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Evaluating the potential of stabilised betacyanins from fermented red dragon fruit (*Hylocereus polyrhizus*) drink: Sustainable colouration and antioxidant enhancement of stirred yoghurt

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A R T I C L E I N F O

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A B S T R A C T

Nowadays, the wide use of synthetic food colourant, Allura Red has elevated negative perceptions among consumers. As food industry players strive towards satisfying consumers' demands, exploring new sustainable natural colourants with multifunctional properties (like betacyanins) has emerged as a novel concept. This study aimed to evaluate the potential of a functional fruit drink, Improved-FRDFD-dH_2O [contained betacyanins of fermented red dragon fruit drink stabilised by 0.4 % xanthan gum and 0.5 % carboxymethyl cellulose hydrocolloid mixture solution and 0.2 % citric acid] to serve as a sustainable and stable functional liquid colourant in yoghurt. The 8-week refrigerated storage study revealed the betacyanins content in the yoghurt added with E162 experienced the lowest stabilities in terms of betacyanins content (>30 % degradation), viscosity (>15 % reduction), pH, LAB viability and antioxidant activity. In contrast, the Improved-FRDFD-dH_2O was able to maintain the yoghurt's viscosity, syneresis, pH and LAB viability without any significant change. The betacyanins of Improved-FRDFD-dH_2O had excellent stability in the yoghurt (<5 % degradation), showing only a minimal difference (ΔE<1.5) in total colour changes and being able to retain >86 % of the initial antioxidant activity by the end of storage. These findings support the Improved-FRDFD-dH_2O as a sustainable functional colourant with antioxidant properties for producing beneficial yoghurt.

1. Introduction

Since its origin (perhaps 10,000–15,000 years ago), yoghurt (and its varieties) has been one of the most popular traditional fermented dairy products that is now regarded as a major contributor to global sales revenue, where the industry of yoghurt is thriving steadily in the international market of fermented milk products and is expected to obtain a huge turnout in the coming decades (Gawai et al., 2017). Yoghurt is a biotechnological food produced from the fermentation of milk by lactic acid bacteria, mainly the cultures of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* (Codex Alimentarius Commission (CAC), 2022; Nontasan et al., 2012). The wide consumer acceptability of yoghurt is contributed by its high nutritional values (such as high in calcium and free of lactose) that could provide many potential health benefits like prevention of gastrointestinal disorders, immune enhancement and improved lactose tolerance (Coïsson et al., 2005; Gawai et al., 2017; Weerathilake et al., 2014). Today, there are a few types of yoghurt that can be commonly found in most developed markets all over the world, mainly the set, stirred, drinking and Greek yoghurts (Gawai et al., 2017). Among these yoghurts, coloured/pigmented and flavoured yoghurt that is usually produced from stirred yoghurt has received great popularity in the current years due to its improved eye appeal and better flavour profile (Coïsson et al., 2005; Gawai et al., 2017; Li et al., 2021). In this context, owing to the features of low cost, high availability and tinctorial strength, synthetic colourant is commonly applied to intensify the colour of the yoghurts, with Allura Red (FD&C Red No. 4) being the most well-known example of certified food colourant in the food industry. However, the production of Allura Red is of low sustainability, especially in the aspect of environment and social responsibility. This is due to it only involves abiotic depletion of

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non-renewable resources, has a low recovery of by-products (aphid residues) and the hazardous starting materials could also impose potentially harmful effects on workers. Moreover, many articles have been published previously on the negative effects of consuming this synthetic colourant, which include inducing/worsening asthma, being carcinogenic (at high concentrations) and triggering hyperactivity in children (Gebhardt et al., 2020; Kobylewski and Jacobson, 2010; Okafor et al., 2016).

Due to the adverse effects of synthetic colourants, the interest and demand of consumers for eco-friendly/non-toxic natural food colourants are increasing as they also aspire more to sustainable food value chains (Devadiga and Ahipa, 2020; Gebhardt et al., 2020). Although anthocyanin (E163, derived from grape skin extract) is a water-soluble natural pigment that can impart a red-purple-blue colour to food products, its application range is often limited to low-pH food products only. This is because anthocyanin is prone to interact with food matrices and is unstable at pH over 3 (tends to shift from red/ purple to blue), hence, it is unsuitable for application in yoghurt with a pH value of around 4.6 or lower. In contrast, betacyanins is a better option as a natural red colourant to replace the artificial colourant Allura Red as it does not only possess high tinctorial strength but also has a wide tolerable range of pH from 3.5 to 7 that allows it to be suitable for applying in sour to neutral foods like yoghurts (Gebhardt et al., 2020; Martins et al., 2017; Strack et al., 2003).

Similar to other natural sources with coloured compounds, red dragon fruit (RDF, *Hylocereus polyrhizus*) and red beetroot have been commonly reported to possess powerful antioxidant activity, thanks to their vivid bioactive pigment betacyanins (Herbach et al., 2006b; Lim et al., 2024). However, the shelf life of natural pigments like betacyanins is normally very low as they are susceptible to degradation upon exposure to heat, oxygen, light and high water activity during food processing, transportation and storage, leading to the loss in colour and bioactivity over time (Herbach et al., 2006b; Stich, 2016). To date, red beetroot is the only betacyanins source that has been approved by the Food and Drug Administration (FDA) as a natural colourant powder (E162) in the food application. Yet, natural colourant usually needs to be applied at higher levels to compensate and achieve the same colour intensity as the artificial colourant. However, this may then affect the physicochemical characteristics of the yoghurt during the storage period (Aguilar et al., 2015; Calva-Estrada et al., 2022; Clydesdale, 1993; Gebhardt et al., 2020; Rodriguez-Amaya, 2019). Moreover, its production process is also considered to be of low sustainability as it requires multiple, additional steps of treatment (such as grinding, extraction, homogenisation, decantation, separation of residues, purification and drying of pigment extract) and high consumption of raw materials, energy and solvent/water, hence resulting in high cost and price (Akbar Hussain et al., 2018; Gebhardt et al., 2020). Furthermore, the heat generated during the spray-drying of beetroot extract can cause a significant reduction in the betacyanins content, while the pigment powder also has the drawbacks of high hygroscopicity, spattering, required redissolved step and low solubility (Cai and Corke, 2000; Herbach et al., 2004; Lim et al., 2022; Xu et al., 2018). The instability of betacyanins was not only present when in their source, but the pigments also experienced a high degradation rate when being incorporated into the drink model such as yoghurt. In a study conducted by Guneser (2021), it was also discovered that the yoghurts containing betacyanins from commercial natural beetroot liquid colourant experienced almost 50 % loss during the 60-day storage at 4 °C. It has been suggested that it is inevitable to explore strategies that can stabilise the betacyanins before applying them as colourant and antioxidant in food systems, with the addition of anionic polysaccharides and the presence of metal chelating agents and organic acids being the most promissory solutions (Schneider-Teixeira et al., 2022).

As there is a need for a new sustainable approach that can ensure high betacyanins production, low betacyanins degradation and lower cost and skills required, fermentation and direct incorporation of food hydrocolloids solution and food additive are potential techniques that could be employed. In the previous study, natural fermentation was found to be able to increase the RDF betacyanins concentration to tenfold (from 11.7 g/L to 131.7 g/L) and produce a fermented red dragon fruit drink (FRDFD) with betacyanins that are stable at 4 °C (< 20 % degradation throughout eight-week storage) (Choo et al., 2018a,b; Lim et al., 2023a). Meanwhile, significant stability improvement was reported, where the incorporation of anionic polysaccharides mixture solution [0.4 % xanthan gum (XG) and 0.5 % carboxymethyl cellulose (CMC)] and 0.2 % citric acid (CA) into the FRDFD was able to synergistically enhance the stability of betacyanins (< 15 % degradation) in liquid matrix throughout the 4-week storage period at 25 °C, thereby producing a potential stable functional drink, Improved-FRDFD-dH_2O (Lim et al., 2023). As there is a significant inclination towards fruit-type stirred yoghurts due to the presence of natural colours and flavours that are widely accepted and welcomed among consumers, the Improved-FRDFD-dH_2O is a potential functional ingredient that can be supplemented into the yoghurt also (Kailasapathy et al., 2008; Sarkar, 2019; Senaka Ranadheera et al., 2012). Hence, the present study is novel and the first that aimed to incorporate Improved-FRDFD-dH_2O as a healthy functional colourant into self-prepared yoghurt (as a drink model) and evaluate the storage stability of the stirred yoghurt through various physicochemical and betacyanins content analyses over 8 weeks at 4 °C. The free radical scavenging capacity of the yoghurts incorporated with or without different natural betacyanins colourants (Improved-FRDFD-dH_2O, FRDFD or E162) were assessed as well to study their ability in enhancing the antioxidant activities to lead to the development of a new functional yoghurt that could fulfil consumer needs. The present study met the food industry's exploration and demand for natural, sustainable functional ingredients (produced from mild approaches, simple to apply and low cost) that can impart multiple advantages of colouring, stable physicochemical properties and nutritional enhancement simultaneously for the production of beneficial yoghurt (Demirkol and Tarakci, 2018; Gawai et al., 2017).

2. Materials and methods

2.1. Raw materials

A total of 10 kg of fresh red dragon fruit (RDF) (*Hylocereus polyrhizus*) (approximately 0.5–0.6 kg each) without physical defects (cracks, cuts and damages) and microbiological spoilage (sunken lesions, dark blotches and mouldy) and 1 kg of white fine sugar (Central Sugars Refinery, CSR, Malaysia) as well as 6 L of pure fresh cow milk (Farm Fresh Milk, Malaysia) were purchased from a local supermarket (Giant Hypermarket, Kuala Lumpur, Malaysia). The RDF were all rinsed under tap water to remove any sand residues and dirt on the peel surface, followed by pat-dry using 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), commercial beetroot red colourant powder (E162) and commercial yoghurt culture mixture of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* (YO 22.10) were purchased from Take It Global Sdn. Bhd. (Malaysia). Potassium persulfate, 2,2′-azino-bis(3-ethyl benzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,2-diphenyl-1–1-picrylhydrazyl (DPPH) and tetramethylchromane-2-carboxylic acid (Trolox) all were obtained from Sigma-Aldrich (USA). MRS (de Man, Rogosa, Sharpe) agar and ST (*Streptococcus thermophilus*) agar were purchased from HiMedia (USA), sodium chloride and absolute ethanol were obtained from HmbG Chemicals (Germany), betanin (TCI, Japan), methanol (HPLC grade) (Merck, Germany), orthophosphoric acid (R&M Chemical, UK) and ultra-pure water (Millipore, USA) were used throughout the study.

2.3. Fermented red dragon fruit drink (FRDFD), improved fermented red dragon fruit drink (improved-FRDFD-dH_2O) and beetroot red colourant solution preparation

The fermented red dragon fruit drink (FRDFD) was prepared using two 2-L airtight fermentation tanks according to the protocols published previously (Lim et al., 2023, 2022). Sterilisation was first carried out by washing and rinsing all the utensils with boiled water. Under a sterile and clean condition, the optimised fermentation was performed by arranging the white fine sugar layer by layer alternately with the pieces of RDF slices in a ratio of 1:10, with the top and bottom layers fully covered by the sugar. After seven days of natural fermentation at room temperature (25 °C), the FRDFD was collected and filtered through a sterilised sieve bag. Subsequently, the Improved Fermented Red Dragon Fruit Drink (Improved-FRDFD-dH_2O) was formed by mixing the FRDFD with XG (0.4 %, w/v) and CMC (0.5 %, w/v) hydrocolloid mixture solution in the ratio of 1:2. Then, 0.2 % (w/v) of citric acid (CA) was added and stirred (1000 rpm) at room temperature with a magnetic stirrer. The potassium sorbate was added as a preservative at 0.1 % (w/v) and stirred at the speed of 1000 rpm for 5 mins in both Improved-FRDFD-dH_2O (pH: 3.87 ± 0.01; a_w: 0.969 ± 0.001) and FRDFD (pH: 3.89 ± 0.01; a_w: 0.963 ± 0.001). Besides, 1 % (w/v) beetroot red colourant solution was prepared by dissolving the commercial beetroot red colourant powder (E162) in distilled water. Lastly, pasteurisation was carried out for all betacyanins colourant samples at 75 °C for 15 s, followed by cooling down to room temperature under running tap water. All the betacyanins colourant samples were stored in the 500-mL Schott Duran® bottles under dark and clean conditions at −20 °C.

2.4. Yoghurt preparation

The preparation of different yoghurts was carried out according to previously published protocols with some modifications (Gengatharan et al., 2017; Ścibisz et al., 2019). Firstly, the pasteurised and homogenised pure cow milk was pre-heated to 42 °C, followed by inoculating and mixing with 0.4 % (w/v) commercial 1:1 mixture of yoghurt culture (*L. bulgaricus* and *S. thermophilus*) thoroughly in the pre-heated milk. Next, the inoculated milk samples were incubated in the 500-mL Schott Duran® bottles at 42 °C for 4 h until they reached pH 4.6 ± 0.1, followed by cooling to ambient temperature. Subsequently, three yoghurt samples with different betacyanins colourant were prepared aseptically by incorporating the Improved FRDFD-dH_2O (at 2 %, v/v), FRDFD (at 0.5 %, v/v) and 1 % (w/v) beetroot red colourant (E162) solution (at a final concentration of 0.05 %, w/v) into three yoghurts separately (namely YO-IF, YO-F and YO-B, respectively) and then stirred evenly to standardise and achieve an absorbance value of 0.70 ± 0.02 at a wavelength of 538 nm using a UV–visible spectrophotometer (Secomam UviLine 9400, France) to obtain similar initial betacyanins concentrations and mimic the colour of commercial yoghurts (with plain yoghurt used as a blank to correct for the light diffusion by the yoghurt milk particles). The plain yoghurt (P-YO) without the addition of any colourant sample was prepared as the negative control. In general, on a quality basis, commercial yoghurts have a shelf life of at least 4–7 weeks (Chandan and O'rell, 2006; Michael et al., 2015). Hence, all the yoghurts formed in the present study were then stored in dark, at 4 °C for eight weeks, and subjected to different food analyses at two-week intervals (from Week 0 to Week 8).

2.5. Food storage analyses

Eight analyses which included five physicochemical characteristics (betacyanins content, colour, pH, viscosity, syneresis), LAB viability and two chemical-based antioxidant activity tests were conducted on all yoghurt samples.

2.5.1. Betacyanins concentration

The aqueous fraction containing betacyanins was separated from the yoghurt samples based on the previously published procedure with minor modifications (Flores-Mancha et al., 2021). Briefly, 10 g of each yoghurt sample was centrifuged (X-22R, Beckman Coulter, USA) at 10, 000 × g, 4 °C for 30 mins. Next, the upper aqueous fraction of the yoghurt sample was filtered through hydrophilic PTFE syringe filters (0.45 μM) into the sample vials (Liu et al., 2020; López-Hernández and de Quirós, 2016; Mochizuki et al., 2009). Betacyanins (betanin and isobetanin) concentration quantification of the four yoghurt samples was carried out using HPLC-DAD according to (Choo et al., 2019; Foong et al., 2012) with slight modifications. The working solutions of the betanin standard that consists of betanin and isobetanin (2000–10,000 mg/L) were prepared. An Agilent 1200 series HPLC system (Agilent, USA) fitted with a G1322A degasser, C1325B injector, G1329A column oven and G1315D diode array detector (DAD) was employed. A reversed-phase ZORBAX Eclipse XDB-C18 Analytical column (4.6 × 150 mm, 5-Micron) was used throughout this analysis. The detection was carried out at the wavelength of 538 nm and indicated by peaks plotted in chromatograms. The concentration of betacyanins (mg/L) was calculated based on the betanin standard equation, $y = 0.1158x$ ($R^2 = 0.997$) and isobetanin standard equation, $y = 0.1137x$ ($R^2 = 0.999$).

2.5.2. Colour analysis

The colour of the yoghurt samples was analysed using a colourimeter (ColourFlex EZ, HunterLab, Australia). The colourimeter was first calibrated with a white and black calibration plate. The yoghurt sample was homogenised and placed in the Colour Flex sample cup to record the mean value of three measurements. The CIELab parameters, L* (lightness), *a** [red(+)/green(−) attribute] and *b** [yellow(+)/blue(−) attribute] were evaluated and the ΔE (total colour difference) was calculated based on Eq. (1) (Schneider-Teixeira et al., 2022).

$$\Delta E = \sqrt{\left[(L*_F - L*_I)^2 + (a*_F - a*_I)^2 + (b*_F - b*_I)^2\right]} \quad (1)$$

Where ΔE is the total colour difference by Week 8; L^*_F is the L* value at final storage time (Week 8); a^*_F is the a* value at final storage time (Week 8); b^*_F is the b* value at final storage time (Week 8); L^*_I is initial L* value at initial storage time (Week 0); a^*_I is the a* value at initial storage time (Week 0); b^*_I is the b* value at initial storage time (Week 0).

2.5.3. pH

The pH of the yoghurt samples was measured using a pH meter (Jenway Model 3505, UK) according to the protocols published previously (Lim et al., 2022).

2.5.4. Viscosity

The viscosity of the yoghurt samples was measured using a viscometer (Brookfield Model DV-11+Pro, USA). The viscometer was autozeroed before use, followed by rinsing and cleaning the LV-3 spindle with distilled water and then dried. The spindle code of the LV-3 spindle was fixed to 63. The rotational speed of the spindle was set at 60 rpm. The reading displayed in centipoise (cP) was recorded when the value shown was stable with no fluctuation (Demirkol and Tarakci, 2018; Lim et al., 2023, 2022). The percentage change in viscosity was calculated according to Eq. (2).

$$Percentage\ change = -\left[(V_I - V_F) / V_I \times 100\right] \quad (2)$$

Where V_I is the viscosity value (cP) at initial storage time (Week 0); V_F is the viscosity value (cP) at final storage time (Week 8).

2.5.5. Syneresis

The syneresis of the yoghurt samples was determined based on the previously published procedure with slight modifications (Golmakani et al., 2021; Wang et al., 2020). 30 g of each stirred yoghurt sample were centrifuged (X-22R, Beckman Coulter, USA) at 222 × *g* for 10 min at 4 °C. The syneresis was calculated according to Eq. (3), which is a measurement of the liquid (whey) separation from the gel matrix (Gengatharan et al., 2017; Hematyar et al., 2012; Wang et al., 2020).

$$Syneresis(\%) = W_S/W_Y \times 100 \quad (3)$$

Where W_S is the weight of supernatant (g); W_Y is the weight of yoghurt.

2.5.6. Viability of lactic acid bacteria (LAB)

The enumeration of *L. delbrueckii ssp. bulgaricus* and *S. thermophilus* in the yoghurt samples were determined by the plate count method. Firstly, each of the yoghurt samples was diluted to 10^{-5} in a sterilised 0.85 % (w/v) physiological salt solution and homogenised with a stomacher (BagMixer 400 W, Interscience, France) for 2 mins at speed 4. The diluted yoghurt samples (1 mL) were pipetted and spread onto MRS agar and ST agar using the spread plate technique for the enumeration of *L. bulgaricus* and *S. thermophilus*, respectively. Subsequently, the MRS plates were incubated anaerobically while the ST agar plates were incubated aerobically at 37 °C for 48 h. The total colonies appearing on the plates were counted, then multiplied by the dilution factor, and the results were expressed and presented as the log colony forming units per millilitre (log CFU/mL) (Gengatharan et al., 2017).

2.5.7. Antioxidant activity analyses

The antioxidant activities of the four yoghurt samples were assessed using 2,20-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays.

The ABTS radical scavenging assay was carried out according to (Choo et al., 2019) with minor modifications. Firstly, the ABTS radical solution was prepared by mixing 10 mL of 7 mM ABTS solution with 10 mL of 2.45 mM potassium persulfate solution and stored under dark conditions at room temperature for 12–16 h. Before carrying out the assay, ethanol was used to adjust the ABTS radical solution to an absorbance of 0.7 ± 0.02 at 734 nm. Then, 25 μL of each yoghurt sample was added to 975 μL of ABTS radical solution, followed by incubation for 6 mins in dark conditions for the reaction to happen at room temperature. The absorbance values were measured using a UV-visible spectrophotometer (Secomam UviLine 9400, France) at 734 nm against a blank (distilled water). The percentage inhibition (%) of ABTS radical was calculated using Eq. (4). The ABTS radical scavenging capacity of the betacyanins in the samples was expressed as millimoles of Trolox equivalents antioxidant capacity (TEAC) and calculated based on the equation, $y = 81.445x + 3.9427$ ($R^2 = 0.999$) obtained from the standard curve of Trolox (0.2–0.8 mM). The percentage retained of the ABTS radical scavenging capacity was calculated according to Eq. (5).

The DPPH radical scavenging assay was carried out according to (Choo et al., 2019) with minor modifications. Briefly, 0.1 mL of each yoghurt sample was added to 3.9 mL of 80 % ethanolic 60 μM DPPH solution in test tubes. The test tubes were vortexed for 15 s, followed by incubation for 30 mins in dark conditions for the reaction to happen at room temperature. The absorbance values were measured using a UV-visible spectrophotometer (Secomam UviLine 9400, France) at 515 nm against a blank (80 % ethanol). The percentage inhibition (%) of DPPH radical was calculated using Eq. (3). The DPPH radical scavenging capacity of the betacyanins in the samples was expressed as millimoles of Trolox equivalents antioxidant capacity (TEAC) and calculated based on the equation, $y = 42.746x + 4.3546$ ($R^2 = 0.991$) obtained from the standard curve of Trolox (0.2–0.8 mM). The percentage retained of the DPPH radical scavenging capacity was calculated according to Eq. (5).

$$Percentage\ inhibition\ of\ free\ radicals\ (\%) = [(A_C - A_s)/A_C] \times 100 \quad (4)$$

Where A_c is the absorbance of the ABTS/DPPH radical solution with negative control (distilled water) at λ_{max} (734 nm/515 nm); A_S is the absorbance of ABTS/DPPH radical solution with test sample at λ_{max} (734 nm/515 nm).

$$Percentage\ retained = A_F/A_I \times 100 \quad (5)$$

Where A_F is the ABTS/DPPH radicals scavenging capacity (mM TEAC) at final storage time (Week 8); A_F is the ABTS/DPPH radicals scavenging capacity (mM TEAC) at final storage time (Week 0).

2.6. Statistical analysis

All the numerical data were measured in triplicate with triplicate samples (*n* = 3) and expressed as mean ± standard deviations. All results were analysed using IBM SPSS Statistics Software (SPSS Version 25). One-way analysis of variance (ANOVA) with Tukey's test was performed to analyse the significant difference ($p < 0.05$) between means.

3. Results and discussion

3.1. Betacyanins concentration over storage period

Betanin and isobetanin are the prominent constituents (75–95 %) of betacyanins which serve as the predominant bioactive compounds that responsible for the red-violet colouring and functional properties in the yoghurts must present at sufficient concentration throughout the 8-week storage period at 4 °C, where the results are shown in Table 1 (Singh and Bharati, 2014; Stintzing and Carle, 2007, 2004). At Week 0, it can be seen that there is no significant difference in the initial concentrations of betanin (0.284–0.287 mM) and isobetanin (0.253–0.256 mM) between the YO-IF, YO-F and YO-B, after standardised to the similar initial absorbance value of 0.70 ± 0.02 at 538 nm. Meanwhile, the P-YO without the addition of any colourant did not contain any betacyanins pigment. During the 8-week storage period at 4 °C, the betanin pigments in the YO-B suffered the highest loss of 31.20 %, with significant degradation that can be observed almost every two weeks. Meanwhile,

Table 1
Betanin and isobetanin concentrations (mM) of yoghurts added with different betacyanins colourants over 8-week storage period at 4 °C.

Betacyanins	Storage period (Week)	YO-IF	YO-F	YO-B	P-YO
Betanin	0	0.287 ± 0.009^{aA}	0.284 ± 0.015^{aA}	0.286 ± 0.013^{aA}	–
	2	0.286 ± 0.011^{aA}	0.290 ± 0.010^{aA}	0.258 ± 0.007^{bB}	–
	4	0.287 ± 0.011^{aA}	0.288 ± 0.010^{aA}	0.244 ± 0.006^{bcB}	–
	6	0.286 ± 0.003^{aA}	0.277 ± 0.008^{aA}	0.237 ± 0.006^{cB}	–
	8	0.277 ± 0.009^{aA}	0.245 ± 0.009^{bB}	0.197 ± 0.005^{dC}	–
	Percentage change	−3.43 %	−13.61 %	−31.20 %	–
Isobetanin	0	0.256 ± 0.011^{aA}	0.253 ± 0.011^{aA}	0.256 ± 0.008^{aA}	–
	2	0.249 ± 0.004^{aA}	0.251 ± 0.003^{aA}	0.253 ± 0.005^{aA}	–
	4	0.248 ± 0.005^{aA}	0.246 ± 0.008^{aA}	0.248 ± 0.006^{aA}	–
	6	0.248 ± 0.010^{aA}	0.246 ± 0.011^{aA}	0.242 ± 0.002^{aA}	–
	8	0.249 ± 0.004^{aA}	0.240 ± 0.007^{aA}	0.202 ± 0.005^{bB}	–
	Percentage change	−2.76 %	−5.17 %	−21.16 %	–

Data is represented as mean ± standard deviation values (*n* = 3). ^{ab}Mean value with different superscript in each column differs significantly ($p < 0.05$). ^{AB}Mean value with different superscript in each row differs significantly ($p < 0.05$). YO-IF, yoghurt with Improved-FRDFD-dH_2O (Improved Fermented Red Dragon Fruit Drink); YO-F, yoghurt with FRDFD (fermented red dragon fruit drink); YO-B, yoghurt with beetroot red (E162), P-YO, plain yoghurt (as control).

the YO-F displayed only a significant betanin content reduction in Week 8, with a total betanin loss of 13.61 %. Among all the yoghurt samples, only the betanin of YO-IF was able to remain stable without any significant change, with the lowest betanin degradation of 3.43 % only by the end of the storage study. Similar trends can be observed in the isobetanin concentration as well. Among all the yoghurt samples, the YO-B experienced significant and the highest isobetanin degradation (21.61 %), as compared to the YO-F and YO-IF which shown no remarkable reductions (5.17 % and 2.76 %, respectively) in the isobetanin concentration. The present results signify better stability of the natural betacyanins colourant derived from the functional drinks of RDF as compared to the commercial E162 produced from the beetroot extract.

Previously, it has been presumed that the degradation of betacyanins in the yoghurt mainly happened through the normal hydrolysis process of aldimine bond during refrigerated storage which resulted in the formation of cycloDopa 5-O-β-glucoside and betalamic acid (Gengatharan et al., 2017). Besides the hydrolytic cleavage of the aldimine bond, the structural change of betanin and isobetanin may also happen via hydrolysis of the glycosidic bond (deglycosylation), dehydrogenation and decarboxylation (Herbach et al., 2006b; Schneider-Teixeira et al., 2022; Stintzing and Carle, 2007). Many factors could be contributing to the different betacyanins stability observed in different yoghurts in the present research, such as food matrix and product formulation, dissolved oxygen in the yoghurts, the presence of metal cations and pH (Guneser, 2021). The mixing of betacyanins colourants with yoghurts can cause the oxygen to be absorbed during the stirring process, while it is also known that metabolic activities of the LAB (which are catalase negative) can result in the production and accumulation of hydrogen peroxide (as mentioned in Section 3.5) and glycosidase enzymes (Fan and Cliff, 2017; Ścibisz et al., 2019). Different from the FRDFD, the commercial E162 colourant (added in the YO-B) produced from the purified beetroot extract lacks a natural drink matrix like the FRDFD. This had possibly caused the betacyanins in the YO-B to be more prone to hydrolytic cleavage and degradation as the pigments are more exposed to dissolved oxygen, hydrogen peroxide and glycosidase enzymes. Moreover, although the pH of YO-B remained within the betacyanins stable pH range (3.5–7) during the storage period, the drastic pH reduction (from 4.49 to 4.13) might also lead to the instability of betacyanins via dehydrogenation and C-17 decarboxylation. In contrast, the YO-F showed better betacyanins stability as compared to YO-B. This could be attributable to the drink matrix which contains plant constituents like sugars, acids, pectic substances and ascorbic acid (as an antioxidant) that can help to stabilise the betacyanins by partially suppressing hydrolytic cleavage of betacyanins aldimine bond and reducing the oxidation of betacyanins in the YO-F (Azeredo, 2009; Herbach et al., 2006b; Martins et al., 2017; Sadowska-Bartosz and Bartosz, 2021). On an interesting note, the FRDFD betanin exhibited higher stability when present in the yoghurt (YO-IF) (13.61 % of betanin loss) as compared to when in its original FRDFD (15.72 % of betanin loss) as observed in the previous study (Choo et al., 2018b). This is possibly due to the interaction and binding of betanin with the C = O, C—N and N—H groups of yoghurt milk protein which could have contributed to the stabilisation of betanin glycosidic bond, leading to a lower risk of betanin and isobetanin degradation through deglycosylation in YO-F (He et al., 2016; Ścibisz et al., 2019; Zhao et al., 2020).

Generally, the betacyanins content in the YO-IF was found to be the most stable among all the coloured yoghurts, thanks to the presence of XG, CMC and CA which had helped to further stabilise and protect the betacyanins, aside from possessing the original FRDFD matrix effect also. In Section 3.1, it has been mentioned that the YO-IF demonstrated the highest storage stability in terms of viscosity and syneresis due to the XG and CMC from the Improved-FRDFD-dH_2O having assisted in binding with the water molecules. This action greatly helped to resist the movement of the water, thereby lowering the rate of hydrolytic cleavage on the betacyanins aldimine bond by the free-moving water molecules. As the anionic charge carboxyl groups of XG and CMC were able to bind with the free cationic betacyanins, this pigment-polysaccharide association also helped to reduce the risk of betacyanins from being attacked by water in the YO-IF. Moreover, due to the relatively stable 3D gel network contributed by the XG and CMC in the YO-IF (as mentioned in Section 3.4) that can provide a better compartmentation effect, the betacyanins can be physically trapped and protected from oxidation and degradation caused by the dissolved oxygen and hydrogen peroxide (Lim et al., 2023, 2022). Furthermore, with protein molecules that are stabilised in the 3D gel network of YO-IF, the interaction and binding of the betacyanins with the yoghurt milk protein can be more stable as compared to YO-F and YO-B. This can then minimise the degradation rate of betanin and isobetanin through deglycosylation by the into betanidin and isobetanidin glycosidase enzymes produced by the LAB in YO-IF (He et al., 2016; Ścibisz et al., 2019; Stintzing and Carle, 2007; Zhao et al., 2020). Additionally, the CA (from the Improved-FRDFD--dH_2O) had also contributed to maintaining the betacyanins stability in YO-IF, by functioning as a good pH buffering agent (to resist drastic pH change in YO-IF, as mentioned in Section 3.3) and metal chelating agent (to bind with metals like Ca, Cr, Cu and Fe that commonly present in the yoghurt). Moreover, CA could also provide aliphatic acid moieties (esterification) and sequestering effects to protect the betacyanins from the nucleophilic attack of any free water molecules in the YO-IF (Lim et al., 2023; Luis et al., 2015). With all these stabilisation mechanisms, the YO-IF (produced from the incorporation of Improved-FRDFD-dH_2O in plain yoghurt) not only showed the best betacyanins stability result ($<$ 5 % degradation over 8-week of refrigerated storage) among all the coloured yoghurt samples in the present study, but the betacyanins in YO-IF were also more stable as compared to the yoghurts added with commercial natural beetroot liquid colourant (49.36 % betacyanins loss after 60-day cold storage), *Alternanthera brasiliana* extract (84.43–91.58 % betacyanins loss after 21-day cold storage) or multiple emulsions (made with gum arabic and maltodextrin) of cactus pear extract (11.54–28.57 % betacyanins loss after 36-day cold storage) (Cenobio-Galindo et al., 2019; Guneser, 2021; Schneider-Teixeira et al., 2022).

3.2. Colour over storage period

Besides the viscosity and syneresis, colour is often another important attribute perceived by consumers that will influence their judgment on the quality of the product, thus colourant is often added to the yoghurts to improve the attractiveness of the product (Schneider-Teixeira et al., 2022). However, natural colourants normally have limitations in terms of colour stability, especially during long storage periods or when there are interferences with other food ingredients occur, hence the colour analysis of the yoghurts added with different betacyanins colourants is conducted and shown in Table 2. At Week 0, it can be seen that the L^* of YO-IF, YO-F and YO-B (91.01–91.35) were significantly lower than that of P-YO (94.33), due to the incorporation of betacyanins pigments colourants (Improved-FRDFD-dH_2O, FRDFD and E162) had reduced the lightness of the yoghurts. The YO-IF, YO-F and YO-B with lower L^* values are expected to be more favourable to consumers as compared to P-YO since it has been reported that consumers usually prefer lower brightness in dairy products (García-Pérez et al., 2005; Hutchings, 2011; Manzoor et al., 2019). Different from P-YO which had a negative a^* value (−1.63), the presence of betacyanins red pigments had resulted in positive a^* values (4.40–4.51) that refers to redness being observed in YO-IF, YO-F and YO-B at Week 0. The red colour properties of betacyanins are implied by the conjugation of a substituted aromatic nucleus to the chromophore (1,7-diazaheptamethinium resonance system with conjugated double bonds of betalamic acid) (Martínez-Rodríguez et al., 2022). There was no significant difference between the a^* of YO-IF, YO-F and YO-B, as three of them had been standardised to the same initial absorbance value and also had similar initial betacyanins concentrations, as mentioned in Section 3.1. Moreover, the addition of betacyanins colourant at Week 0 had significantly caused lower b^*

Table 2
Colour parameters of yoghurts added with different betacyanins colourants over 8-week storage period at 4 °C.

Parameters	Storage Period (Week)	YO-IF	YO-F	YO-B	P-YO
L*	**0**	91.35 ± 0.72^{aB}	91.01 ± 0.89^{bB}	91.29 ± 0.67^{bB}	94.33 ± 0.39^{aA}
	2	91.23 ± 0.88^{aB}	91.55 ± 0.89^{abB}	91.75 ± 0.78^{abB}	94.35 ± 0.56^{aA}
	4	91.41 ± 0.50^{aB}	91.82 ± 0.39^{abB}	92.02 ± 0.50^{abB}	94.58 ± 0.50^{aA}
	6	91.98 ± 0.39^{aC}	92.89 ± 0.28^{aBC}	92.78 ± 0.22^{aB}	94.59 ± 0.39^{aA}
	8	92.02 ± 0.78^{aB}	92.59 ± 0.39^{abB}	93.04 ± 0.29^{aB}	94.43 ± 0.40^{aA}
a*	**0**	4.42 ± 0.11^{aA}	4.51 ± 0.16^{aA}	4.40 ± 0.21^{aA}	−1.63 ± 0.06^{aB}
	2	4.33 ± 0.05^{aA}	3.93 ± 0.15^{bB}	3.81 ± 0.03^{bB}	−1.58 ± 0.06^{aC}
	4	4.35 ± 0.15^{aA}	4.11 ± 0.04^{bB}	2.78 ± 0.06^{cC}	−1.57 ± 0.06^{aD}
	6	3.70 ± 0.18^{bA}	3.55 ± 0.06^{cA}	1.42 ± 0.04^{dB}	−1.63 ± 0.06^{aC}
	8	3.56 ± 0.10^{bA}	2.59 ± 0.06^{dB}	1.37 ± 0.03^{dC}	−1.61 ± 0.01^{aD}
b*	**0**	5.30 ± 0.15^{bB}	5.35 ± 0.10^{cB}	5.40 ± 0.27^{dB}	8.35 ± 0.03^{cA}
	2	5.33 ± 0.15^{bC}	5.21 ± 0.12^{cC}	5.99 ± 0.03^{cB}	8.34 ± 0.02^{cA}
	4	5.31 ± 0.17^{bC}	5.34 ± 0.05^{cC}	6.01 ± 0.23^{cB}	8.57 ± 0.02^{aA}
	6	5.62 ± 0.03^{abD}	5.77 ± 0.03^{bC}	7.35 ± 0.10^{bB}	8.42 ± 0.01^{bA}
	8	5.87 ± 0.06^{aD}	6.58 ± 0.03^{aC}	7.92 ± 0.15^{aB}	8.39 ± 0.02^{bcA}
ΔE	**0–8**	1.24 ± 0.01^{C}	2.80 ± 0.26^{B}	4.32 ± 0.10^{A}	0.12 ± 0.01^{D}

Data is represented as mean ± standard deviation values ($n = 3$). ^{ab}Mean value with different superscript in each column of each parameter differs significantly ($p < 0.05$). ^{AB}Mean value with different superscript in each row differs significantly ($p < 0.05$). YO-IF, yoghurt with Improved-FRDFD-dH_2O (Improved Fermented Red Dragon Fruit Drink); YO-F, yoghurt with FRDFD (fermented red dragon fruit drink); YO-B, yoghurt with beetroot red (E162); P-YO, plain yoghurt (as control); L*, lightness/luminosity; *a**, red (+) / green (-) coordinate; *b**, blue (-) / yellow (+) coordinate; *ΔE*, total colour differences over 8-week storage.

values (yellowness) in YO-IF, YO-F and YO-B (5.30–5.40) as compared to P-YO (8.35).

Throughout the 8-week storage study at 4 °C, the P-YO remained stable without a significant change in *L*, a** and *b** colour parameters. However, remarkable increases in the L* and b* values and significant decreases in the a* values can be observed from Table 2 for both YO-F and YO-B during 8-week storage. This can be attributed to the degradation and structural modifications of red pigments betacyanins into betalamic acids (by hydrolysis) and neobetacyanins (by dehydrogenation) that are yellow in colour as well as orange-red 17-decarboxybetacyanins (by C-17 decarboxylation) which resulted in the loss of redness and increase of brightness and yellowness in the YO-F and YO-B, as commonly reported in the past studies (Gengatharan et al., 2017; Guneser, 2021; Herbach et al., 2006b, 2006a; Schneider-Teixeira et al., 2022). Although there was no significant loss of betacyanins being observed in YO-IF (as shown in Section 3.1), the minor betacyanins degradation had slightly contributed to the *a** and *b** values change also, but without affecting the *L** over the 8-week refrigerated storage. Nonetheless, as compared to YO-F and YO-B which experienced significant reductions in the *a** values almost every two weeks, the red colour of YO-IF was the most stable. This is because YO-IF was able to maintain the *a** value without any significant change until Week 4, then only experienced a significant change for once during Week 6. Meanwhile, similar trends of stability can also be observed in the *b** values. From Table 2, higher changes can be observed in the *a** values of all three yoghurts added with betacyanins colourants as compared to that of the *L** and *b** values, which is in accordance with the study of (Guneser, 2021) which had reported that the reaction rate for changes in the *a** values of the yoghurt added with commercial beetroot liquid colourant was higher than those for the *L** and *b** values at all storage temperatures, as a consequence of the changes in chemical structures and spectral properties of betacyanins. The total colour difference (*ΔE*) between the time of manufacture (Week 0) and the end of the storage (Week 8) is a vital indicator of the changes in food quality as it infers information on the perceivable changes in colour during processing and storage. By the end of this storage study, significant differences can be found in the *ΔE* values between all the yoghurts samples, whereby the P-YO had the lowest *ΔE* value (0.12), followed by the YO-IF (1.24), YO-F (2.80) and lastly the YO-B demonstrated the highest *ΔE* value (4.32). Generally, when the total colour difference is in the range of $3.5 < \Delta E < 5$, it can be considered as there is a very distinct colour difference in the analysed sample, in other words, a clear colour difference is noticeable by the eye of a standard observer (Mokrzycki and Tatol, 2011). Therefore, it can be deduced that the colour change in the YO-B is evident to the human eyes, while there is not much colour change in the YO-IF, YO-F and P-YO after eight weeks of storage at 4 °C and the human eyes would not be able to differentiate it.

3.3. pH over storage period

In general, the inoculation of milk with the yoghurt starter cultures (*L. bulgaricus* and *S. thermophilus*) helps to perform milk fermentation by consuming the lactose and producing lactic acid that aids in destabilising the casein micelles complex and denaturing the whey proteins through the solubilisation of the calcium and phosphate ions (Fan and Cliff, 2017). As these two yoghurt cultures work in synergy and bring down the pH to 4.6 (the isoelectric point of casein), the casein micelles would aggregate to form a cumulative/continuous gel network surrounded by fat and serum globules, thus, coagulating the milk proteins, entrapping the whey and the yoghurt is formed (Bahrami et al., 2013; Fan and Cliff, 2017; Sieuwerts et al., 2010; Soukoulis et al., 2007). It has been emphasised the importance of studying the yoghurt pH after incorporating with natural colourants (especially those made from juice) as it may have an effect not only on the colourant stability but also on the composition and properties of the resulting yoghurt product, where the results are shown in Table 3 (Cais-Sokolińska and Walkowiak-Tomczak, 2021). From Table 3, it can be observed that the addition of different betacyanins colourants did not cause a significant change in the pH of all

Table 3
pH of yoghurts added with different betacyanins colourants over 8-week storage period at 4 °C.

Storage Period (Week)	YO-IF	YO-F	YO-B	P-YO
0	4.51 ± 0.03^{aA}	4.50 ± 0.04^{aA}	4.49 ± 0.02^{aA}	4.61 ± 0.10^{aA}
2	4.50 ± 0.07^{aAB}	4.45 ± 0.04^{abBC}	4.39 ± 0.01^{bC}	4.56 ± 0.02^{abA}
4	4.54 ± 0.02^{aAB}	4.44 ± 0.03^{abB}	4.20 ± 0.02^{cC}	4.57 ± 0.06^{abA}
6	4.51 ± 0.01^{aA}	4.42 ± 0.02^{abB}	4.14 ± 0.03^{cdC}	4.49 ± 0.02^{abA}
8	4.53 ± 0.03^{aA}	4.41 ± 0.01^{bB}	4.13 ± 0.03^{dC}	4.46 ± 0.01^{bB}

Data is represented as mean ± standard deviation values ($n = 3$). ^{ab}Mean value with different superscript in each column differs significantly ($p < 0.05$). ^{AB}Mean value with different superscript in each row differs significantly ($p < 0.05$). YO-IF, yoghurt with Improved-FRDFD-dH_2O (Improved Fermented Red Dragon Fruit Drink); YO-F, yoghurt with FRDFD (fermented red dragon fruit drink); YO-B, yoghurt with beetroot red (E162), P-YO, plain yoghurt (as control).

the yoghurts (ranging from 4.49 to 4.61) at Week 0. During the refrigerated storage, although there is a small pH reduction in P-YO and YO-F from Week 0 to Week 6, the changes are statistically not significant. Instead, remarkable pH reductions were observed at Week 8 for both P-YO and YO-F. This observation could be associated with the post-acidification effect caused by the lactic acid produced from the metabolic activities of *L. bulgaricus* and *S. thermophilus* through the consumption of lactose in the yoghurts during refrigerated storage (Gengatharan et al., 2017; Guneser, 2021; Schneider-Teixeira et al., 2022). Meanwhile, YO-B demonstrated the largest change in the pH value among all the yoghurt samples, where its pH value experienced a significant reduction almost every two weeks, leaving only pH 4.13 by the end of the storage study at 4 °C. Besides the factor of post-acidification effect, the drastic pH reduction in YO-B could be mainly attributed to the betalamic acid produced from the high degradation rate of betacyanins, as shown in Section 3.1. In contrast, only YO-IF was able to remain stable without any significant change in the pH values (ranging from 4.50 to 4.54) throughout the 8-week storage at 4 °C. This could be due to the betacyanins content (both betanin and isobetanin) in the YO-IF being stable (as discussed in Section 3.1), hence free of extra betalamic acid production that can contribute to the pH reduction. Besides that, the presence of CA from Improved-FRDFD-dH_2O in YO-IF could have also served as an effective buffering agent to resist drastic pH change caused by the post-acidification effects of the lactic acid (Lulek et al., 2022; Ścibisz et al., 2019; Tamime and Deeth, 1980). Although all the yoghurt samples exhibited pH values between 4.13 to 4.61 which were within the limit (pH 4.6 or below) prescribed by the Food and Drug Administration (FDA) in 2023, the Improved-FRDFD--dH_2O is still considered the best betacyanins colourant that can be applied to produce a functional coloured yoghurt (YO-IF) with the most stable pH in the present study (FDA, 2023).

3.4. Viscosity and syneresis over storage period

Generally, the yoghurt's viscosity (the resistance of a fluid to flow) can be affected by many factors like homogenisation, heat treatment, pH, parameters of processing and storage conditions (Hematyar et al., 2012; Miller, 2012). As a typical oil-in-water emulsion, the fat in the yoghurt milk tends to separate upon standing (Tamime and Robinson, 2007). Hence, in the present study, the homogenised and pasteurised milk was used to produce the yoghurt as it contained fat globules that were reduced in size (through homogenisation), with promoted interaction between whey proteins and casein as well as free of other microorganisms' competitions (through pasteurisation) that may limit the growth of starter LAB culture, hence able to enhance the yoghurt viscosity, stabilise the gel (lower whey separation) and reduce creaming (separation of the fat-enriched layer from the aqueous phase) especially during the incubation and storage period (Chandan, 2011; Fan and Cliff, 2017; Soukoulis et al., 2007). It has been suggested that the colourants from natural sources like fruits and vegetables to be added into the yoghurt must be easily dispersible in yoghurt without causing texture defects like drastic changes in viscosity and syneresis (Hui, 1993). From Table 4, it can be seen that the addition of different betacyanins natural colourants did not cause significant change to the viscosity of the yoghurts at Week 0 (ranging from 1218.67 cP to 1243.90 cP). The 8-week storage study at 4 °C revealed that the P-YO experienced a significant viscosity change at Week 4 but was able to remain stable until the end of the study, with a total of 12.98 % reduction in the viscosity value. On the other hand, although there was a slight decrement in the viscosity values during the storage, there was no significant viscosity change observed for both YO-IF and YO-F. This indicates that the incorporation of Improved-FRDFD-dH_2O and FRDFD was able to improve the yoghurt's viscosity storage stability, especially the YO-IF which had the lowest viscosity change of −0.54 % only. In contrast, the YO-B experienced the highest reduction of 17.52 % in the viscosity value (from 1218.67 cP to 1005.20 cP) among all the yoghurt samples in the present study.

Table 4
Viscosity (cP) of yoghurts added with different betacyanins colourants over 8-week storage period at 4 °C.

Storage Period (Week)	YO-IF	YO-F	YO-B	P-YO
0	1236.00 ± 69.74^{aA}	1243.90 ± 17.44^{aA}	1218.67 ± 42.77^{aA}	1221.13 ± 72.61^{aA}
2	1242.67 ± 32.08^{aA}	1238.67 ± 43.14^{aA}	1110.67 ± 10.07^{bB}	1160.00 ± 20.00^{abB}
4	1235.33 ± 36.07^{aA}	1206.67 ± 17.24^{aA}	1048.00 ± 28.00^{bcB}	1075.33 ± 8.33^{bB}
6	1228.00 ± 21.17^{aA}	1174.67 ± 58.29^{aAB}	1013.13 ± 10.07^{cC}	1058.67 ± 64.29^{bBC}
8	1229.33 ± 59.91^{aA}	1132.00 ± 76.21^{aAB}	1005.20 ± 40.97^{cB}	1062.67 ± 49.69^{bB}
Percentage change	−0.54 %	−9.00 %	−17.52 %	−12.98 %

Data is represented as mean ± standard deviation values ($n = 3$). ^{ab}Mean value with different superscript in each column differs significantly ($p < 0.05$). ^{AB}Mean value with different superscript in each row differs significantly ($p < 0.05$). YO-IF, yoghurt with Improved-FRDFD-dH_2O (Improved Fermented Red Dragon Fruit Drink); YO-F, yoghurt with FRDFD (fermented red dragon fruit drink); YO-B, yoghurt with beetroot red (E162), P-YO, plain yoghurt (as control).

Besides viscosity, syneresis which refers to the whey separation from the yoghurt's gel matrix (due to the reduction of whey proteins' connection power caused by the shrinkage of the three-dimensional structure of a protein network in yoghurt) is also another common primary defect of yoghurt during storage that can influence the shelf life of yoghurt as it becomes visible on the surface and affect the yoghurt's appearance and texture, hence serving as an important quality indicator of yoghurt (as shown in Fig. 1) (Gengatharan et al., 2017; Sigdel et al., 2018). At Week 0, it can be observed that all the yoghurts samples added with different betacyanins natural colourants (YO-IF, YO-F and YO-B) had significantly lower syneresis percentages (3.4–4.5 %) as compared to P-YO (9.3 %). This is in accordance with the results of Gengatharan et al. (2017), which suggested that the presence of betacyanins could help retain the yoghurts' gel firmness like other phenolic compounds through hydrogen bonding and electrostatic interaction. Besides that, it has been suggested that the incorporation of natural colourants especially from fruit juice/drink like FRDFD and Improved-FRDFD-dH_2O that contain mucilage may increase the total solid and hence the water-holding capacity of the yoghurts, contributing to the stable viscosity and reduced syneresis of YO-F and YO-IF (Aleman et al., 2023). During the 8-week storage period, all three coloured yoghurts, YO-IF, YO-F and YO-B also showed lower syneresis percentages (ranging from 3.4 % to 6.4 %) compared to that of P-YO (9.3–10.3 %). However, the YO-F, YO-B and P-YO experienced significant syneresis increments during the storage study, indicating that there were more releases of whey caused by the decrease in water holding capacity (Gengatharan et al., 2017). Similar observations of increasing syneresis percentages in all yoghurt samples (plain and supplemented with E162 or RDF extract) were reported by Gengatharan et al. (2017). This was attributable to the reduction in the pH values of these three yoghurts (as discussed in Section 3.3) which had caused a contraction effect in the casein micelle matrix and disorganization of the casein network, leading to an increasing whey elimination from the yoghurt gel network (Flores-Mancha et al., 2021; Gengatharan et al., 2017; Golmakani et al., 2021). Moreover, the reduction in the betacyanins (especially betanin) concentration (as mentioned in Section 3.1) could have resulted in the loss of hydrogen bonding and electrostatic interaction to retain the gel firmness of the yoghurts, thus leading to relatively greater syneresis percentage increments in both YO-F (from 3.5 % to 4.7 %), YO-B (from 4.5 % to 6.4 %) as compared to P-YO (from 9.3 % to 10.3 %) by the end of the storage study.

It is worth noting that, only YO-IF was able to remain no significant change in the syneresis percentages (3.4–3.9 %) throughout this 8-week

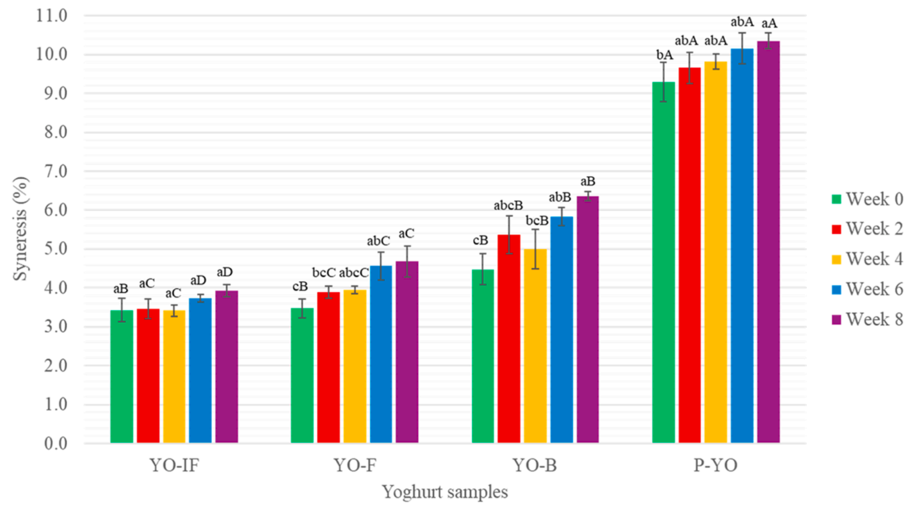

Fig. 1. Syneresis (%) of yoghurts added with different betacyanins colourants over 8-week storage period at 4 °C. Data is represented as mean ± standard deviation values ($n = 3$). ^{ab}Mean value with different superscript indicate significant difference of a yoghurt sample over different week ($p < 0.05$). ^{AB}Mean value with different superscript indicate significant difference between different yoghurt samples at the same week ($p < 0.05$). YO-IF, yoghurt with Improved-FRDFD-dH$_2$O (Improved Fermented Red Dragon Fruit Drink); YO-F, yoghurt with FRDFD (fermented red dragon fruit drink); YO-B, yoghurt with beetroot red (E162), P-YO, plain yoghurt (as control).

storage study at refrigerated temperature. This syneresis result is in accordance with the viscosity result of YO-IF which revealed that the incorporation of Improved-FRDFD-dH$_2$O is the best choice in the present study to produce a potential functional coloured yoghurt with the most stable and improved texture properties (specifically, viscosity and syneresis). Many factors could have contributed to these observations. Aside from the aspects of having a stable betacyanins content (as discussed in Section 3.1) and pH (as discussed in Section 3.3) to help retain the yoghurts' gel firmness, the presence of XG and CMC from Improved-FRDFD-dH$_2$O in the YO-IF could have played vital roles as well. In the realm of food development, it is very common for food industry players to include at least one or combination of more than one type of hydrocolloids (like XG and CMC) as the stabilisers and thickeners to combat the yoghurt storage defects of variation in viscosity and syneresis (Hematyar et al., 2012; Norziah et al., 2006). The present viscosity and syneresis results are comparable with the previous studies which found that the incorporation of 0.5 % CMC was able to produce a yoghurt with low syneresis (90.66 % stability), while the addition of XG even at low concentrations (0.005–0.01 %) was also able to increase the firmness and reduce the syneresis of the yoghurt (Hematyar et al., 2012; Sebayang, 2019). The ability of YO-IF to possess stable viscosity and low syneresis percentage during the 8-week storage at 4 °C is attributable to the interaction between the XG and CMC (from Improved-FRDFD-dH$_2$O) with the yoghurt milk portion through a few mechanisms. Firstly, both XG and CMC have hydroxyl (–OH) groups that can bind with water molecules through hydrogen bonds and maintain the viscosity of the yoghurt's continuous phase (Hansen, 1994; Tamime and Robinson, 2007). This will then help to contribute to the mesh effect in the three-dimensional (3D) gel network formed in the yoghurt, thereby trapping the water in the structure and favouring water retention (increasing the water holding capacity) (Flores-Mancha et al., 2021; Ren et al., 2024). By retarding the free movement of water, the protein molecules can be stabilised in the form of a network (Tamime and Robinson, 2007). Moreover, the hydrocolloid mixture solution of XG and CMC can also trap the casein aggregates within the viscous hydrocolloid mixture solution (Hematyar et al., 2012). Furthermore, the food hydrocolloids are also capable of reacting with the milk constituents (mainly the proteins) to elevate their hydration level (Tamime and Robinson, 2007). In this context, both XG and CMC as the anionic polysaccharides are able to interact with the positive charges on the surface of casein micelles as well to form soluble complexes that are stable to storage, thereby strengthening the casein network and minimising syneresis (Hansen, 1994; Hematyar et al., 2012; Tamime and Robinson, 2007).

3.5. *Viability of lactic acid bacteria (LAB) over storage period*

The LAB (*L. bulgaricus* and *S. thermophilus*) inoculated in the milk does not only function to produce lactic acid and form the yoghurt with a unique sour taste but also plays an important role in producing antimicrobial compounds (such as hydrogen peroxide, acetaldehyde and bacteriocins) that can inhibit the growth of other microorganisms to improve the quality and safety of the yoghurts, therefore the viability of the LAB in the yoghurts is important to be assessed after adding with natural colourants (Table 5) (Fan and Cliff, 2017). The results in the present study showed that the incorporation of different betacyanins colourant did not cause a significant effect on the LAB counts in all the yoghurt samples at Week 0. The total LAB counts (sums of *L. bulgaricus* and *S. thermophilus*) of all the yoghurt samples (8.52–8.66 log CFU/mL) at Week 0 were higher than the minimum level (≥ 8 log CFU/mL) required by the National Yoghurt Association (USA) for the yoghurts to

Table 5
Total viable counts (log CFU/mL) of lactic acid bacteria (LAB) of yoghurts added with different betacyanins colourants over 8-week storage period at 4 °C.

Storage Period (Week)	YO-IF	YO-F	YO-B	P-YO
0	8.52 ± 0.20^{aA}	8.54 ± 0.15^{aA}	8.53 ± 0.23^{aA}	8.66 ± 0.17^{aA}
2	8.48 ± 0.16^{aA}	8.35 ± 0.23^{abA}	8.53 ± 0.13^{aA}	8.47 ± 0.14^{aA}
4	8.40 ± 0.18^{aA}	8.30 ± 0.14^{abA}	8.42 ± 0.21^{aA}	8.29 ± 0.15^{aA}
6	8.30 ± 0.14^{aA}	8.27 ± 0.10^{abA}	8.10 ± 0.13^{abA}	8.36 ± 0.15^{aA}
8	8.30 ± 0.16^{aA}	8.05 ± 0.13^{bAB}	7.92 ± 0.06^{bB}	8.35 ± 0.10^{aA}

Data is represented as mean ± standard deviation values ($n = 3$). ^{ab}Mean value with different superscript in each column differs significantly ($p < 0.05$). ^{AB}Mean value with different superscript in each row differs significantly ($p < 0.05$). YO-IF, yoghurt with Improved-FRDFD-dH$_2$O (Improved Fermented Red Dragon Fruit Drink); YO-F, yoghurt with FRDFD (fermented red dragon fruit drink); YO-B, yoghurt with beetroot red (E162), P-YO, plain yoghurt (as control).

be considered as "contains live and active cultures" at the time of manufacture (Gengatharan et al., 2017; Mani-López et al., 2014).

However, it can be noted that during the refrigerated storage, the viability of LAB in YO-F and YO-B declined significantly at Week 8, with YO-B experiencing the highest reduction (−0.61 log CFU/mL) in microbial counts among all the yoghurt samples. It has been suggested that the viability of LAB can be influenced by many factors, such as the pH, food matrix, food ingredients and additives, storage duration and temperature (Tripathi and Giri, 2014). The significant and highest decrement of the LAB count in YO-B was mainly attributed to the remarkable pH reduction (caused by betalamic acid as mentioned in Section 3.2) that has hindered the growth and stability of LAB (Tripathi and Giri, 2014). Meanwhile, it has been reported that the LAB culture was metabolically more active in natural yoghurt like P-YO as compared to yoghurt that was blended/mixed with fruit samples like YO-F as a consequence of the presence of additional acids such as acetic acids from the FRDFD might inhibit the growth of LAB (Do Espírito Santo et al., 2011; Foong et al., 2012; Ścibisz et al., 2019; Xia et al., 2022). On the other hand, the LAB viability in YO-IF remained stable (without a significant change) throughout the 8-week storage study and also had no significant difference compared to P-YO. Since both YO-IF and YO-F were able to maintain LAB viability > 8 log CFU/mL without any significant difference as compared to P-YO throughout the 8-week refrigerated storage study, this indicated that the potassium sorbate from the Improved-FRDFD-dH$_2$O and FRDFD would not cause a significant antimicrobial effect on the LAB, possibly due to the final concentration of potassium sorbate in the yoghurts was too low. Therefore, the findings in the present study revealed that the incorporation of Improved-FRDFD-dH$_2$O did not cause adverse effects on the LAB viability but instead could sustain the viability of both LAB culture steadily in the YO-IF during the prolonged (8-week) storage at 4 °C, possibly through the provision of a stable pH environment (thanks to the buffering agent, CA) (Ścibisz et al., 2019; Tamime and Deeth, 1980). Nonetheless, during the entire storage study, the LAB counts in all the yoghurt samples were maintained at 7.92–8.66 log CFU/mL, which met the level recommended by the FDA (≥ 6 log CFU/mL) and the Codex Standard For Fermented Milks (CXS 243–2003) (≥ 7 log CFU/mL) for a yoghurt that is to be considered as "contains live and active cultures" throughout the storage period (Codex Alimentarius Commission (CAC), 2022; FDA, 2021). With these minimum counts of live and active cultures, all the yoghurts including YO-IF in the current study are considered as being able to provide the benefits of balancing the normal intestinal microbiota levels, overcoming lactose intolerance and reducing the risk of colon cancer (Anuyahong et al., 2020).

3.6. Antioxidant activity over storage period

In recent years, yoghurt has been discovered as a good carrier for functional food ingredients and antioxidant compounds (O'Sullivan et al., 2016). This has led to a rising interest in enhancing the antioxidant characteristics of yoghurt through the incorporation of fruit byproducts, drinks/juices or extracts that are rich in natural pigments and beneficial bioactive compounds (Balasundram et al., 2006). Hence, the ABTS and DPPH assays were employed to investigate the potential of the incorporation of different natural betacyanins colourants to improve the free radical scavenging capacity of the yoghurt, where the results are shown in Table 6. Before the addition of any betacyanins colourant, it can be observed that the P-YO was also exhibiting scavenging capacity against the ABTS and DPPH radicals throughout the 8-week refrigerated storage study. The capability of plain/natural yoghurt (like P-YO) to exert antioxidant effects is mainly contributed by the whey and casein proteins as well as many bioactive amino acids and low-molecular-weight peptides (that were released as a result of milk fermentation) which possess the ability to donate electrons and hydrogen atoms. Moreover, previously it has been commonly reported that the presence of many other compounds like vitamins C, A and E,

Table 6
Free radicals scavenging capacity (mM TEAC) of yoghurts added with different betacyanins colourants over 8-week storage period at 4 °C.

Assays	Storage Period (Week)	YO-IF	YO-F	YO-B	P-YO
ABTS	**0**	0.90 ± 0.04^{aA}	0.88 ± 0.01^{aA}	0.87 ± 0.02^{aA}	0.78 ± 0.01^{aB}
	2	0.93 ± 0.02^{aA}	0.86 ± 0.01^{abB}	0.83 ± 0.02^{aB}	0.71 ± 0.02^{bC}
	4	0.88 ± 0.03^{aA}	0.82 ± 0.03^{bcAB}	0.75 ± 0.04^{bB}	0.74 ± 0.03^{abB}
	6	0.86 ± 0.03^{aA}	0.78 ± 0.02^{cB}	0.68 ± 0.02^{cC}	0.66 ± 0.00^{cC}
	8	0.78 ± 0.03^{bA}	0.72 ± 0.00^{dB}	0.61 ± 0.02^{dC}	0.59 ± 0.01^{dC}
	Percentage retained	86.07 %	81.51 %	69.65 %	75.98 %
DPPH	**0**	1.37 ± 0.03^{aAB}	1.42 ± 0.08^{aA}	1.35 ± 0.03^{aAB}	1.23 ± 0.01^{aB}
	2	1.31 ± 0.07^{aA}	1.27 ± 0.01^{bAB}	1.21 ± 0.00^{bB}	1.21 ± 0.01^{abB}
	4	1.26 ± 0.04^{abA}	1.26 ± 0.06^{bA}	1.15 ± 0.05^{bA}	1.13 ± 0.06^{bA}
	6	1.30 ± 0.05^{abA}	1.22 ± 0.01^{bB}	0.86 ± 0.02^{cC}	0.81 ± 0.03^{cC}
	8	1.19 ± 0.03^{bA}	1.01 ± 0.02^{cB}	0.79 ± 0.01^{cC}	0.76 ± 0.04^{cC}
	Percentage retained	86.69 %	71.55 %	58.24 %	61.88 %

Data is represented as mean ± standard deviation values ($n = 3$). ^{ab}Mean value with different superscript in each column differs significantly ($p < 0.05$). ^{AB}Mean value with different superscript in each row differs significantly ($p < 0.05$). YO-IF, yoghurt with Improved-FRDFD-dH$_2$O (Improved Fermented Red Dragon Fruit Drink); YO-F, yoghurt with FRDFD (fermented red dragon fruit drink); YO-B, yoghurt with beetroot red (E162), P-YO, plain yoghurt (as control).

uric acid and carotenoids were also responsible for the total antioxidant capacity of a plain yoghurt (P-YO) (Papadaki and Roussis, 2022; Perna et al., 2014; Stobiecka et al., 2022). The incorporation of Improved-FRDFD-dH$_2$O, FRDFD and E162 had further enhanced the antioxidant activity of the yoghurts, producing the YO-IF, YO-F and YO-B which possessed higher free radical scavenging capacities against the ABTS (0.61–0.90 mM TEAC) and DPPH (0.79–1.42 mM TEAC) radicals compared to that of the YO-P (ABTS: 0.59–0.78 mM TEAC; DPPH: 0.76–1.23 mM TEAC) throughout the whole 8-week storage study. This is appreciable to the presence of the bioactive pigments betacyanins which are known to be potent antioxidants (Choo et al., 2019; Gengatharan et al., 2017; Ravichandran et al., 2013). Similar improved antioxidant capacities were also reported for the yoghurts added with RDF pulps water extract, E162 or mashed RDF in past studies (Gengatharan et al., 2017; Zainoldin and Baba, 2009).

The free radical scavenging capacities of all the yoghurts were found to decrease over the storage period, which is corroborated by the study of (Gengatharan et al., 2017). Among all the yoghurt samples, the ABTS and DPPH radicals scavenging capacity of YO-B declined the most, retaining only 69.65 % and 58.24 % of its initial antioxidant activities in ABTS and DPPH assays, respectively. This is corresponding to the highest degradation rate of betacyanins observed in YO-B (as mentioned in Section 3.1), leading to the loss in the antioxidant activities. In contrast, only YO-IF was able to maintain its free radicals scavenging capacity without any significant change for 6 weeks continuously (Week 0 to Week 6), retaining ≥ 86 % of its initial antioxidant activities in both ABTS and DPPH assays at the end, which was also the highest retainment percentage among all the yoghurt samples. Moreover, by the end of the 8-week storage period, the ABTS and DPPH radicals scavenging capacities (0.78 mM TEAC and 1.19 mM TEAC, respectively) of the YO-IF were also the highest compared to the other three yoghurt samples. This is contributed by the highly stable betacyanins of

Improved-FRDFD-dH_2O in YO-IF to serve as the hydrogen and electron donor steadily throughout the storage study. Interesting to note that, the YO-IF that contains Improved-FRDFD-dH_2O was found to have higher retaining ($\geq$ 86 %) of its initial antioxidant activity as compared to that of the yoghurt added with multiple emulsions (made with gum arabic and maltodextrin) of cactus pear extract ($>$ 70 %) in the study of (Cenobio-Galindo et al., 2019). This justifies the Improved-FRDFD-dH_2O developed from the direct application of 0.4 % XG and 0.5 % CMC hydrocolloid mixture solution and 0.2 % CA in FRDFD was able to better protect and maintain the antioxidant activity of the betacyanins as compared to the multiple emulsions method for producing a stable functional colourant. The present study also proves that having a natural colourant with stabilised betacyanins is not only important for maintaining the colour stability of the yoghurt (as discussed in Section 3.2) but also very crucial for improving and sustaining the antioxidant activity of the yoghurt that is to be claimed as a "functional yoghurt".

4. Conclusion

In a nutshell, the innovative potential of Improved-FRDFD-dH_2O [a functional drink produced from fermented red dragon fruit drink (FRDFD) added with 0.4 % XG and 0.5 % CMC hydrocolloid mixture solution and 0.2 % citric acid] as a sustainable, effective and stable functional colourant in yoghurt (as a drink model) was successfully evaluated in this work. The results in the present study revealed that the syneresis of the yoghurt (YO-IF) incorporated with Improved-FRDFD-dH_2O was significantly lowered, while the presence of betacyanins from Improved-FRDFD-dH_2O remarkably increased the redness and enhanced the free radical scavenging activities of the yoghurt. Most importantly, the 8-week storage study at 4 °C showed that owing to the highly stable betacyanins of YO-IF ($<$ 5 % of degradation), there was only a very small difference ($\Delta E < 1.5$) in the total colour changes of YO-IF (unnoticeable by a standard observer) and $>$ 86 % of its initial free radical scavenging capacities was retained by the end of the storage. Moreover, YO-IF was able to maintain its pH, viscosity, syneresis and LAB viability without any significant change throughout the 8-week refrigerated storage. The Improved-FRDFD-dH_2O undoubtedly showed the best performance as a low-cost, natural and sustainable functional ingredient that was not only can impart the colouring function but also able to improve the yoghurt's physicochemical characteristics stability and produce a functional yoghurt with enhanced antioxidant activity. This study successfully addressed various goals of the United Nations 2030 Agenda for Sustainable Development.

CRediT authorship contribution statement

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

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.

Data availability

Data will be made available on request.

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Ethical statement

The authors declare that there is no ethics requirement related to this research.

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