Heliyon 9 (2023) e21025

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Synergistic enhancing effect of xanthan gum, carboxymethyl cellulose and citric acid on the stability of betacyanins in fermented red dragon fruit (*Hylocereus polyrhizus*) drink during storage

Teck Wei Lim [a,b], Renee Lay Hong Lim [a], Liew Phing Pui [b], Chin Ping Tan [c], Chun Wai Ho [b,*]

[a] *Department of Biotechnology, Faculty of Applied Sciences, UCSI University, No. 1, Jalan Menara Gading, UCSI Heights, 56000 Cheras, Kuala Lumpur, Malaysia*
[b] *Department of Food Science and Nutrition, Faculty of Applied Sciences, UCSI University, No. 1, Jalan Menara Gading, UCSI Heights, 56000 Cheras, Kuala Lumpur, Malaysia*
[c] *Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia (UPM), 43400 Serdang, Selangor, Malaysia*

ARTICLE INFO

Keywords:
Concentrated betacyanins
Stability
Red dragon fruit
Xanthan gum
Carboxymethyl cellulose
Citric acid

ABSTRACT

Nowadays, the demand for using healthy natural pigments (betacyanins) in the food industry is increasing. The present study aimed to overcome the circumstances that render the betacyanins instability in the red dragon fruit drink using mild approaches. These included optimised fermentation, incorporation of anionic polysaccharide mixture solution [xanthan gum (XG, 0.30–0.40 %, w/v) and carboxymethyl cellulose (CMC, 0.50–0.90 %, w/v)] and also addition of citric acid (CA, 0.05–0.20 %, w/v). The results of this study showed that the hydrocolloid mixture solution of XG and CMC significantly increased the samples' viscosity, pH and °Brix but reduced the a_w, while betacyanins concentration had no significant change. The incorporation of CA at increasing concentration only reduced the samples' pH significantly without affecting the viscosity, a_w and °Brix. Among all fermented samples, Formulation 3E (0.40 % XG + 0.50 % CMC + 0.20 % CA) had achieved the desired commercial reference viscosity while also successfully minimised betacyanins degradation from 60.18 % to 14.72 %, had the best pH stability and no significant change in viscosity, a_w and °Brix values after 4-week storage at 25 °C. The fermented red dragon fruit drink with betacyanins stabilised by Formulation 3E can be produced and served as an independent functional drink product and as a stable, functional ingredient (natural colourant) for the food industry.

1. Introduction

Natural pigments such as betalains, anthocyanins, chlorophylls and carotenoids have sparked an increasing interest in food industries due to their economic, environmental and health-beneficial properties [1,2]. Betalains are water-soluble nitrogen-containing pigments comprised of betacyanins and betaxanthins. In appearance, betacyanins have red-violet colour, which is different from the

* Corresponding author.
E-mail address: cwho@ucsiuniversity.edu.my (C.W. Ho).

https://doi.org/10.1016/j.heliyon.2023.e21025
Received 16 September 2023; Received in revised form 23 September 2023; Accepted 13 October 2023
Available online 14 October 2023
2405-8440/© 2023 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

betaxanthins that are yellow-orange. Compared to the betaxanthins, betacyanins were found to have higher stability at acidic pH (3–7) and less prone to oxidation, thanks to the glycosylated structure providing a higher oxidation–reduction potential [3–5]. There are a few main sources where betacyanins can be found, such as the red beetroot, cactus/prickly pear and red dragon fruit. Red dragon fruit is a commercial fruit and has high value to be further studied as it was found to have the highest betacyanins content (7.9–11.7 mg/mL) as compared to the red beetroot (5 mg/g) and cactus/prickly pear (0.393 mg/g) [6–8]. However, it is well known that the greatest limitation of betacyanins is the high degradation rate due to various factors such as heat, light, oxygen and water activity which in turn alter the structure, affecting the properties and functions of betacyanins, and subsequently compromise the quality of the final product [3]. Thus, appropriate preservation of betacyanins is a critical issue and of utmost importance in order to maximise their potential in serving as food colourants and functional bioactive components. In this context, various extraction approaches and downstream processing of betacyanins have been studied and proposed. These include certain conventional methods (such as maceration, Soxhlet extraction, hydrodistillation), microwave-assisted extraction, ultrasound-assisted extraction, supercritical fluid extraction, pulsed electric field extraction, spray drying and freeze drying [9]. Though these approaches are very different from each other, all of them have two common ultimate aims, which are to maximise the betacyanins concentration and to minimise the betacyanins degradation. In a past study [10], it had been proven that optimised fermentation technique was able to concentrate betanin in the red dragon fruit (*Hylocereus polyrhizus*) (RDF) drink to ten-fold higher amount (131.7 g/L) as compared to 11.7 g/L in normal red dragon fruit juice (RDFJ) reported by another study in 2009 [8]. Moreover, different from the RDFJ which only had the betacyanins (betanin and isobetanin) retention proportion of 0.35, the fermented red dragon fruit drink (FRDFD) stored at 25 °C was found to have a higher retention proportion of betacyanins (0.50). In other words, the FRDFD had better betacyanins stability than RDFJ [11].

Aside from that, due to the betacyanins' unstable properties, it is advised to apply betacyanins in short shelf-life foods that are produced under minimum heat treatment, packaged and marketed in a dry condition away from high oxygen levels. Though these recommendations seem effective, they are not a long-term solution and are particularly impractical for most food manufacturers who usually apply multi-use packaging or have specific requirements for thermal processing. Therefore, a more realistic and robust solution was suggested by including food additives like food hydrocolloids, chelating agents and antioxidants as stabilisers in the products containing betacyanins [12].

Food hydrocolloids are macromolecules with high molecular weights that play crucial roles as food ingredients/additives in the food science and technology field to improve the viscosity and quality of foods, thanks to their rheological and physical properties to serve as stabilisers, thickening and gelling agents. Nowadays, it is becoming more often and preferred for the use of binary hydrocolloids as the synergistic interaction among the hydrocolloid mixture can help to overcome the weakness of individual hydrocolloids, thereby achieving the desired functionality, quality and economic benefit of the end product [13,14]. For instance, xanthan gum (XG, a water-soluble, anionic polysaccharide composed of 1,4-linked β-D-glucose residues) and carboxymethyl cellulose (CMC, an anionic, water-soluble cellulose derivative) that were added together into different types of juices such as asparagus, orange, carrot and apple juices have been shown to increase the viscosity, maintain turbidity and suspension as well as to improve the stability of fruit juice particle in the dispersion [15–17]. Besides functioning as stabilisers in fruit juice, it has also become a dynamic field for using food hydrocolloids to protect the bioactive compounds and nutrients in the food industry through various mechanisms. In this context, anionic polysaccharides are particularly useful and effective in protecting cationic natural pigments like betacyanins. In the previous study [18], the incorporation of a hydrocolloid mixture solution of 0.30 % (w/v) XG and 0.50 % (w/v) CMC into FRDFD to produce improved fermented red dragon fruit drink (Improved-FRDFD-dH_2O) had shown promising results in reducing the degradation of betacyanins significantly from 60.55 % to 30.66 %.

Besides that, due to the matrix nature of Improved-FRDFD-dH_2O being a type of fermented fruit drink, the sample also contains some naturally present metal cations that can cause the degradation and reduction in the half-life of the betacyanins even at trace amounts, leading to possible decolouration of the Improved-FRDFD-dH_2O as compared to a pure betanin solution [19,20]. As such, food additives like ascorbic acid (AA) and citric acid (CA) may be a good option for stabilising betacyanins. Although AA was found to be effective in increasing the stability of betacyanins in past studies [4,21], the metal cations can to catalyse the oxidation of AA via molecular oxygen, therefore will reduce the effectiveness of AA [22]. As an alternative, CA can be added to enhance the stability of the betacyanins further.

To the best of our knowledge, research related to the effects of different concentrations of CA on betacyanins stability during long-term storage is still scarce and has had no new progress/update for a long time. Moreover, the viscosity of Improved-FRDFD-dH_2O (0.3 % XG and 0.5 % CMC) in the above mentioned study [18] (210.45 cP) was still below the desired commercial reference viscosity (308.30 cP) due to insufficient amount of XG and CMC added, and the betacyanins degradation rate (30.66 %) might not yet be fully minimised. Therefore, the present investigation aims to improve the previous study [18], whereby two different sets of hydrocolloid formulations comprised of different ratios of XG and CMC were proposed and studied with the intention to achieve the desired reference viscosity in the FRDFD. Subsequently, the present study, for the first time, included the CA at different concentrations in the selected best formulations of Improved-FRDFD-dH_2O in order to examine the enhancing effects of CA towards the stability of betacyanins and the physicochemical characteristics of the Improved-FRDFD-dH_2O. The present work is novel and unique in combining three approaches (that are rather mild, with less technical skills required and more applicable at the industrial scale in the future) together, which are: optimised fermentation, food hydrocolloids (XG and CMC) and food additive (CA) to concentrate and stabilise the red dragon fruit betacyanins throughout four weeks storage period at room temperature (25 °C). With these approaches, the present research successfully and significantly improved the stability of the red dragon fruit drink betacyanins (<15 % degradation). The Improved-FRDFD-dH_2O produced in this research can serve as a functional drink that contains high concentrations of betacyanins (which are proven to have high antioxidant activity) that are stable at room temperature for potential application as natural red colourants as well.

Table 1
Formulations of hydrocolloid mixture solutions.

Hydrocolloid Solution Formulations	XG Concentration (% w/v)	CMC Concentration (% w/v)
1	0.30	0.50
2	0.35	0.50
3	0.40	0.50
4	0.45	0.50
5	0.50	0.50
6	0.30	0.50
7	0.30	0.60
8	0.30	0.70
9	0.30	0.80
10	0.30	0.90

CMC, carboxymethyl cellulose; XG, xanthan gum; Final volume = 100 mL.

2. Materials and methods

2.1. Raw materials

Approximately 10 kg of fresh red dragon fruit (RDF) were bought from a local supermarket (Giant Hypermarket, Kuala Lumpur, Malaysia), and 1 kg of white fine sugar (Central Sugars Refinery, CSR) was purchased from a local market (99 Speedmart). Each RDF was selected with an average weight of 0.5–0.6 kg and ensured that it was fresh and free from physical defects (cracks, damages and cuts) and microbiological spoilage (dark blotches, sunken lesions and mouldy) based on observation. All the RDF were rinsed with tap water to remove any dirt and sand residues on the surface and followed by pat-dry with towel paper.

2.2. Chemicals

Chemicals used in this study were xanthan gum (food grade, CP Kelco, USA), carboxymethyl cellulose (food grade, ProFood Products Outlet, USA), citric acid, potassium sorbate (Scifex, Malaysia) and Reserve™ by Jeunesse Global®.

2.3. Fermented red dragon fruit drink (FRDFD) preparation

The optimised fermentation process was carried out using four 2-L airtight fermentation tanks based on previously published protocols [18]. All of the utensils used were sterilised with boiled water, and the whole process was conducted in a sterilised and clean condition to avoid contaminations. Briefly, the optimised fermentation was carried out by arranging the white fine sugar layer by layer alternately with the pieces of RDF slices in the ratio of 1:10, where both of the top and bottom layers were fully covered with the sugar. Then, the fermentation tank was closed tightly and stored in a clean environment for natural fermentation to be carried out for seven days at room temperature (25 °C). After seven days, the fermented red dragon fruit drink (FRDFD) was collected and filtered through a sterilised sieve bag. The pasteurisation of FRDFD was conducted at 75 °C for 15 s and the FRDFD was stored in a 500-mL Schott Duran® bottle.

2.4. Phase 1: Formulation of improved fermented red dragon fruit drink (Improved-FRDFD-dH_2O)

Different formulations of hydrocolloid mixture solutions of XG and CMC were prepared to mix with FRDFD to produce Improved-FRDFD-dH_2O.

2.4.1. XG and CMC hydrocolloid mixture solutions preparation

The XG and CMC hydrocolloid mixture solution were prepared according to previously published procedures [18] with slight modifications. Ten formulations of hydrocolloid mixture solutions were prepared with different concentrations of XG (0.30–0.50 %, w/v) and CMC (0.50–0.90 %, w/v), as shown in Table 1. Formulations 1 to 5 were using a fixed amount of CMC (0.50 %) with varying amounts of XG while formulations 6 to 10 were using a fixed amount of XG (0.30 %) at varying concentrations of CMC.

2.4.2. Formation of improved fermented red dragon fruit drink (Improved-FRDFD-dH_2O)

Ten formulations of Improved-FRDFD-dH_2O were formed by mixing the FRDFD with each of the ten formulations of hydrocolloid mixture solutions in the ratio of 1:2. The diluted FRDFD (FRDFD-dH_2O) was prepared for each set of formulation as the control by mixing FRDFD with distilled water in the ratio of 1:2. 0.10 % (w/v) of potassium sorbate was added and stirred at 1000 rpm for 5 min in all samples. Next, pasteurisation was performed for all samples at 75 °C for 15 s and cooled down to room temperature under running tap water, followed by conducting physiochemical analyses for all samples which include viscosity, betacyanin concentration, pH, water activity and total soluble solids [18]. Two formulations that had the closest viscosities to the reference product, Reserve™ by Jeunesse Global® (308.3 ± 0.99 cP) were selected for the next Phase 2 storage stability study.

Table 2
Different formulations of red dragon fruit samples for Phase 2 storage stability study.

Samples	FRDFD (mL)	Hydrocolloid solution (mL)	Distilled water (mL)	Citric acid (% w/v)
FRDFD-dH_2O	150	–	300	–
Improved-FRDFD-dH_2O				
Formulation 3A	150	300 (Formulation 3)	–	0.00
Formulation 3B	150	300 (Formulation 3)	–	0.05
Formulation 3C	150	300 (Formulation 3)	–	0.10
Formulation 3D	150	300 (Formulation 3)	–	0.15
Formulation 3E	150	300 (Formulation 3)	–	0.20
Formulation 10A	150	300 (Formulation 10)	–	0.00
Formulation 10B	150	300 (Formulation 10)	–	0.05
Formulation 10C	150	300 (Formulation 10)	–	0.10
Formulation 10D	150	300 (Formulation 10)	–	0.15
Formulation 10E	150	300 (Formulation 10)	–	0.20

FRDFD-dH_2O, Diluted fermented red dragon fruit drink; Improved-FRDFD-dH_2O, Improved fermented red dragon fruit drink; Final volume = 450 mL.

2.5. Phase 2: Storage stability study of Improved-FRDFD-dH_2O (containing betacyanins) with citric acid (CA)

The best formulations (3 and 10) closest to the reference product were selected for the Phase 2 storage stability study with CA. The control (FRDFD-dH_2O) and five samples of each best formulation of Improved-FRDFD-dH_2O were prepared with the same procedure described in Section 2.4 but in a larger volume (450 mL) until the step before adding potassium sorbate. The CA was weighed and added into the Improved-FRDFD-dH_2O at different concentrations (0–0.20 %, w/v) according to Table 2 and stirred at the speed of 1000 rpm at room temperature with a magnetic stirrer. Subsequently, 0.10 % (w/v) of potassium sorbate was added and stirred at 1000 rpm for 5 min in all samples, followed by pasteurisation (75 °C for 15 s) and cooled down to room temperature. All the samples were transferred and stored in the 100-mL Schott Duran® bottles under dark and clean conditions at room temperature (25 °C) for four weeks. All the samples were subjected to the same physiochemical analyses at one week intervals, starting from Week 0 to Week 4.

2.6. Physicochemical and betacyanins content analysis

In both phases (Sections 2.4 and 2.5), the analyses of betacyanins content and four physicochemical characteristics were conducted on all the samples and controls.

2.6.1. Viscosity

The viscosity of the samples was measured using a viscometer (Brookfield Model DV-11+Pro, USA) according to previously published procedures [18].

2.6.2. Betacyanins concentration

The betacyanins concentration of the samples was quantified using a UV–visible spectrophotometer (Secomam Model UviLine 9400, Germany). All the samples were subjected to 20x dilution with distilled water, followed by taking the measurements at 538 nm for each sample (distilled water was used as zero blank). The betacyanins concentration was calculated according to Equation (1) [18].

$$B_c = (A \times DF \times MW \times 1000) \,/\, (\varepsilon \times L) \quad (1)$$

Where B_C is the betacyanin concentration in milligrams per litre (mg/L); A is the absorption value at λ_{max} (538 nm); DF is the dilution factor; MW is the molecular weight of betacyanin (550 g/mol); ε is the molar extinction coefficient of betacyanin (60000 L/mol × cm); L is the path length of the cuvette (1 cm).

2.6.3. pH

The pH meter's calibration was first performed with pH 7.0 and pH 4.0 buffer solutions prior to the analysis of samples. The pH of the samples was measured using a pH meter (Jenway Model 3505, UK) according to previously published protocols [18].

2.6.4. Water activity (a_w)

The water activity of the samples was determined using an electronic dew-point water activity meter (AquaLab Pre Benchtop Water Activity Meter, USA) at 25 °C. Measurements were taken in triplicate based on previously published procedures [18].

2.6.5. Total soluble solids (TSS)

The total soluble solids (TSS) of the samples were measured using a handheld refractometer with detection degree Brix (°Brix) range of 0–32 °Brix (Fisher Scientific, USA) [18].

Table 3
Viscosity (cP) of two different sets of hydrocolloids (XG and CMC) formulations of Improved-FRDFD-dH_2O and the control FRDFD-dH_2O.

CMC Concentration (% w/v)	XG Concentration (% w/v)					FRDFD-dH_2O
	0.30	0.35	0.40	0.45	0.50	
0.50	220.46 ± 14.44^{e}	274.15 ± 14.25^{d}	316.46 ± 13.94^{c}	362.28 ± 14.72^{b}	403.73 ± 13.83^{a}	1.55 ± 0.13^{f}
XG Concentration (% w/v)	**CMC Concentration (% w/v)**					**FRDFD-dH_2O**
	0.50	**0.60**	**0.70**	**0.80**	**0.90**	
0.30	222.25 ± 14.15^{d}	243.2 ± 14.40^{cd}	261.65 ± 11.75^{bc}	285.60 ± 9.90^{ab}	308.40 ± 10.40^{a}	1.65 ± 0.10^{e}

Data is represented as mean ± standard deviation values (n = 2). ^{ab}Mean value with different superscript in each row differs significantly ($p < 0.05$). CMC, carboxymethyl cellulose; XG, xanthan gum; FRDFD-dH_2O, diluted fermented red dragon fruit drink (as control).

Table 4
Betacyanins concentration (mg/L) of two different sets of hydrocolloids (XG and CMC) formulations of Improved-FRDFD-dH_2O and also the control FRDFD-dH_2O.

CMC Concentration (% w/v)	XG Concentration (% w/v)					FRDFD-dH_2O
	0.30	0.35	0.40	0.45	0.50	
0.50	83.99 ± 2.30^{a}	83.64 ± 2.52^{a}	85.60 ± 1.28^{a}	88.59 ± 2.01^{a}	90.70 ± 0.80^{a}	88.27 ± 2.03^{a}
XG Concentration (% w/v)	**CMC Concentration (% w/v)**					**FRDFD-dH_2O**
	0.50	**0.60**	**0.70**	**0.80**	**0.90**	
0.30	85.39 ± 3.59^{a}	86.30 ± 2.62^{a}	86.95 ± 1.05^{a}	88.69 ± 2.23^{a}	90.98 ± 1.90^{a}	90.06 ± 1.96^{a}

Data is represented as mean ± standard deviation values (n = 2). ^{ab}Mean value with different superscript in each row differs significantly ($p < 0.05$). CMC, carboxymethyl cellulose; XG, xanthan gum; FRDFD-dH_2O, diluted fermented red dragon fruit drink (as control).

2.7. Statistical analysis

All the experimental results were analysed using IBM SPSS Statistics Software (SPSS Version 25). All the numerical data were measured in triplicate with replicate samples (n = 2) and expressed as mean ± standard deviations. One-way analysis of variance (ANOVA) with Tukey's test was applied to analyse the significant difference ($p < 0.05$) between means.

3. Results and discussion

3.1. Phase 1: Formulation of improved fermented red dragon fruit drink (Improved-FRDFD-dH_2O)

3.1.1. Viscosity

It has been proposed that the production of an improved functional drinks is possible via the application of novel technologies, such as addition of food hydrocolloids. The effects of the addition of different concentrations of XG and CMC towards the viscosity of the samples are shown in Table 3.

Table 3 shows that the viscosities of all the formulations of Improved-FRDFD-dH_2O were significantly different from the control FRDFD-dH_2O, in which the viscosity of the Improved-FRDFD-dH_2O increased with increasing concentration of XG or CMC added. This phenomenon can be explained by the higher concentration of XG or CMC provided a higher number of hydroxyl (-OH) groups to interact, collide and form hydrogen bonds with the water molecules in the drink, therefore leading to a rapid rise in the viscosity [17,23,24]. At fixed 0.50 % CMC, increasing XG concentration from 0.30 to 0.50 % successfully elevated the viscosity of the drink between 142-fold and 260-fold (from 1.55 cP to minimally 220.46 cP and maximally 403.73 cP). Meanwhile, the incorporation of hydrocolloid mixture solution of 0.50–0.90 % CMC at fixed 0.30 % XG possessed a considerable effect of 134-fold–186-fold increase in the drink's viscosity (from 1.65 cP to minimally 222.25 cP and maximally 308.40 cP). This inferred that the percentage increment of the drink's viscosity was higher when increasing XG concentration compared to that of CMC. The present results are in accordance with the previous studies [15,18], which also revealed that XG can produce a higher viscosity sample (apple juice and Improved-FRDFD-dH_2O, respectively) at lower concentration compared to that of CMC. The reasons behind this could be due to the XG being a type of branched hydrocolloid that consists of a higher number and longer branches allowing it to form more hydrogen bonds with the water molecules in the drink, thereby able to produce a more viscous sample. In comparison, CMC has fewer branches and mainly depends on the degree of polymerisation of CMC for a greater hydration effect. Since the degree of polymerisation is affected by the mass fraction of CMC, a higher concentration of CMC is usually needed to reach the critical concentration (molecular weight and intrinsic viscosity increase) in order for molecules to start interacting with each other and thereby producing a significantly higher viscosity product [13,24]. In the previous study [18], the viscosity of Improved-FRDFD-dH_2O was still below the reference viscosity. In the present study, both sets of hydrocolloids formulations with relatively higher concentrations of XG and CMC have effectively produced higher viscosities of Improved-FRDFD-dH_2O compared to that in the past study [18]. Two best formulations that have the closest viscosity to the

Table 5
pH values of two different sets of hydrocolloids (XG and CMC) formulations of Improved-FRDFD-dH_2O and also the control FRDFD-dH_2O.

CMC Concentration (% w/v)	XG Concentration (% w/v)					FRDFD-dH_2O
	0.30	0.35	0.40	0.45	0.50	
0.50	4.94 ± 0.01[a]	4.95 ± 0.00[a]	4.95 ± 0.01[a]	4.95 ± 0.01[a]	4.96 ± 0.02[a]	4.71 ± 0.01[b]
XG Concentration (% w/v)	**CMC Concentration (% w/v)**					**FRDFD-dH_2O**
	0.50	**0.60**	**0.70**	**0.80**	**0.90**	
0.30	4.85 ± 0.02[a]	4.85 ± 0.02[a]	4.85 ± 0.00[a]	4.87 ± 0.01[a]	4.87 ± 0.01[a]	4.71 ± 0.01[b]

Data is represented as mean ± standard deviation values (n = 2). [ab]Mean value with different superscript in each row differs significantly ($p < 0.05$). CMC, carboxymethyl cellulose; XG, xanthan gum; FRDFD-dH_2O, diluted fermented red dragon fruit drink (as control).

Table 6
Water activity of two different sets of hydrocolloids (XG and CMC) formulations of Improved-FRDFD-dH_2O and also the control FRDFD-dH_2O.

CMC Concentration (% w/v)	XG Concentration (% w/v)					FRDFD-dH_2O
	0.30	0.35	0.40	0.45	0.50	
0.50	0.975 ± 0.001[b]	0.974 ± 0.000[b]	0.973 ± 0.002[b]	0.969 ± 0.001[c]	0.968 ± 0.002[c]	0.982 ± 0.001[a]
XG Concentration (% w/v)	**CMC Concentration (% w/v)**					**FRDFD-dH_2O**
	0.50	**0.60**	**0.70**	**0.80**	**0.90**	
0.30	0.975 ± 0.001[b]	0.975 ± 0.002[b]	0.974 ± 0.002[b]	0.970 ± 0.002[bc]	0.968 ± 0.003[c]	0.983 ± 0.001[a]

Data is represented as mean ± standard deviation values (n = 2). [ab]Mean value with different superscript in each row differs significantly ($p < 0.05$). CMC, carboxymethyl cellulose; XG, xanthan gum; FRDFD-dH_2O, diluted fermented red dragon fruit drink (as control).

Table 7
Total soluble solids (°Brix) of two different sets of hydrocolloids (XG and CMC) formulations of Improved-FRDFD-dH_2O and also the control FRDFD-dH_2O.

CMC Concentration (% w/v)	XG Concentration (% w/v)					FRDFD-dH_2O
	0.30	0.35	0.40	0.45	0.50	
0.50	7.00 ± 0.10[a]	7.00 ± 0.00[a]	7.00 ± 0.10[a]	7.00 ± 0.10[a]	7.00 ± 0.00[a]	6.40 ± 0.10[b]
XG Concentration (% w/v)	**CMC Concentration (% w/v)**					**FRDFD-dH_2O**
	0.50	**0.60**	**0.70**	**0.80**	**0.90**	
0.30	6.80 ± 0.00[a]	6.80 ± 0.10[a]	6.90 ± 0.10[a]	6.90 ± 0.00[a]	6.90 ± 0.10[a]	6.50 ± 0.10[b]

Data is represented as mean ± standard deviation values (n = 2). [ab]Mean value with different superscript in each row differs significantly ($p < 0.05$). CMC, carboxymethyl cellulose; XG, xanthan gum; FRDFD-dH_2O, diluted fermented red dragon fruit drink (as control).

reference product, Reserve™ by Jeunesse Global® (308.3 ± 0.99 cP) were successfully produced and identified to be comprised of two different ratios of XG and CMC for the Phase 2 storage stability study, which are Formulation 3 (0.40 % XG + 0.50 % CMC, viscosity: 316.46 ± 13.94 cP) and Formulation 10 (0.30 % XG + 0.90 % CMC, viscosity: 308.40 ± 10.40 cP).

3.1.2. Betacyanins concentration

The different concentrations of hydrocolloids towards the betacyanins for the Improved-FRDFD-dH_2O in this study are depicted in Table 4. It can be seen that all the formulations of Improved-FRDFD-dH_2O have no significant difference from each other and also to the control FRDFD-dH_2O in terms of betacyanins concentration. The current results are in line with the previous study [18], which revealed no significant changes in the betacyanins content after the addition of 0.15–0.30 % XG and 0.30–0.50 % CMC solutions into the FRDFD. This indicates that the addition of XG and CMC, even at higher concentrations in the present study, also did not exert any considerable negative effects on the betacyanins content in the FRDFD. Furthermore, it can be seen that the maximum degradation rate of the betacyanins content among all the formulations was only 5.25 % (from 88.27 mg/L to 83.64 mg/L) in the present study. This minor degradation value is relatively lower as compared to the previous study which reported 15.16 % of betacyanins degraded after employing the freeze-drying technique to encapsulate the RDF peel betacyanins extract with different ratios of polysaccharides including CMC [25].

3.1.3. pH, water activity (a_w) and total soluble solids (TSS)

The pH, water activity and total soluble solids (TSS) of two different sets of formulations of Improved-FRDFD-dH_2O and the control FRDFD-dH_2O were measured and shown in Table 5, Table 6 and Table 7, respectively.

In Table 5, it can be seen that the increase of either XG (0.30–0.50 %) or CMC (0.50–0.90 %) concentration (at fixed 0.50 % CMC or

Table 8
Viscosity (cP) of selected two formulations of Improved-FRDFD-dH_2O with different concentrations of CA and control FRDFD-dH_2O over 4-week storage period at 25 °C.

Improved-FRDFD-dH_2O	Storage Period (Week)	Citric Acid Concentration (%)					FRDFD-dH_2O
		A-0.00	B-0.05	C-0.10	D-0.15	E−0.20	
Formulation 3	**0**	315.40 ± 18.50^{aA}	316.80 ± 18.80^{aA}	314.45 ± 18.00^{aA}	317.30 ± 19.00^{aA}	316.71 ± 17.02^{aA}	2.62 ± 0.54^{aB}
	1	311.10 ± 19.90^{aA}	313.3 ± 19.00^{aA}	310.80 ± 20.00^{aA}	315.90 ± 19.50^{aA}	314.80 ± 18.80^{aA}	2.31 ± 0.61^{aB}
	2	306.70 ± 15.50^{aA}	309.82 ± 17.20^{aA}	308.10 ± 17.40^{aA}	311.40 ± 15.50^{aA}	311.13 ± 25.00^{aA}	1.95 ± 0.60^{aB}
	3	302.10 ± 16.80^{aA}	306.16 ± 22.70^{aA}	305.70 ± 15.30^{aA}	308.90 ± 19.50^{aA}	308.92 ± 18.92^{aA}	1.88 ± 0.42^{aB}
	4	300.30 ± 17.00^{aA}	303.68 ± 16.32^{aA}	302.87 ± 16.53^{aA}	306.40 ± 16.00^{aA}	306.52 ± 20.10^{aA}	1.67 ± 0.50^{aB}
Formulation 10	**0**	308.15 ± 17.75^{aA}	310.90 ± 17.20^{aA}	307.65 ± 17.50^{aA}	310.40 ± 17.00^{aA}	309.65 ± 17.35^{aA}	2.40 ± 0.45^{aB}
	1	303.40 ± 17.00^{aA}	308.15 ± 18.10^{aA}	305.90 ± 23.50^{aA}	307.65 ± 16.35^{aA}	307.15 ± 15.90^{aA}	2.10 ± 0.55^{aB}
	2	299.65 ± 19.90^{aA}	303.90 ± 16.90^{aA}	301.40 ± 23.00^{aA}	303.40 ± 17.90^{aA}	304.15 ± 16.90^{aA}	1.90 ± 0.30^{aB}
	3	295.40 ± 15.90^{aA}	299.65 ± 20.15^{aA}	298.90 ± 19.40^{aA}	300.90 ± 24.00^{aA}	301.15 ± 21.90^{aA}	1.70 ± 0.26^{aB}
	4	293.15 ± 11.90^{aA}	297.65 ± 16.95^{aA}	295.40 ± 15.50^{aA}	299.40 ± 20.10^{aA}	298.90 ± 18.50^{aA}	1.60 ± 0.75^{aB}

Data is represented as mean ± standard deviation values (n = 2). ^{ab}Mean value with different superscript in each column of each Improved-FRDFD-dH_2O formulation differs significantly ($p < 0.05$). ^{AB}Mean value with different superscript in each row differs significantly ($p < 0.05$). Improved-FRDFD-dH_2O, Improved Fermented Red Dragon Fruit Drink; FRDFD-dH_2O, diluted fermented red dragon fruit drink (as control); Formulation 3, 0.40 % XG + 0.50 % CMC; Formulation 10, 0.30 % XG + 0.90 % CMC.

0.30 % XG) did not lead to a significant change in the pH of the Improved-FRDFD-dH_2O. However, in both formulation sets, the pH values of all the Improved-FRDFD-dH_2O were significantly higher than the control FRDFD-dH_2O. The increase in the pH might be due to the dilution of FRDFD with higher concentrations of hydrocolloid mixture solution of XG and CMC, which might have higher pH values (5–6.11 and 6.77–8.25, respectively) than the distilled water [14,26,27]. A similar trend was observed in the previous study [28] where the pH of the tomato-carrot juice was increased following the addition of hydrocolloid mixture solution of XG and CMC. Nonetheless, all the pH values of the formulations (4.85–4.96) were under the recommended pH range (<5) to inhibit the microbial growth for better preservation of acid food products and at the same time are still within the pH range of betacyanins (pH 3.5–7.0), XG (pH 3–10) and CMC (pH 4–10) for optimum stability and functionality [29–31].

The water activity of all the formulations of Improved-FRDFD-dH_2O (that are with relatively higher concentrations range of XG and CMC) in the current study were significantly different from the control FRDFD-dH_2O (Table 6). The results obtained agree with the previously published studies [32,33], whereby the incorporation of hydrocolloids will affect the competition for the water between the solutes. As the hydrocolloid concentrations increased, more hydroxyl groups were present, which subsequently increased the competition between the added hydrocolloids and the hydrophilic molecules in the drink, thereby inter-chain bonding promoted and significantly decreased the water activity in the fruit juice or filling [17,23]. Nonetheless, the water activity range (0.968–0.975) of all the Improved-FRDFD-dH_2O formulations in this study remained near to that of a standard fruit juice/drink, around 0.97 [34].

The addition of two different sets of hydrocolloids (XG and CMC) solution formulations into the FRDFD led to significantly higher TSS content in the Improved-FRDFD-dH_2O than in the FRDFD-dH_2O control, as shown in Table 7. This can be explained by the hydrocolloids in all the Improved-FRDFD-dH_2O that have elevated the solid fraction in the drink, thereby increasing TSS levels [32]. Similarly, a study reported in 2015 [28] also found that the addition of hydrocolloids XG and CMC could cause slight increases in the TSS of the tomato-carrot juice. Nevertheless, the TSS values of all the formulations of Improved-FRDFD-dH_2O (6.80–7.00 °Brix) had met the minimum TSS level as the RDF itself (6.00–8.20 °Brix), which enabled the Improved-FRDFD-dH_2O to be considered as a reconstituted juice based on the General Standard for Fruit Juices and Nectars (CODEX STAN 247–2005) [35–37].

3.2. Phase 2: Storage stability study of Improved-FRDFD-dH_2O (containing betacyanins) with citric acid

3.2.1. Viscosity over the storage period

The changes of viscosity in the two selected formulations of Improved-FRDFD-dH_2O with different concentrations of CA and control (FRDFD-dH_2O) over four weeks of storage period at 25 °C are shown in Table 8. Based on the obtained results, the viscosities of all the samples of Formulations 3 and 10 of Improved-FRDFD-dH_2O (ranging from 293.15 cP to 317.30 cP) were similar to those in Phase 1 in which all were higher than the FRDFD-dH_2O control (1.60–2.62 cP) throughout the whole storage study. Although a relatively higher concentration of XG, CMC and CA were added into the FRDFD in the present study, all samples of Formulations 3 and 10 of Improved-FRDFD-dH_2O and the FRDFD-dH_2O control had no significant change in terms of viscosity throughout the 4-week storage period.

Table 9
Betacyanins concentration (mg/L) of selected two formulations of Improved-FRDFD-dH_2O with different concentrations of CA and control FRDFD-dH_2O over 4-week storage period at 25 °C.

Improved-FRDFD-dH_2O	Storage Period (Week)	Citric Acid Concentration (%)					FRDFD-dH_2O
		A-0.00	B-0.05	C-0.10	D-0.15	E−0.20	
Formulation 3	**0**	101.39 ± 1.01^{aC}	116.63 ± 3.90^{aB}	121.18 ± 4.92^{aB}	127.23 ± 2.23^{aAB}	133.47 ± 7.53^{aA}	102.08 ± 3.92^{aC}
	1	95.69 ± 2.31^{bD}	111.57 ± 5.07^{abC}	116.74 ± 1.10^{abBC}	123.36 ± 2.50^{aAB}	130.46 ± 2.69^{aA}	95.77 ± 2.77^{aD}
	2	88.87 ± 2.13^{cD}	104.56 ± 3.96^{bcC}	109.93 ± 3.10^{bcBC}	116.29 ± 1.01^{bAB}	123.28 ± 4.40^{abA}	71.65 ± 1.15^{bE}
	3	81.86 ± 0.80^{dD}	96.89 ± 2.01^{cC}	102.91 ± 2.41^{cdBC}	109.22 ± 3.30^{cAB}	115.73 ± 4.62^{bA}	55.78 ± 1.00^{cE}
	4	77.56 ± 1.94^{dD}	94.75 ± 4.05^{cC}	100.87 ± 1.83^{dBC}	107.14 ± 1.50^{cAB}	113.82 ± 3.72^{bA}	40.65 ± 1.82^{dE}
Percentage Change		−23.50 %	−18.77 %	−16.76 %	−15.79 %	−14.72 %	−60.18 %
Formulation 10	**0**	103.86 ± 1.91^{aAB}	107.43 ± 3.22^{aAB}	110.28 ± 5.50^{aAB}	112.66 ± 2.23^{aAB}	114.58 ± 7.10^{aA}	101.12 ± 3.50^{aB}
	1	97.35 ± 4.20^{abBC}	102.12 ± 1.91^{abAB}	105.14 ± 3.09^{abAB}	107.89 ± 2.00^{abA}	109.18 ± 2.30^{abA}	92.23 ± 3.03^{bC}
	2	90.48 ± 1.54^{bcB}	96.16 ± 4.13^{bcAB}	98.27 ± 4.18^{bcA}	116.29 ± 1.01^{bcA}	101.13 ± 1.50^{bcA}	79.85 ± 1.20^{cC}
	3	84.61 ± 3.10^{cdC}	90.66 ± 1.68^{cdBC}	93.87 ± 1.20^{cAB}	109.22 ± 3.30^{cdAB}	98.45 ± 2.22^{cA}	57.84 ± 2.05^{dD}
	4	79.02 ± 2.55^{dC}	86.35 ± 3.37^{dB}	89.93 ± 1.00^{cAB}	107.14 ± 1.50^{dAB}	95.43 ± 3.26^{cA}	42.16 ± 1.32^{eD}
Percentage Change		−23.92 %	−19.62 %	−18.45 %	−17.42 %	−16.71 %	−58.31 %

Data is represented as mean ± standard deviation values (n = 2). ^{ab}Mean value with different superscript in each column of each Improved-FRDFD-dH_2O formulation differs significantly ($p < 0.05$). ^{AB}Mean value with different superscript in each row differs significantly ($p < 0.05$). Improved-FRDFD-dH_2O, Improved Fermented Red Dragon Fruit Drink; FRDFD-dH_2O, diluted fermented red dragon fruit drink (as control); Formulation 3, 0.40 % XG + 0.50 % CMC; Formulation 10, 0.30 % XG + 0.90 % CMC.

3.2.2. Betacyanins concentration over the storage period

The status of the betacyanins concentration in the two selected formulations of Improved-FRDFD-dH_2O with different concentrations of CA and control FRDFD-dH_2O over four weeks of storage period at 25 °C is the most pivotal aspect to be analysed and studied in the present work, in which the results are shown in Table 9. According to Table 9 and it can be seen that at Week 0, both Formulations 3A and 10A of Improved-FRDFD-dH_2O had similar results as in Phase 1 in which there was no significant difference found in the betacyanins content compared to the FRDFD-dH_2O control. In the present study, the FRDFD-dH_2O control suffered the highest betacyanins loss (58.31–60.18 %) during the 4-week storage stability study at room temperature, which corroborated with the highest degradation rate of 60.55 % for betacyanins previously observed for FRDFD-dH_2O control [18]. By comparison, the betacyanins content in Formulations 3A and 10A had no significant change from Week 3 to Week 4, compared to the FRDFD-dH_2O, which experienced a significant reduction in betacyanins concentration each week. In addition, Formulations 3A and 10A significantly reduced the betacyanins degradation from 60.18 % to 23.15 % and from 58.31 % to 23.92 %, respectively. This demonstrated that the higher concentrations of XG and CMC in Formulation 3A and 10A in the present study had successful in further reducing the betacyanins degradation as compared to the previous study that showed a relatively higher loss (30.66 %) of betacyanins in the Improved-FRDFD-dH_2O added with 0.30 % XG and 0.50 % CMC solution [18]. This is because the higher concentrations of XG and CMC had further elevated the viscosity by binding with more water molecules to resist their movement, thereby further reducing the rate of betacyanins aldimine bond cleavage/hydrolysis caused by the free-moving water molecules [3]. At higher concentrations of XG and CMC, more anionic charge carboxyl groups could be contributed by both hydrocolloids to bind with the free cationic betacyanins and this pigment-polysaccharide association can therefore prevent more betacyanins from being attacked by water. Usually, at low concentrations, the hydrocolloids may not be able to cover the entire surface of the droplets, leading to coalescence and instability. However, the higher concentrations of XG and CMC in Formulations 3 and 10 of this study are believed to have a better electrostatic repulsion effect between both hydrocolloids and wider coverage in the aqueous phase of Improved-FRDFD-dH_2O when forming the three-dimensional network. This could then result in a more stable network (to impede the droplet migration), better compartmentation effect and more betacyanins can be physically trapped and protected from oxidation in the hydrocolloid matrices throughout the storage period [12,18].

In general, the betacyanins degradation rates of all the samples of Formulation 3 of Improved-FRDFD-dH_2O (ranging from 14.72 % to 23.50 %) are lower compared to that of Formulation 10 (ranging from 16.71 % to 23.92 %). This could be due to the slight dysfunctional of the less stable CMC in all the samples of Formulation 10 (that are with higher CMC concentration, 0.90 % and lower XG concentration, 0.30 %), which subsequently led to slightly higher reduction rates in the viscosity and pH levels throughout the storage period (as shown in Sections 3.2.1 and 3.2.3), thus unable to provide greater protection to betacyanins compared to the samples of Formulation 3 (that are with lower CMC concentration, 0.50 % but higher XG concentration, 0.40 %) [18,24,27,28,38].

Table 10
pH of selected two formulations of Improved-FRDFD-dH_2O with different concentrations of CA and control FRDFD-dH_2O over 4-week storage period at 25 °C.

Improved-FRDFD-dH_2O	Storage Period (Week)	Citric Acid Concentration (%)					FRDFD-dH_2O
		A-0.00	B-0.05	C-0.10	D-0.15	E−0.20	
Formulation 3	**0**	4.86 ± 0.01^{aA}	4.64 ± 0.04^{aB}	4.53 ± 0.02^{aC}	4.39 ± 0.05^{aD}	4.29 ± 0.04^{aE}	4.51 ± 0.02^{aC}
	1	4.83 ± 0.01^{abA}	4.62 ± 0.01^{abB}	4.51 ± 0.01^{abC}	4.38 ± 0.01^{aD}	4.27 ± 0.02^{aE}	4.08 ± 0.03^{bF}
	2	4.80 ± 0.02^{bcA}	4.59 ± 0.01^{bcB}	4.48 ± 0.02^{bcC}	4.34 ± 0.01^{abD}	4.22 ± 0.05^{abE}	4.00 ± 0.01^{cF}
	3	4.78 ± 0.02^{cA}	4.55 ± 0.00^{cdB}	4.45 ± 0.01^{cdC}	4.31 ± 0.02^{bD}	4.20 ± 0.02^{bE}	3.92 ± 0.03^{dF}
	4	4.72 ± 0.03^{dA}	4.52 ± 0.01^{dB}	4.42 ± 0.02^{dC}	4.29 ± 0.06^{bD}	4.20 ± 0.01^{bE}	3.80 ± 0.00^{eF}
Percentage Change		−2.88 %	−2.59 %	−2.42 %	−2.27 %	−2.10 %	−15.74 %
Formulation 10	**0**	4.63 ± 0.01^{aA}	4.47 ± 0.01^{aB}	4.36 ± 0.00^{aD}	4.24 ± 0.00^{aE}	4.15 ± 0.00^{aF}	4.45 ± 0.01^{aC}
	1	4.60 ± 0.00^{bA}	4.45 ± 0.01^{abB}	4.33 ± 0.03^{aC}	4.22 ± 0.02^{aD}	4.13 ± 0.01^{aF}	4.18 ± 0.01^{bE}
	2	4.53 ± 0.02^{cA}	4.42 ± 0.03^{bcB}	4.29 ± 0.01^{bC}	4.17 ± 0.03^{abD}	4.10 ± 0.03^{abE}	4.01 ± 0.00^{cF}
	3	4.49 ± 0.01^{dA}	4.39 ± 0.01^{cdB}	4.27 ± 0.01^{bcC}	4.16 ± 0.01^{bD}	4.07 ± 0.03^{bE}	3.86 ± 0.01^{dF}
	4	4.46 ± 0.02^{eA}	4.37 ± 0.02^{dB}	4.25 ± 0.02^{cC}	4.14 ± 0.01^{bD}	4.06 ± 0.02^{bE}	3.78 ± 0.02^{eF}
Percentage Change		−3.67 %	−2.24 %	−2.52 %	−2.36 %	−2.17 %	−15.06 %

Data is represented as mean ± standard deviation values (n = 2). ^{ab}Mean value with different superscript in each column of each Improved-FRDFD-dH_2O formulation differs significantly ($p < 0.05$). ^{AB}Mean value with different superscript in each row differs significantly ($p < 0.05$). Improved-FRDFD-dH_2O, Improved Fermented Red Dragon Fruit Drink; FRDFD-dH_2O, diluted fermented red dragon fruit drink (as control); Formulation 3, 0.40 % XG + 0.50 % CMC; Formulation 10, 0.30 % XG + 0.90 % CMC.

Besides that, previous study [12] also suggested that the relatively linear conformation of the XG compared to other food hydrocolloids provided more opportunities for stable intramolecular interactions (such as hydrophobic associations) together with intermolecular hydrogen bonding, thus formulation with higher XG concentration is expected to give lower steric hindrances to the betacyanins pigments.

For the first time, CA is added together with the hydrocolloid mixture solution of XG and CMC to examine the combined stabilisation effect on the betacyanins that were concentrated from fermentation. At Week 0, significant increments in the betacyanins content were observed in both Formulations 3 and 10 of Improved-FRDFD-dH_2O following the addition of CA (0–0.20 %) at increasing concentration, as shown in Table 9. This could be due to the acidification effect caused by the CA, which had led to the recondensation of betalamic acid and cyclo-Dopa-5-*O*-β-glucoside, which then formed more betacyanins pigments in the samples [3]. During 4-week storage at 25 °C, all Formulations 3 and 10 (B to E) of Improved-FRDFD-dH_2O added with CA had lower betacyanins loss (ranging from 14.72 % to 18.77 % and 16.71 %–19.62 %, respectively) than that of Formulations 3A and 10A which were without CA (23.50 % and 23.92 % of betacyanins loss, respectively). It can be observed that there is a decreasing trend in the betacyanins degradation as the concentration of CA increased from 0.05 % to 0.20 % for both Formulations 3 and 10. Different from the present study's results, previously, it was found that the addition of 0.01 % and 0.10 % of CA had no impact on betacyanins stability [20]. This could be due to the concentration of CA applied in that study being too low which led to the inconsiderable protective effect of CA on betacyanins. The positive protective results shown in the present research are supported and in line with the previously published studies that showed that the addition of 1 % CA had increased the half-life of betanin by 1.5 times [39] and significantly retained the betacyanins content by approximately 55 % [4].

From Table 9 and it can be observed that only Formulations 3E (with 0.20 % CA) of Improved-FRDFD-dH_2O maintained the slightest significant changes in betacyanins concentration throughout the four weeks of storage study at room temperature. The protective effect of CA presented in this study is attributable to a number of functions and mechanisms. Firstly, the CA, being a good pH regulator and pH buffering agent has helped in resisting drastic pH change (as discussed in Section 3.2.3) that can lead to the instability of betacyanins in the drink [40,41]. Besides that, as mentioned before, the stability of the betacyanins could be impaired by some metal cations such as Al^{3+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+} and Sn^{2+} that are naturally present in the food constituents and matrix of the Improved-FRDFD-dH_2O through reducing the half-life of the betacyanins significantly [19,20]. The addition of CA into the Improved-FRDFD-dH_2O in the present study helped to further enhance the stability of the betacyanins by serving as a metal cation chelating agent to bind with the metal cations [3,19,22]. Moreover, it has been suggested that the CA can provide sequestering effects by partially neutralising the electrophilic centre of betacyanins via the association around the quaternary amino nitrogen that is positively charged, thereby blocking the nucleophilic attack of water molecules towards the betacyanins [39,42]. In addition to that, the incorporation of CA into the Improved-FRDFD-dH_2O might have also led to the esterification of aliphatic acid (CA) with betacyanins which subsequently improved the betacyanins stability, thanks to the protection provided by the aliphatic acid moieties to the aldimine bond to reduce its susceptibility to hydrolytic cleavage [3]. Furthermore, as CA is a well-known green crosslinking agent, minor crosslinking of the polysaccharides XG and CMC with the aid of CA might have occurred in the Improved-FRDFD-dH_2O via the interaction and attachment of the polyfunctional carboxyl groups of the CA with the hydroxyl groups of XG and CMC in two esterification reactions during the minor heating process (pasteurisation of the samples). This has then helped in further enhance the strength and stability of the abovementioned three-dimensional network/matrix formed by the XG and CMC in the aqueous phase, thereby providing more stable protection to the betacyanins in the Improved-FRDFD-dH_2O [43]. However, optimising the concentrations of each hydrocolloid and CA used is crucial to foster and achieve optimum synergising effects on stabilising the betacyanins. In

Table 11
Water activity of selected two formulations of Improved-FRDFD-dH_2O with different concentrations of CA and control FRDFD-dH_2O over 4-week storage period at 25 °C.

Improved-FRDFD-dH_2O	Storage Period (Week)	Citric Acid Concentration (%)					FRDFD-dH_2O
		A-0.00	B-0.05	C-0.10	D-0.15	E−0.20	
Formulation 3	**0**	0.974 ± 0.000^{aB}	0.973 ± 0.001^{aB}	0.971 ± 0.005^{aB}	0.970 ± 0.000^{aB}	0.973 ± 0.004^{aB}	0.981 ± 0.000^{aA}
	1	0.975 ± 0.002^{aAB}	0.977 ± 0.003^{aA}	0.972 ± 0.001^{aAB}	0.972 ± 0.002^{aAB}	0.971 ± 0.001^{aB}	0.977 ± 0.004^{aA}
	2	0.973 ± 0.001^{aA}	0.975 ± 0.001^{aA}	0.973 ± 0.002^{aA}	0.971 ± 0.001^{aA}	0.968 ± 0.003^{aA}	0.975 ± 0.005^{aA}
	3	0.973 ± 0.005^{aAB}	0.973 ± 0.003^{aAB}	0.970 ± 0.001^{aAB}	0.968 ± 0.001^{aB}	0.972 ± 0.002^{aAB}	0.976 ± 0.003^{aA}
	4	0.972 ± 0.001^{aB}	0.972 ± 0.002^{aB}	0.969 ± 0.001^{aB}	0.969 ± 0.000^{aB}	0.970 ± 0.001^{aB}	0.977 ± 0.001^{aA}
Formulation 10	**0**	0.975 ± 0.003^{aB}	0.974 ± 0.001^{aB}	0.972 ± 0.003^{aB}	0.971 ± 0.002^{aB}	0.970 ± 0.000^{aB}	0.981 ± 0.000^{aA}
	1	0.972 ± 0.004^{aA}	0.970 ± 0.003^{aA}	0.971 ± 0.001^{aA}	0.972 ± 0.003^{aA}	0.971 ± 0.001^{aA}	0.976 ± 0.005^{aA}
	2	0.968 ± 0.003^{aB}	0.971 ± 0.000^{aAB}	0.968 ± 0.001^{aB}	0.969 ± 0.000^{aAB}	0.969 ± 0.002^{aAB}	0.975 ± 0.005^{aA}
	3	0.970 ± 0.004^{aB}	0.968 ± 0.004^{aB}	0.970 ± 0.001^{aB}	0.971 ± 0.000^{aB}	0.968 ± 0.001^{aB}	0.979 ± 0.001^{aA}
	4	0.971 ± 0.001^{aB}	0.970 ± 0.001^{aB}	0.969 ± 0.002^{aB}	0.968 ± 0.003^{aB}	0.970 ± 0.001^{aB}	0.978 ± 0.001^{aA}

Data is represented as mean ± standard deviation values (n = 2). ^{ab}Mean value with different superscript in each column of each Improved-FRDFD-dH_2O formulation differs significantly ($p < 0.05$). ^{AB}Mean value with different superscript in each row differs significantly ($p < 0.05$). Improved-FRDFD-dH_2O, Improved Fermented Red Dragon Fruit Drink; FRDFD-dH_2O, diluted fermented red dragon fruit drink (as control); Formulation 3, 0.40 % XG + 0.50 % CMC; Formulation 10, 0.30 % XG + 0.90 % CMC.

this study, Formulation 3E (0.30 % XG and 0.50 % CMC with 0.20 % CA) had shown the best protective effect on the betacyanins (only suffered 14.72 % of loss) in the Improved-FRDFD-dH_2O among all the other samples throughout the 4-week storage at 25 °C.

3.2.3. pH over the storage period

The pH storage results of the Improved-FRDFD-dH_2O that are added with and without CA are shown in Table 10. At Week 0, adding CA at increasing concentration from 0.05 % to 0.20 % significantly decreased the pH values of both Formulations 3 and 10 of Improved-FRDFD-dH_2O. During the 4-week storage period, the FRDFD-dH_2O control in both sets of formulation not only had a significant reduction each week but also showed the highest percentage reduction of around 15 % in terms of the pH levels at the end of Week 4. All the samples, including the control, had undergone pasteurisation and added with potassium sorbate to preserve the samples for longer shelf-life stability. As there was no significant reduction in the TSS value shown in Section 3.2.5, the significant pH reduction in the FRDFD-dH_2O control of this study was unlikely to be caused by the growth of lactic acids microbes such as *Leuconostoc* and *Lactobacillus* that are commonly present in acidic food products (especially unpasteurised) and produce acidic waste products such as acetic and formic acids [44,45]. Therefore, the reduction in the pH values of FRDFD-dH_2O control could be due to the degradation of betacyanins caused by the hydrolysis action of the extra water molecules on the aldimine bond which resulted in the formation of cyclo-Dopa-*O*-β-glucoside and a large amount of betalamic acid, thereby leading to the increase in acidity [3]. Although significant pH reductions were also observed in Formulations 3A and 10A during the storage study, the percentage decrease of pH was greatly reduced in both Formulations 3A and 10A (−2.88 % and −3.67 %, respectively) as compared to that of the FRDFD-dH_2O control (around −15 %), indicating that the addition of hydrocolloid mixture solution had significantly helped in lowering the formation of betalamic acid by stabilising the betacyanins in the drink, as discussed in Section 3.2.2.

Meanwhile, the addition of CA from 0.05 % to 0.20 % in the Improved-FRDFD-dH_2O (Formulations 3B-E and 10B-E) effectively minimised pH reduction from around 3 % reduction in Formulation 3A and 10A to approximately 2.50 % (or lower) reduction only. The present results are in accordance with the previous report which discovered the mixing of orange juice that contains CA with beet juice (which contains betacyanins) was able to assist in stabilising the pH value of the juice throughout the 30-day storage study [46]. These findings are appreciable to the CA being a good buffering agent had increased the buffering capacity of the Improved-FRDFD-dH_2O, thus preventing drastic pH reduction that can cause a high degradation rate of betacyanins as well as structural dysfunctional of CMC (as discussed in Section 3.2.2) in the samples [40,41]. In general, all the Improved-FRDFD-dH_2O samples of Formulation 3 (with and without CA) have relatively lower pH reduction than that of Formulation 10, indicating a higher XG concentration can produce a better pH stability product compared to the product with a higher CMC concentration. Among all the samples, Formulation 3E had shown the best pH stability, where its pH value started to remain stable from Week 2 until Week 4 while also having the lowest pH reduction rate of only 2.10 % by the end of this storage stability study.

3.2.4. Water activity (a_w) over the storage period

Water activity is also an important weekly assessment to provide valuable information about the perishability, quality and stability

Table 12
Total soluble solids (°Brix) of selected two formulations of Improved-FRDFD-dH_2O with different concentrations of CA and control FRDFD-dH_2O over 4-week storage period at 25 °C.

Improved-FRDFD-dH_2O	Storage Period (Week)	Citric Acid Concentration (%)					FRDFD-dH_2O
		A-0.00	B-0.05	C-0.10	D-0.15	E−0.20	
Formulation 3	**0**	7.00 ± 0.00^{aA}	7.00 ± 0.10^{aA}	7.00 ± 0.20^{aA}	7.00 ± 0.00^{aA}	7.00 ± 0.15^{aA}	6.60 ± 0.20^{aB}
	1	7.00 ± 0.10^{aA}	7.00 ± 0.10^{aA}	7.00 ± 0.10^{aA}	7.00 ± 0.00^{aA}	7.00 ± 0.10^{aA}	6.60 ± 0.10^{aB}
	2	6.80 ± 0.20^{aA}	7.00 ± 0.10^{aA}	7.00 ± 0.10^{aA}	7.00 ± 0.00^{aA}	7.00 ± 0.10^{aA}	6.40 ± 0.10^{aB}
	3	6.80 ± 0.10^{aA}	6.80 ± 0.10^{aA}	7.00 ± 0.00^{aA}	7.00 ± 0.10^{aA}	7.00 ± 0.10^{aA}	6.40 ± 0.00^{aB}
	4	6.80 ± 0.10^{aA}	6.80 ± 0.00^{aA}	6.80 ± 0.10^{aA}	7.00 ± 0.10^{aA}	7.00 ± 0.00^{aA}	6.40 ± 0.15^{aB}
Formulation 10	**0**	7.00 ± 0.00^{aA}	7.00 ± 0.10^{aA}	7.00 ± 0.10^{aA}	7.00 ± 0.15^{aA}	7.00 ± 0.00^{aA}	6.40 ± 0.10^{aB}
	1	7.00 ± 0.10^{aA}	7.00 ± 0.10^{aA}	7.00 ± 0.10^{aA}	7.00 ± 0.15^{aA}	7.00 ± 0.10^{aA}	6.40 ± 0.10^{aB}
	2	7.00 ± 0.10^{aA}	7.00 ± 0.00^{aA}	7.00 ± 0.10^{aA}	7.00 ± 0.00^{aA}	7.00 ± 0.00^{aA}	6.20 ± 0.10^{aB}
	3	6.80 ± 0.10^{aA}	7.00 ± 0.10^{aA}	7.00 ± 0.15^{aA}	7.00 ± 0.00^{aA}	7.00 ± 0.10^{aA}	6.20 ± 0.00^{aB}
	4	6.80 ± 0.00^{aA}	6.80 ± 0.00^{aA}	6.80 ± 0.20^{aA}	7.00 ± 0.10^{aA}	7.00 ± 0.00^{aA}	6.20 ± 0.10^{aB}

Data is represented as mean ± standard deviation values (n = 2). ^{ab}Mean value with different superscript in each column of each Improved-FRDFD-dH_2O formulation differs significantly ($p < 0.05$). ^{AB}Mean value with different superscript in each row differs significantly ($p < 0.05$). Improved-FRDFD-dH_2O, Improved Fermented Red Dragon Fruit Drink; FRDFD-dH_2O, diluted fermented red dragon fruit drink (as control); Formulation 3, 0.40 % XG + 0.50 % CMC; Formulation 10, 0.30 % XG + 0.90 % CMC.

of betacyanins pigment in the Improved-FRDFD-dH_2O, where the results are shown in Table 11. At Week 0, Formulations 3A and 10A of Improved-FRDFD-dH_2O showed similar results as in Phase 1 where the water activity values of both formulations are significantly lower than that of control FRDFD-dH_2O. Interestingly, the addition of 0.05 %–0.20 % CA in Formulations 3B-E and 10B-E did not cause any significant change to the water activity of the Improved-FRDFD-dH_2O at Week 0. Similarly, previously it was reported that the mixing different concentrations of XG and CMC with citric acid-sucrose solution did not produce to a wide range of water activity values [14]. From this, it can be deduced that the incorporation of higher concentrations of XG and CMC solution and also the addition of CA into the FRDFD in the present research are still able to produce the Improved-FRDFD-dH_2O samples with stable water activity values and hence stable viscosity (as shown in Section 3.2.1) and quality throughout the 4 weeks storage period at 25 °C.

3.2.5. Total soluble solids (TSS) over the storage period

TSS is a vital attribute to reflect the quality and shelf-life stability of the selected formulations of Improved-FRDFD-dH_2O (that are with and without CA) in comparison with the control FRDFD-dH_2O over four weeks at room temperature, where the results are depicted in Table 12. Referring to Table 12, the addition of CA from 0.05 % to 0.20 % in the Formulations 3 and 10 of Improved-FRDFD-dH_2O did not result in a significant change in the TSS values of the samples (ranging from 6.80 to 7.00 °Brix). The current work agrees with the results and statement of [14] where the use of XG and CMC in the citric acid–sucrose model system will not show any effect on the TSS of the final product. At the same time, all samples including the control are relatively stable, with no significant change in the TSS content over four weeks of storage duration in the present study. Overall, the results have implied that higher hydrocolloids (XG and CMC) concentration and CA incorporation would not exert any considerable effect while able to retent the TSS content of the Improved-FRDFD-dH_2O within the TSS range of RDF over long-term storage for preservation of good reconstituted juice quality.

4. Conclusion

In general, the present study successfully fulfilled the two main focuses when applying natural pigment in the food industry, which are maximising the extracted pigment concentration and reducing the pigment degradation, in a more realistic way for easier scaling up in food industries by the combination of three approaches: optimised fermentation, anionic polysaccharides (XG and CMC) and food additive (CA) addition to concentrate and stabilise the RDF betacyanins via various mechanisms. The present study significantly improved and filled in the gap of the past study, where the desired commercial reference viscosity (308.3 cP) was achieved in this study by the Improved-FRDFD-dH_2O Formulation 3A (0.40 % XG and 0.50 % CMC) and Formulation 10A (0.30 % XG and 0.90 % CMC). The storage study results showed that both Formulations 3A and 10A of Improved-FRDFD-dH_2O successfully reduced the betacyanins loss from around 60 %–23.50 % and 23.92 %, respectively. The incorporation of CA further improved the betacyanins stability, with only 14.72 % and 16.71 % of betacyanins degradation being observed in Formulations 3E (0.40 % XG + 0.50 % CMC + 0.20 % CA) and 10E (0.40 % XG + 0.50 % CMC + 0.20 % CA) respectively by the end of the 4-week storage period. Taken together, the evidence from this study highlights that Formulation 3E provided the best protection and stabilisation effects on betacyanins, while also showing the best pH stability and no significant change in viscosity, water activity and TSS values after 4-week storage at 25 °C. Yet, further research is recommended to be conducted on studying the degradation kinetics of betacyanins and discovering the activation energy and half-life of betacyanins in Improved-FRDFD-dH_2O. Besides that, it is also suggested to study further the long-term (eight weeks) storage stability of Improved-FRDFD-dH_2O as well as the stabilisation effects of green tea extract as the antioxidant towards the betacyanins in Improved-FRDFD-dH_2O.

Data availability statement

The data associated with the present study has not been deposited into a publicly available repository. The data associated with the present study is included/referenced in article.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Teck Wei Lim: Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Visualization, Writing – original draft, Writing – review & editing. **Renee Lay Hong Lim:** Conceptualization, Supervision, Writing – review & editing. **Liew Phing Pui:** Conceptualization, Supervision, Writing – review & editing. **Chin Ping Tan:** Conceptualization, Supervision. **Chun Wai Ho:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to express their gratitude to the Ministry of Higher Education Malaysia for funding this research project through Fundamental Research Grant Scheme (FRGS) with project code: [FRGS/1/2022/STG02/UCSI/02/1]; and UCSI University through Research Excellence & Innovative Grant: [REIG-FAS-2021/011].

References

[1] S. Jurić, M. Jurić, Ż. Król-Kilińska, K. Vlahoviček-Kahlina, M. Vinceković, V. Dragović-Uzelac, F. Donsì, Sources, stability, encapsulation and application of natural pigments in foods, Food Rev. Int. 38 (2022) 1735–1790, https://doi.org/10.1080/87559129.2020.1837862.
[2] C. Carrillo, G. Nieto, L. Martínez-Zamora, G. Ros, S. Kamiloglu, P.E.S. Munekata, M. Pateiro, J.M. Lorenzo, J. Fernández-López, M. Viuda-Martos, J.Á. Pérez-Álvarez, F.J. Barba, Novel approaches for the recovery of natural pigments with potential health effects, J. Agric. Food Chem. (2021), https://doi.org/10.1021/ACS.JAFC.1C07208/ASSET/IMAGES/LARGE/JF1C07208_0002.JPEG.
[3] K.M. Herbach, F.C. Stintzing, R. Carle, Betalain stability and degradation - structural and chromatic aspects, J. Food Sci. 71 (2006) R41–R50, https://doi.org/10.1111/j.1750-3841.2006.00022.x.
[4] K.M. Herbach, M. Rohe, F.C. Stintzing, R. Carle, Structural and chromatic stability of purple pitaya (Hylocereus polyrhizus [Weber] Britton & Rose) betacyanins as affected by the juice matrix and selected additives, Food Res. Int. 39 (2006) 667–677, https://doi.org/10.1016/J.FOODRES.2006.01.004.
[5] H.M.C. Azeredo, Betalains: properties, sources, applications, and stability–a review, Int. J. Food Sci. Technol. 44 (2009) 2365–2376, https://doi.org/10.1111/j.1365-2621.2007.01668.x.
[6] H. Ahmadi, Z. Nayeri, Z. Minuchehr, F. Sabouni, M. Mohammadi, Betanin purification from red beetroots and evaluation of its anti-oxidant and anti-inflammatory activity on LPS-activated microglial cells, PLoS One 15 (2020), e0233088, https://doi.org/10.1371/journal.pone.0233088.
[7] C. Albano, C. Negro, N. Tommasi, C. Gerardi, G. Mita, A. Miceli, L. De Bellis, F. Blando, Betalains, phenols and antioxidant capacity in cactus pear [opuntia ficus-indica (L.) mill.] fruits from apulia (south Italy) genotypes, Antioxidants 4 (2015) 269–280, https://doi.org/10.3390/antiox4020269.
[8] D. Phebe, M.K. Chew, A.A. Suraini, O.M. Lai, O.A. Janna, Red-fleshed pitaya (Hylocereus polyrhizus) fruit colour and betacyanin content depend on maturity, Int. Food Res. J. 16 (2009). http://mymedr.afpm.org.my/publications/46342. (Accessed 20 June 2021).
[9] S.J. Calva-Estrada, M. Jiménez-Fernández, E. Lugo-Cervantes, Betalains and their applications in food: the current state of processing, stability and future opportunities in the industry, Food Chem. Mol. Sci. 4 (2022), 100089, https://doi.org/10.1016/J.FOCHMS.2022.100089.
[10] K.Y. Choo, C. Kho, Y.Y. Ong, Y.Y. Thoo, L.H. Lim, C.P. Tan, C.W. Ho, Fermentation of red dragon fruit (Hylocereus polyrhizus) for betalains concentration, Int. Food Res. J. 25 (2018) 2539–2546. http://psasir.upm.edu.my/id/eprint/66325/1/%2840%29.pdf. (Accessed 21 June 2021).
[11] K.Y. Choo, C. Kho, Y.Y. Ong, Y.Y. Thoo, R.L.H. Lim, C.P. Tan, C.W. Ho, Studies on the storage stability of fermented red dragon fruit (Hylocereus polyrhizus) drink, Food Sci. Biotechnol. 27 (2018) 1411–1417, https://doi.org/10.1007/s10068-018-0367-4.
[12] M. Marchuk, M.J. Selig, G.B. Celli, P. Lawrence, D.M. Smilgies, A. Abbaspourrad, Mechanistic investigation via QCM-D into the color stability imparted to betacyanins by the presence of food grade anionic polysaccharides, Food Hydrocolloids 93 (2019) 226–234, https://doi.org/10.1016/j.foodhyd.2019.01.057.
[13] H.D. Goff, Q. Guo, Chapter 1: the role of hydrocolloids in the development of food structure, in: Handb. Food Struct. Dev., 2019, pp. 1–28, https://doi.org/10.1039/9781788016155-00001.
[14] A. Ozgur, M. Dogan, S. Karaman, Rheological interactions of the xanthan gum and carboxymethyl cellulose as alternative to pectin in organic acid–sucrose model system: simplex lattice mixture design approach, Eur. Food Res. Technol. 243 (2017) 1041–1056, https://doi.org/10.1007/s00217-016-2809-7.
[15] G.E. Ibrahim, I.M. Hassan, A.M. Abd-Elrashid, K.F. El-Massry, A.H. Eh-Ghorab, M.R. Manal, F. Osman, Effect of clouding agents on the quality of apple juice during storage, Food Hydrocolloids 25 (2011) 91–97, https://doi.org/10.1016/J.FOODHYD.2010.05.009.
[16] C. Liang, X. Hu, Y. Ni, J. Wu, F. Chen, X. Liao, Effect of hydrocolloids on pulp sediment, white sediment, turbidity and viscosity of reconstituted carrot juice, Food Hydrocolloids 20 (2006) 1190–1197, https://doi.org/10.1016/J.FOODHYD.2006.01.010.
[17] T.V.L. Nguyen, T.N.M. Vuong, T.Y.N. Tran, D.L. Tri, Interactions of xanthan gum and carboxymethyl cellulose on physical and sensory of cloudy Asparagus juice using response surface methodology, Asian J. Chem. 31 (2019) 2345–2350, https://doi.org/10.14233/AJCHEM.2019.22146.
[18] T.W. Lim, C.J. Lim, C.A. Liow, S.T. Ong, L.H. Lim, L.P. Pui, C.P. Tan, C.W. Ho, Studies on the storage stability of betacyanins from fermented red dragon fruit (Hylocereus polyrhizus) drink imparted by xanthan gum and carboxymethyl cellulose, Food Chem. 393 (2022), 133404, https://doi.org/10.1016/J.FOODCHEM.2022.133404.
[19] I. Sadowska-Bartosz, G. Bartosz, Biological properties and applications of betalains, Molecules 26 (2021) 2520, https://doi.org/10.3390/MOLECULES26092520.

[20] S. Wybraniec, K. Starzak, A. Skopińska, M. Szaleniec, J. Słupski, K. Mitka, P. Kowalski, T. Michałowski, Effects of metal cations on betanin stability in aqueous-organic solutions, Food Sci. Biotechnol. 22 (2013) 353–363, https://doi.org/10.1007/S10068-013-0088-7.
[21] E.L. Attoe, J.H. von Elbe, Oxygen involvement in betanine degradation: effect of antioxidants, J. Food Sci. 50 (1985) 106–110, https://doi.org/10.1111/j.1365-2621.1985.tb13287.x.
[22] S.J. Schwartz, J.L. Cooperstone, M.J. Cichon, H. Joachim, M.M. Giusti, Colorants, in: Fennema's Food Chem., CRC Press, 2017, pp. 681–752. https://www.taylorfrancis.com/chapters/edit/10.1201/9781315372914-12/colorants-steven-schwartz-jessica-cooperstone-morgan-cichon-joachim-von-elbe-monica-giusti. (Accessed 11 November 2021).
[23] D. Saha, S. Bhattacharya, Hydrocolloids as thickening and gelling agents in food: a critical review, J. Food Sci. Technol. 47 (2010) 587–597, https://doi.org/10.1007/s13197-010-0162-6.
[24] S. Akkarachaneeyakorn, S. Tinrat, Effects of types and amounts of stabilizers on physical and sensory characteristics of cloudy ready-to-drink mulberry fruit juice, Food Sci. Nutr. 3 (2015) 213–220, https://doi.org/10.1002/FSN3.206.
[25] W.D.R. Putri, S.R. Nurbaya, E.S. Murtini, Microencapsulation of betacyanin extract from red dragon fruit peel, Curr. Res. Nutr. Food Sci. 9 (2021) 953–960, https://doi.org/10.12944/CRNFSJ.9.3.22.
[26] C.E. Brunchi, M. Bercea, S. Morariu, M. Dascalu, Some properties of xanthan gum in aqueous solutions: effect of temperature and pH, J. Polym. Res. 23 (2016) 1–8, https://doi.org/10.1007/S10965-016-1015-4.
[27] I. Hercules, AQUALON Sodium Carboxymethlcellulose Physical and Chemical Properties, Wilmington, 1999, https://doi.org/10.1016/B978-008055232-3.60229-6.
[28] O.G. Nwaokoro, C.T. Akanbi, Effect of the addition of hydrocolloids to tomato-carrot juice blend, J. Nutr. Heal. Food Sci. 3 (2015) 1–10, https://doi.org/10.4172/2155-9600.1000212.
[29] J.N. BeMiller, Cellulose and cellulose-based hydrocolloids, in: Carbohydr. Chem. Food Sci., third ed., Elsevier Inc., 2019, pp. 223–240, https://doi.org/10.1016/C2016-0-01960-5.
[30] J.N. BeMiller, Xanthan, in: Carbohydr. Chem. Food Sci., third ed., Elsevier Inc., 2019, pp. 261–269, https://doi.org/10.1016/C2016-0-01960-5.
[31] Z. Gao, E.B.M. Daliri, J.U.N. Wang, D. Liu, S. Chen, X. Ye, T. Ding, Inhibitory effect of lactic acid bacteria on foodborne pathogens: a review, J. Food Protect. 82 (2019) 441–453, https://doi.org/10.4315/0362-028X.JFP-18-303.
[32] M. Javanmard, N.L. Chin, S.H. Mirhosseini, J. Endan, Characteristics of gelling agent substituted fruit jam: studies on the textural, optical, physicochemical and sensory properties, Int. J. Food Sci. Technol. 47 (2012) 1808–1818, https://doi.org/10.1111/J.1365-2621.2012.03036.X.
[33] J. Cropotova, U. Tylewicz, N. Dellarosa, L. Laghi, S. Romani, M. Dalla Rosa, Effect of freezing on microstructure and degree of syneresis in differently formulated fruit fillings, Food Chem. 195 (2016) 71–78, https://doi.org/10.1016/J.FOODCHEM.2015.03.056.
[34] S.J. Schmidt, A.J. Fontana Jr., E: water activity values of select food ingredients and products, in: G.V. Barbosa-Cánovas, A.J. Fontana Jr., S.J. Schmidt, T. P. Labuza (Eds.), Water Act. Foods Fundam. Appl., second ed., John Wiley & Sons, 2020, pp. 573–591. https://onlinelibrary.wiley.com/doi/pdf/10.1002/9781118765982#page=573. (Accessed 14 July 2021).
[35] Codex Alimentarius Commission (Cac), Codex general standard for fruit juices and Nectars (CODEX STAN 247-2005), in: Codex Aliment., United States Food and Agriculture Organization, FAO, 2005. https://www.fao.org/fao-who-codexalimentarius/sh-proxy/es/?lnk=1&url. (Accessed 13 July 2021).
[36] B. Jamilah, C.E. Shu, M. Kharidah, M.A. Dzulkifly, A. Noranizan, Physico-chemical characteristics of red pitaya (Hylocereus polyrhizus) peel, Int. Food Res. J. 18 (2011) 279–286. http://psasir.upm.edu.my/id/eprint/16208/1/16208.pdf. (Accessed 13 July 2021).
[37] C. Tamby Chik, S. Bachok, N. Baba, N. Abdullah, Quality characteristics and acceptability of three types of pitaya fruits in a consumer acceptance test, J. Tour. Hosp. Culin. Arts. 3 (2011) 89–98. https://ir.uitm.edu.my/id/eprint/44412/. (Accessed 13 July 2021).
[38] F. Wang, Z. Sun, X. Li, K. Wang, D. Chen, Z. Li, Study on factors influencing the viscosity of sodium carboxymethyl cellulose used in capsule-mucous sealers, Geofluids (2022) 2022, https://doi.org/10.1155/2022/4198311.
[39] J.H. Pasch, J.H. von Elbe, Betanine stability in buffered solutions containing organic acids, metal cations, antioxidants, or sequestrants, J. Food Sci. 44 (1979) 72–75, https://doi.org/10.1111/J.1365-2621.1979.TB10007.X.
[40] R. Ciriminna, F. Meneguzzo, R. Delisi, M. Pagliaro, Citric acid: emerging applications of key biotechnology industrial product, Chem. Cent. J. 11 (2017) 1–9, https://doi.org/10.1186/S13065-017-0251-Y/TABLES/4.
[41] J. Lulek, A. Voelkel, M. Skotnicki, M. Lambros, T. Tran, Q. Fei, M. Nicolaou, Citric acid: a multifunctional pharmaceutical excipient, Pharmaceutics 14 (2022) 972, https://doi.org/10.3390/PHARMACEUTICS14050972.
[42] D.D. Castro-Enríquez, B. Montaño-Leyva, C.L. Del Toro-Sánchez, J.E. Juaréz-Onofre, E. Carvajal-Millan, S.E. Burruel-Ibarra, J.A. Tapia-Hernández, C. G. Barreras-Urbina, F. Rodríguez-Félix, Stabilization of betalains by encapsulation-a review, J. Food Sci. Technol. 57 (2020) 1587–1600, https://doi.org/10.1007/s13197-019-04120-x.
[43] B.M. Simões, C. Cagnin, F. Yamashita, J.B. Olivato, P.S. Garcia, S.M. de Oliveira, M.V. Eiras Grossmann, Citric acid as crosslinking agent in starch/xanthan gum hydrogels produced by extrusion and thermopressing, LWT 125 (2020), 108950, https://doi.org/10.1016/J.LWT.2019.108950.
[44] K.R. Aneja, R. Dhiman, N.K. Aggarwal, V. Kumar, M. Kaur, Microbes associated with freshly prepared juices of citrus and carrots, Int. J. Food Sci. 2014 (2014), https://doi.org/10.1155/2014/408085.
[45] J.C. Oliveira, P. Setti-Perdigão, K.A.G. Siqueira, A.C. Santos, M.A.L. Miguel, Microbiological characteristics of orange juices, Food Sci. Technol. 26 (2006) 241–245, https://doi.org/10.1590/S0101-20612006000200002.
[46] M.R.A. Porto, V.S. Okina, T.C. Pimentel, S.H. Prudencio, Physicochemical stability, antioxidant activity, and acceptance of beet and orange mixed juice during refrigerated storage, Beverages 3 (2017) 36, https://doi.org/10.3390/BEVERAGES3030036.