to chew. Patomchaiviwat et al. (2022) also described that increasing the sodium alginate concentration resulted in a slight increase in shell thickness.

Interestingly, the bead at the lowest sodium alginate concentration solution (0.5% w/v) presented just about right consideration in appearance, sweetness and bead membrane texture, which be recommended not to be improved (**Table 4**). The result of just about right scale was in accordance to the 9-hedonic scale that respondents preferred sensory attributes of the sample at the lowest concentration to those of the rest samples at 1.0% w/v and 1.5% w/v concentration.

Table 4: Just-about-right (JAR) consideration of vitamin C butterfly pea juice beads

Sodium alginate concentration (%w/v)	Attributes	Intensity (%)			Net score	Consideration
		Too weak	JAR	Too strong		
0.5	Blue color	3	63	34	31	Decrease
	Appearance	15	75	10	-5	JAR
	Sweetness	15	70	15	0	JAR
	Sourness	36	64	0	-36	Increase
	Flavor	35	65	0	-35	Increase
	Bead membrane texture	19	67	14	-5	JAR
1.0	Blue color	0	54	46	46	Decrease
	Appearance	7	70	23	16	JAR
	Sweetness	44	56	0	-44	Increase
	Sourness	40	60	0	-40	Increase
	Flavor	44	53	3	-41	Increase
	Bead membrane texture	0	54	46	46	Decrease
1.5	Blue color	6	60	34	28	Decrease
	Appearance	13	74	13	0	JAR
	Sweetness	37	52	11	-26	Increase
	Sourness	49	51	0	-49	Increase
	Flavor	35	61	4	-31	Increase
	Bead membrane texture	0	48	52	52	Decrease

The percentage of acceptance score of three vitamin C juice beads at different sodium alginate concentration solutions (0.5% w/v to 1.5% w/v) displayed in Picture 1. The beads, soaked in the lower sodium alginate concentration solution, indicated more acceptance score. Remarkably, the sample at 0.5% w/v concentration showed the highest acceptance score (82%) than those two concentrations. On the other side, few panelists about sixteen percent (8 from 50 panelists) did not accept this product (0.5% w/v concentration) by reasoning of dark blue color, little sourness and odorless of the bead. They mentioned that the product needed to further developed and improved.



Picture 1: The acceptance of vitamin C butterfly pea juice beads

Conclusion

The frozen reverse spherification technique was used to practically prepare edible vitamin C beads of calcium lactate for encapsulating juice comprising 1 L blue butterfly pea tea, 20 g erythritol, 50 ml lime juice and 1.2 g ascorbic acid. The beads were created by freezing calcium lactate-containing vitamin C drinks and dropping them into sodium alginate solution.

The comparison of three juice beads immersed with different sodium alginate concentration solutions revealed that the utilization of lower concentration of sodium alginate, the higher *b* value, brix, preference score of sweetness, bead membrane texture, overall liking and acceptance of the bead. The vitamin C butterfly pea juice bead immersed at the lowest concentration (0.5% w/v) was outstandingly the highest preference score of appearance, sweetness, bead membrane texture and overall liking. The respondents liked this bead slightly. Eighty-two percent of respondents (41 from 50 panelists) accepted the product. However, the additional study on the development of this bead is needed.

References

Carr, A.C. & Frei, B. (1999). Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. *The American Journal of Clinical Nutrition*, *69*, 1086–1087.

Carvalho, G.R.de, Milani, T.M.G., Trinca, N.R.R., Nagai, L.Y. & Barretto, A.C.da.S. (2017). Textured soy protein, collagen and maltodextrin as extenders to improve the physicochemical and sensory properties of beef burger. *Food Science and Technology*, *37*(1), 10–16.

Damodaran, T., Tan, B.W.L., Liao, P., Ramanathan, S., Lim, G.K. & Hassan, Z. (2018). *Clitoria Ternatea* L. root extract ameliorated the cognitive and hippocampal long-term potentiation deficits induced by chronic cerebral hypoperfusion in the rat. *Journal of Ethnopharmacol*. *5*(224), 381-390.

Dhrubo J. S. (2017). Cross linking of calcium ion in alginate produce spherification in molecular gastronomy by pseudoplastic flow. *World Journal of Pharmaceutical Sciences*, *5*(1), 1-10.

Institute of Medicine. (2000). *Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids*. Washington, DC: National Academy Press.

Kamprawet, P. & Vatthanakul, S. (2018). Producing passion fruit beads by reverse spherification technique. *Science and Technology*, *26*(8), 1381-1393.

Li, Y. & Schellhorn, H.E. (2007). New developments and novel therapeutic perspectives for vitamin C. The *Journal of Nutrition*, *137*, 2171-84.

Mukherjee, P.K., Kumar, V., Kumar, N.S. & Heinrich, M. (2008). The Ayurvedic medicine Alitoria ternatea-from traditional use to scientific assessment. *Journal of Ethnopharmacology*, *120*, 291–301.

Patomchaiviwat, V., Sriamornsak, P., Chansiri, G., Limmatvapirat, S., Supawattanakul, A., Chonganon, T., Keattiteerachai, A. & Piriyaprasarth, S. (2022). Development of edible bubbles of calcium alginate for encapsulating energy drinks. *Science, Engineering and Health Studies*, 1-10.

Pullar, J.M., Carr, A.C. & Vissers, M.C.M. (2017). The roles of vitamin C in skin health. *Nutrients*, *9*(8), 866.

Samard, S. & Ryu, G-H. (2019). A comparison of physicochemical characteristics, texture, and structure of meat analogue and meats. *Journal of the Science of Food and Agriculture*, 99, 2708-2715.

Singh, R., Yu, C.C., Chen, G.W., Chen, C.H., Sinaki, N.Y., Lin, J. & Koksel, F. (2022). Butterfly pea flower as a novel ingredient to produce antioxidant-enriched yellow pea-based breakfast cereals. *Foods*, *9*(7), 896.

Siripatrawan, U. & Kaewklin, P. (2016). Fabrication and characterization of chitosan-titanium dioxide nanocomposite film as ethylene scavenging and antimicrobial active food packaging. *LWT-Food Science and Technology*, *68*, 356-363.

Sullivan, V. (2023, May,3). *Spherification*. https://vinsullivan.com/index.php?route=journal2/blog/post&journal_blog_post_id=9

Witkowski, M., Nemet, I., Alamri, H., Wilcox, J., Gupta, N., Nimer, N., Haghikia, A., Li, X.S., Wu, Y., Saha, P.P., Demuth, I., König, M., Steinhagen-Thiessen, E., Cajka, T., Fiehn, O., Landmesser, U., Tang, W.H.W. & Hazen, S.L. (2023). The artificial sweetener erythritol and cardiovascular event risk. *National Library of Medicine*, *29*(3).710-718.