International Food Research Journal 30(1): 216 - 228 (February 2023)
Journal homepage: *http://www.ifrj.upm.edu.my*

Production of yoghurt with *Clitoria ternatea* flower extract supplementation, and its stability during storage

[1,2]*Prastowo, I., [1]Sundari, W., [1]Hanifah, M. R., [3]Octaviana, S., [4,5]Ahda, M., [1]Moro, H. K. E. P. and [1]Narusman, A. A.

[1]*Department of Biology Education, Faculty of Teacher Training and Education, Universitas Ahmad Dahlan, Jalan Ahmad Yani, Tamanan, Banguntapan, Bantul, Yogyakarta 55191, Indonesia*
[2]*Research Center for Food Fermentation Studies, Universitas Ahmad Dahlan, Jalan Ahmad Yani, Tamanan, Banguntapan, Bantul, Yogyakarta 55191, Indonesia*
[3]*Research Center for Biotechnology, National Research and Innovation Agency, Cibinong Sciences Center, Jalan Raya Jakarta-Bogor km 46, Cibinong, Kabupaten, Bogor 16911, Indonesia*
[4]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Ahmad Dahlan, Jalan Prof. Dr. Soepomo, S.H., Warungboto, Yogyakarta 55164, Indonesia*
[5]*Ahmad Dahlan Halal Center, Universitas Ahmad Dahlan, Jalan Prof. Dr. Soepomo, S.H., Warungboto, Yogyakarta 55164, Indonesia*

Article history

Received:
10 March 2022
Received in revised form:
24 May 2022
Accepted:
15 July 2022

Keywords

yoghurt,
stability,
microbial growth,
antioxidant activity,
Clitoria ternatea flower extract

Abstract

The aim of the present work was to analyse 24 h yoghurt fermentation supplemented with *Clitoria ternatea* flower (CTF) extracts (0 - 10%); especially elucidating the relationship between antioxidant activity, carbohydrate constituent, and microbial growth which has never been reported. Carbohydrate constituent in the CTF was also investigated for the first time. Colour changes was also assessed during yoghurt production. Furthermore, the stability of yoghurt was studied during the 7 d storage under low temperature (4°C). The supplementation of CTF extracts (0 - 10%) into yoghurt increased the antioxidant activity (up to 46.65 ± 0.29%) and carbohydrate concentration (glucose, up to 9.63 ± 0.3%; sucrose, up to 7.8 ± 0.5%; inulin, up to 5.7 ± 0.8%; and pectin, up to 7.5 ± 0.3%), but decreased dissolved oxygen (DO) down to 0.65 ± 0.023 mg/L in the medium during fermentation. Surprisingly, prebiotic sugars of inulin and pectin were discovered in CTF. The presence of higher carbohydrate concentration and more anaerobic condition enabled *Lactobacillus delbrueckii* subsp. *bulgaricus* to grow up to 7.74 ± 0.1 log CFU/mL. In contrast, the final cell concentration of *Streptococcus thermophillus* decreased up to 8.12 times as the extract concentrations increased. However, the viability of both bacteria still met the international standards (≥ 7 log CFU/mL). The yoghurt colour turned from light turquoise to purple (L* = 69.47 ± 0.2; a* = 14.78 ± 0.15; b* = - 21.77 ± 0.2) as the pH decreased to 4.5 ± 0.11, and the lactic acid concentration increased up to 1.74 ± 0.37%. Furthermore, the quality of yoghurt in all parameters was relatively stable during storage for antioxidant activity, microbial growth, carbohydrate constituent, DO, lactic acid concentration, anthocyanin content, and pH; meanwhile colour changes only decreased 0 - 0.39 times.

DOI
https://doi.org/10.47836/ifrj.30.1.18

Introduction

Since the outbreak of COVID-19 pandemic, there is a major cultural shift from the conventional lifestyle to healthy lifestyle, including the consumption of healthy foods (Qureshi, 2021). One of the healthy foods is yoghurt, a probiotic food made by bacterial fermentation of milk which has a long history in some cuisines as dips, dressings, and drinks (Szołtysik *et al*., 2020). The consumption of yoghurt shows positive benefits since it aids the digestive system and promotes the immune system (Hamad *et al*., 2020).

In recent days, innovations and modifications have been carried out to enrich the nutritional value of yoghurt. One of which is the supplementation of natural ingredients into yoghurt. Researchers reported that the supplementation of plants extract into yoghurt

*Corresponding author.
Email: papa_fadin@yahoo.com

has shown significant impacts on the yoghurt nutrition (Szołtysik *et al*., 2020). Szołtysik *et al*. (2021) reported that the supplementation of fruit extract into yoghurt increased polyphenol concentration which is related to the antioxidant activity. Meanwhile, the supplementation of plants extract into yoghurt also increased the concentration of sugars which play important role in microbial growth in the yoghurt (Hamad *et al*., 2020). However, studies elucidating the effect of the work of antioxidant activity on microbial growth in a yoghurt fermentation process are quite limited.

Another plant extract that could potentially enrich the yoghurt nutritional value is flower extract. One of the flowers that has gained popularity due to its potential nutritional value is *Clitoria ternatea*, a flowering plant belongs to the Fabaceae family, and native to equatorial South and Southeast Asia (Nair *et al*., 2015). The flower extract of *C. ternatea* has been used in many purposes, ranging from medicines to foods (Oguis *et al*., 2019). For instance, Chusak *et al*. (2018) reported the use of *C. ternatea* flower (CTF) extract in bread fortification. Lakshan *et al*. (2019) developed a non-alcoholic drink based on the CTF extract. Butelase 1, an enzyme mediated the protein ligation, was discovered from the CTF extract that would be advantageous for future protein engineering (Hemu *et al*., 2019). It is also reported that CTF has substances (triterpenoids, anthocyanin, flavonols, cliotides, *etc*.) that potentially have good benefits for the health. Further, CTF extract was reported to show high antioxidant activity that could potentially inhibit the growth of cancer cells (Oguis *et al*., 2019). Additionally, flowers naturally contain a wide variety of carbohydrates (glucose, sucrose, cellulose, pectin, *etc*.) (Marchyshyn *et al*., 2020; Carillo *et al*., 2022). However, information related to the carbohydrate constituent in CTF remain scarce.

Therefore, in the present work, we developed a yoghurt supplemented with CTF extract in order to enhance the functional value of yoghurt. Carbohydrate constituent in tCTF was also investigated. To the best of our knowledge, carbohydrate constituent in the CTF has not been reported elsewhere. The present work also elucidated the relationship between carbohydrate constituent, antioxidant activity, and the growth of *Streptococcus thermophillus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in yoghurt during fermentation as a result of the CTF extract supplementation, which also has not been reported elsewhere. Some researchers reported the supplementation of CTF extract into yoghurt without clearly discussing on the relationship between those three components (Suharman *et al*., 2021; Sutakwa *et al*., 2021). The colour changes in yoghurt were also observed during fermentation as the pH decreased. In food science, other than attracting consumers' attention, colour could also be an indicator for a particular chemical reaction in foods. The stability of yoghurt properties (antioxidant activity, anthocyanin content, colour, DO, pH, lactic acid concentration, carbohydrate concentration, and microbial growth) was also observed during storage at low temperature. Low temperature in storage systems has long been carried out to maintain product quality (Szołtysik *et al*., 2021).

Materials and methods

Materials

C*litoria ternatea* flowers (CTF) were obtained from a local farm in Bantul, the Special Region of Yogyakarta, Indonesia in June 2021 (after harvest). The flowers were subsequently identified by Dr. Hadi Sasongko (M.Si) from the Department of Biology, Faculty of Applied Science and Technology, Universitas Ahmad Dahlan, Indonesia. Pasteurised milk was obtained from PT Ultrajaya Milk Industry (Bandung, Indonesia). Starter culture (containing *Streptococcus thermophillus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) was obtained from PT Cisarua Mountain Diary (Bogor, Indonesia). MRS media, M-17 media, Folin-Ciocalteu reagent, Na_2CO_3, 1-diphenyl-2-picrylhydrazyl (DPPH), carbazole, and ascorbic acid were purchased from Merck KgaA (Darmstadt, Germany).

C*litoria ternatea flower extract preparation*

The extract preparation was conducted according to Oliveira *et al*. (2018) with some modifications. *Clitoria ternatea* flowers were firstly dried, and then pulverised using an electric pulveriser (Mitochiba CH 100, Japan). The powder was later sifted using an 85 mesh-electrical vibrating sifter (Kencana MS 3Mn, Indonesia). The powder (glucose: 9.22 - 9.5%, sucrose: 7.8 - 8%, inulin: 5.7 - 6%, and pectin: 7.5 - 8.2%) was sterilised at 125°C for 10 min. The powder (0, 2, 4, 6, 8, and 10 g) was subsequently macerated in sterilised water (100 mL) at room temperature for 5 min. The mixtures were filtered, and later stored at 5°C until used.

Yoghurt production

The production of yoghurt was carried out according to Szołtysik *et al*. (2020) with some modifications. The yoghurt starter (20 mL) was added into 200 mL of pasteurised milk (lactose: 4.51 – 4.59 g/L, protein: 6 – 6.8 g/L). About 100 mL of the CTF extracts (0, 2, 4, 6, 8, and 10% [w/v]) were added into the mixture, and then incubated at 44°C and 60 rpm for 24 h. Polyphenolic compounds, antioxidant activity, microbial growth (*S. thermophillus* and *L. bulgaricus*), carbohydrate constituent, dissolved oxygen (DO), total acid production, pH, anthocyanin content, and colour changes were later determined every 6 h of 24 h yoghurt fermentation. The control was a yoghurt added with glucose (2.1 g/L) and sucrose (3 g/L) with no antioxidant supplementation, and a yoghurt supplemented with 2% ascorbic acid.

Stability during storage

The stability analysis of yoghurt during storage was carried out according to Szołtysik *et al*. (2021) with some modifications. After 24 h fermentation, the yoghurts were stored at 4°C for 7 d. Antioxidant activity, lactic acid concentration, carbohydrate concentration, anthocyanin content, DO, the growth of *S. thermophillus* and *L. bulgaricus*, as well as colour and pH changes were subsequently evaluated at days 1, 3, and 7.

Analyses

Total phenolic content

The total phenolic content (TPC) was determined according to Szołtysik *et al*. (2021) using Folin-Ciocalteu reagent. The TPC were expressed as a mg of gallic acid equivalents (GAE) per gram of sample.

Anthocyanin content

The anthocyanin content was determined according to Veazie *et al*. (2020) using a pH differential method at 520 and 700 nm, and expressed as a mg of cyanidin-3-glucoside equivalent (CGE) per gram of sample.

Antioxidant activity

The antioxidant activity was determined according to Hamad *et al*. (2020) using DPPH as the free radical, and expressed as the percentage of antioxidant capability to scavenge DPPH.

Microbial growth

The microbial growth was analysed according to Szołtysik *et al*. (2020). Samples were diluted in a serial dilutions and then poured into M-17 and MRS bacterial medium for *S. thermophillus* and *L. bulgaricus*, respectively. The bacterial cells of *S. thermophillus* and *L. bulgaricus* were incubated in an anaerobic condition at pH 5.4 and 37°C, for 48 and 72 h, respectively. The bacterial colonies were counted at the end of cultivation.

Carbohydrate constituent

Glucose and sucrose were determined according to Widyaningrum *et al*. (2016) using HPLC (Shimadzu, Japan). Pectin was determined according to Gomez *et al*. (2014) using HPLC (Shimadzu, Japan). Lactose was determined according to Khabibullaev *et al*. (2019) using HPLC (Shimadzu, Japan). Inulin was determined at 560 nm according to Winarti *et al*. (2011) using carbazol as the reagent.

pH changes and total acid production

The pH changes were determined using a pH meter (Xylem Analytics Lab 865, Germany). The total acid concentration was determined by titrating the samples using NaOH solution (0.1 N), and expressed as the percentage of lactic acid (gram) per litre of the yoghurt (Szołtysik *et al*., 2021). Phenolphthalein was used as the indicator. The results were confirmed using GC (Shimadzu, Japan).

Colour changes

The colour changes were determined according to Szołtysik *et al*. (2021) using a colorimeter (Konica Minolta CR-10 Plus, Japan) which was connected to a computer (PC) installed with Colour Research Lab Tools software developed by Frepik and the Leizer Colour Analysis software version 4 (Leizer Soft). The colours were expressed as L* (the lightness); a* (a positive value [+] indicates the redness, a negative value [-] indicates the greenness); and b* (a positive value [+] indicates the yellowness, a negative value [-] indicates the blueness). The colour measurement of the samples was carried out using a white plate background.

Dissolved oxygen

The dissolved oxygen (DO) was determined according to Talwalkar *et al*. (2004) using a DO meter (Xylem Analytics Lab 745, Germany) during fermentation and storage.

Statistical analysis

All experiments were conducted in triplicate. Figures, tables, means, and standard errors were produced using the Microsoft Excel version 2016. Data were statistically analysed by one-way ANOVA using the SPSS software version 23. The differences between means were analysed by the Duncan's Test ($p < 0.05$).

Results and discussion

The supplementation of CTF extracts (0 - 10%) into yoghurt increased TPC (up to 37.75 ± 2.1 mg GAE/100 g) and antioxidant activity (up to 44.43 ± 4.1%) at the beginning of fermentation (Figure 1A). Other authors also reported an increased in TPC and antioxidant activity in the yoghurt due to the supplementation of plant extracts (Oszmianski *et al*., 2016; Raikos *et al*., 2019; Hamad *et al*., 2020; Szołtysik *et al*., 2021). Even the TPC of yoghurt with 10% CTF extract (37.75 ± 2.1 mg GAE/100 g) was higher than the TPC reported by Rupasinghe *et al*. (2015) and Szołtysik *et al*. (2021). Meanwhile, the antioxidant activity of yoghurt with 10% CTF extract (44.43 ± 4.1%) was also higher than that reported by Hamad *et al*. (2020). The supplementation of CTF extracts (0 - 10%) into yoghurt increased the concentrations of glucose (up to 9.63 ± 0.3 g/L) and sucrose (up to 7.8 ± 0.5 g/L) at the beginning of fermentation (Figure 1B). One of the novelties of the present work was the discovery of inulin and pectin in *C. ternatea* flower. The supplementation of CTF extracts (up to 10%) into yoghurt increased inulin and pectin concentrations up to 5.7 ± 0.8 and 7.5 ± 0.3 g/L, respectively, at the beginning of fermentation (Figure 1B). Inulin is a long chain carbohydrate comprising of the fructose molecules which is produced in some flowers, tubers, and fruits in a wide range of concentrations (Zbikowska *et al*., 2017). Meanwhile, pectin is a complex heteropolysaccharides consisting of galactose, arabinose, rhamnose, fucose, xylose, and apiose molecules linked with an α-1,4-D-galacturonic acid backbone, mostly obtained from some fruits in different concentrations (Millan-Linares *et al*., 2021). However, there have been no publications on the presence of inulin and pectin in *C. ternatea* flower. This would then be advantageous for those working in the nutritional science area.

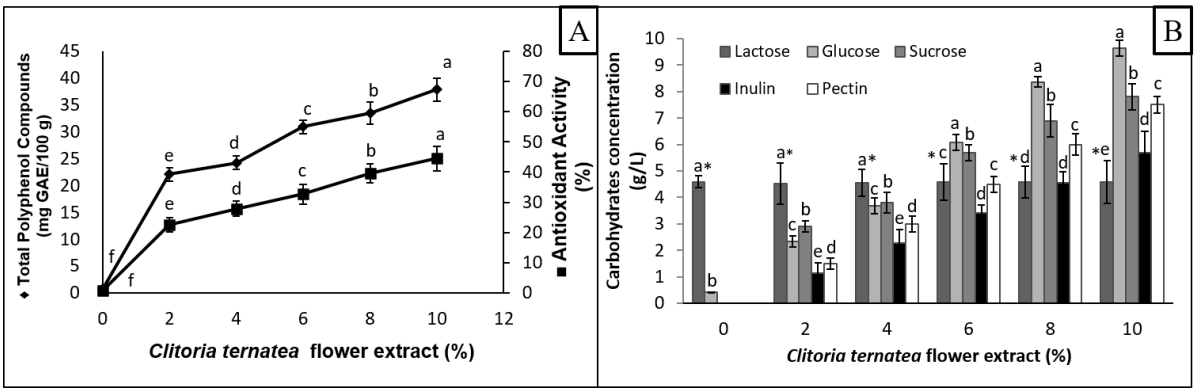

Figure 1. Total polyphenolic compounds (TPC) and antioxidant activity **(A)**; and carbohydrate concentration **(B)** at the beginning of fermentation in the yoghurt production supplemented with CTF extracts (0 - 10%). Results are mean ± standard error of the mean of triplicates ($n = 3$). Means followed by different lowercase letters in the CTF extract supplementation-wise comparison **(A)** and in the carbohydrate group-wise comparison **(B)** are significantly different ($p < 0.05$). Means with asterisk (*) in the CTF extracts supplementation-wise comparison **(B)** are non-significantly different ($p > 0.05$).

Figure 2A shows that the concentrations of glucose and lactose decreased (from 0.41 ± 0.02 to 0.2 ± 0.02 g/L; and from 4.59 ± 0.1 to 2.1 ± 0.3 g/L, respectively) as consumed by the starter cultures (*S. thermophillus* and *L. bulgaricus*) during fermentation. *S. thermophillus* secretes lactase to facilitate the degradation of lactose into glucose and galactose (Nurhartadi *et al*., 2017). Carbohydrate consumption increased bacterial cell growth (*S. thermophillus* and *L. bulgaricus*) up to 7.55 ± 0.22 and 6.7 ± 0.17 log CFU/mL, respectively (Figure 3A). Meanwhile, DO did not show significant decrease (Figure 3A). The antioxidant activities in yoghurts with no supplementation of extract and ascorbic acid were also relatively low. The antioxidant activities were suggested to have come from the milk's peptide

activity (Szołtysik *et al*., 2021). On the other hand, the supplementation of higher carbohydrate concentration into yoghurt (2.1 g/L of glucose and 3 g/L of sucrose) resulted in a drastic increase in the cell growth of *S. thermophillus* and *L. bulgaricus* up to 8.42 ± 0.22 and 6.87 ± 0.11 log CFU/mL, respectively (Figures 2B and 3B). Meanwhile, the carbohydrate supplementation into yoghurt did not affect the DO, in which the DO was statistically similar to the DO of the yoghurt supplemented with 0% CTF extract (Figures 3A and 3B). Carbohydrate supplementation provides energy for bacteria to build and maintain their cells (Nurhartadi *et al*., 2017). Higher concentration of glucose is an important element in fermentation which promotes microbial growth (Hamad *et al*., 2020). Meanwhile, supplementation of sucrose into yoghurt also impacts microbial growth (Nurhartadi *et al*., 2017). Some authors reported that *Streptococcus* spp. could metabolise sucrose since the bacteria produce invertase, an enzyme that facilitates the degradation of sucrose into glucose and fructose (Hu *et al*., 2011; Ahn *et al*., 2012). Similar to those reports, *S. thermophillus* employed in the present work produced invertase with the activity of 5.78 ± 0.02 U/g. Therefore, this bacterium could indeed metabolise sucrose during fermentation. In yoghurt supplemented with 2% antioxidant (ascorbic acid), the carbohydrate consumption was at the same level as the carbohydrate consumption in the yoghurt with no supplementation (Figures 2A and 2C). On the other hand, the DO decreased down to 1.35 ± 0.07 mg/L while the antioxidant activity increased up to 24.98 ± 0.5% at the end of fermentation (Figure 3C). This final DO (1.35 ± 0.07 mg/L) was also 1.25 times lower than the final DO of the yoghurt with no supplementation (Figures 3A and 3C). Both bacteria (*S. thermophillus* and *L. bulgaricus*) grew up to 7.2 ± 0.26 and 6.9 ± 0.19 log CFU/mL, respectively (Figure 3C). However, the final cell concentration of *S. thermophillus* was 2.2 times lower than the final cell concentration of that in yoghurt with no supplementation (Figures 3A and 3C). In contrast, the final cell concentration of *L. bulgaricus* was 1.6 times higher than the final cell concentration of that in yoghurt with no supplementation (Figures 3A and 3C). These phenomena were also found in yoghurts supplemented with CTF extracts (2 - 10%) (Figures 2D - 2H and 3D - 3H). In general, there were higher carbohydrate concentrations in the yoghurt medium at the beginning of fermentation as the extracts were supplemented (2 - 10%) (Figure 1B). Furthermore, as both bacteria (*S. thermophillus* and *L. bulgaricus*) metabolised glucose, lactose, and sucrose at different levels (Figures 2D - 2H), the cell concentrations increased considerably (Figures 3D - 3H). However, the final cell concentration of *S. thermophillus* decreased from 8.11 ± 0.25 to 7.2 ± 0.4 log CFU/mL (8.12 times) as the CTF extracts (2 - 10%) were supplemented into yoghurt (Figures 3D - 3H). In contrast, the final cell concentration of *L. bulgaricus* increased from 7.1 ± 0.21 to 7.74 ± 0.1 log CFU/mL (4.45 times) (Figures 3D - 3H). This suggested that the presence of antioxidant in a fermentation system might have decreased the oxygen which shifted the fermentation condition to be more anaerobic (La Scola *et al*., 2014; Dione *et al*., 2016). Figures 3D to 3H show that the DO of the yoghurts decreased down to 0.65 ± 0.023 mg/L as the extracts were supplemented (2 - 10%). Meanwhile, antioxidant activities increased during fermentation, possibly indicating the capture of oxygen species. Facultative anaerobic bacteria like *S. thermophillus* naturally grow in both aerobic and anaerobic conditions (Dione *et al*., 2016). In a more aerobic condition, the facultative anaerobic bacteria metabolise glucose and generate more ATP molecules (Dione *et al*., 2016). In contrast, those bacteria generate less energy (ATP) in a more anaerobic condition (Dione *et al*., 2016). Therefore, the decrease in oxygen concentration in the fermentation system may turn the condition to be more anaerobic in which *S. thermophillus* may generate less cellular energy to grow (La Scola *et al*., 2014; Dione *et al*., 2016). Consequently, the final cell concentration of *S. thermophillus* (Figures 3D - 3H) decreased as the extracts increased (2 - 10%). However, in general, the bacterial density still increased during fermentation due to the consumption of higher carbohydrate concentration (Figures 3D - 3H). In contrast, obligate anaerobic bacteria like *L. bulgaricus* grow rapidly in a more anaerobic condition. Therefore, *L. bulgaricus* increased drastically up to 7.74 ± 0.1 log CFU/mL as the extracts were supplemented (2 - 10%), due to not only the presence of a higher carbohydrate concentration but also the anaerobic condition (Figures 3D - 3H). In addition, the presence of antioxidant in the yoghurt changed the final cell ratio of *S. thermophillus* to *L. bulgaricus* from 7.07:1 to 0.28:1 (Figure 3). However, the final microbial cell concentrations of both bacteria were still higher than the minimum level

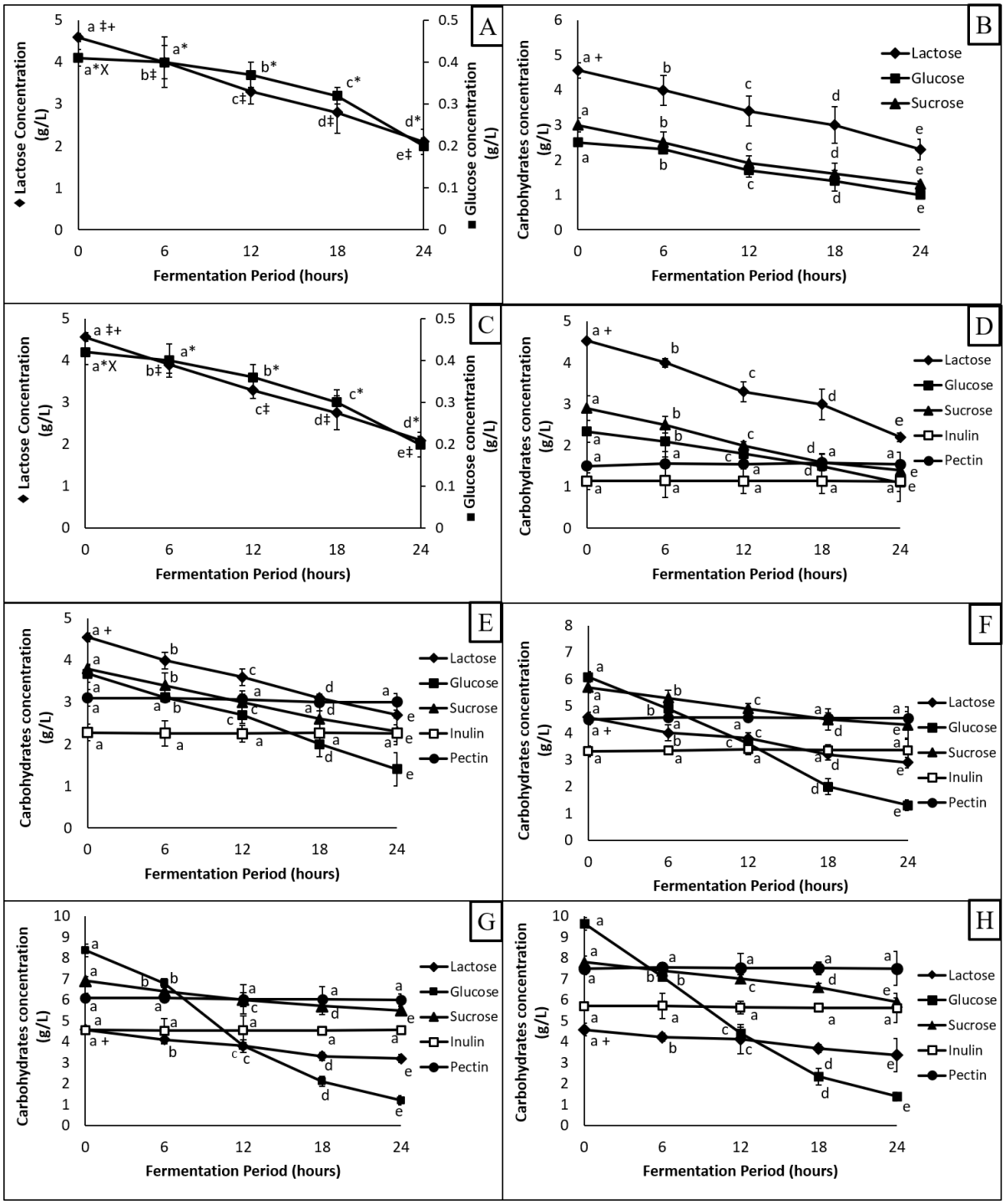

Figure 2. Carbohydrate concentrations during 24 h fermentation of the yoghurt supplemented with 0% **(A)**, 2% **(D)**, 4% **(E)**, 6% **(F)**, 8% **(G)**, and 10% **(H)** of CTF extracts. The control was yoghurt supplemented with glucose (2.5 g/L) and sucrose (3 g/L), with no antioxidant supplementation **(B)**; and supplemented with 2% ascorbic acid with an initial antioxidant activity of 23.18 ± 0.02% **(C)**. Values are mean ± standard error of the mean of triplicates (n = 3). Means followed by different lowercase letters in the fermentation period-wise comparison are significantly different ($p < 0.05$). Means with the same symbols in **(A)** and **(C)** comparison (*, ‡) and in the CTF extracts supplementation-wise comparison (+, X) are non-significantly different ($p > 0.05$).

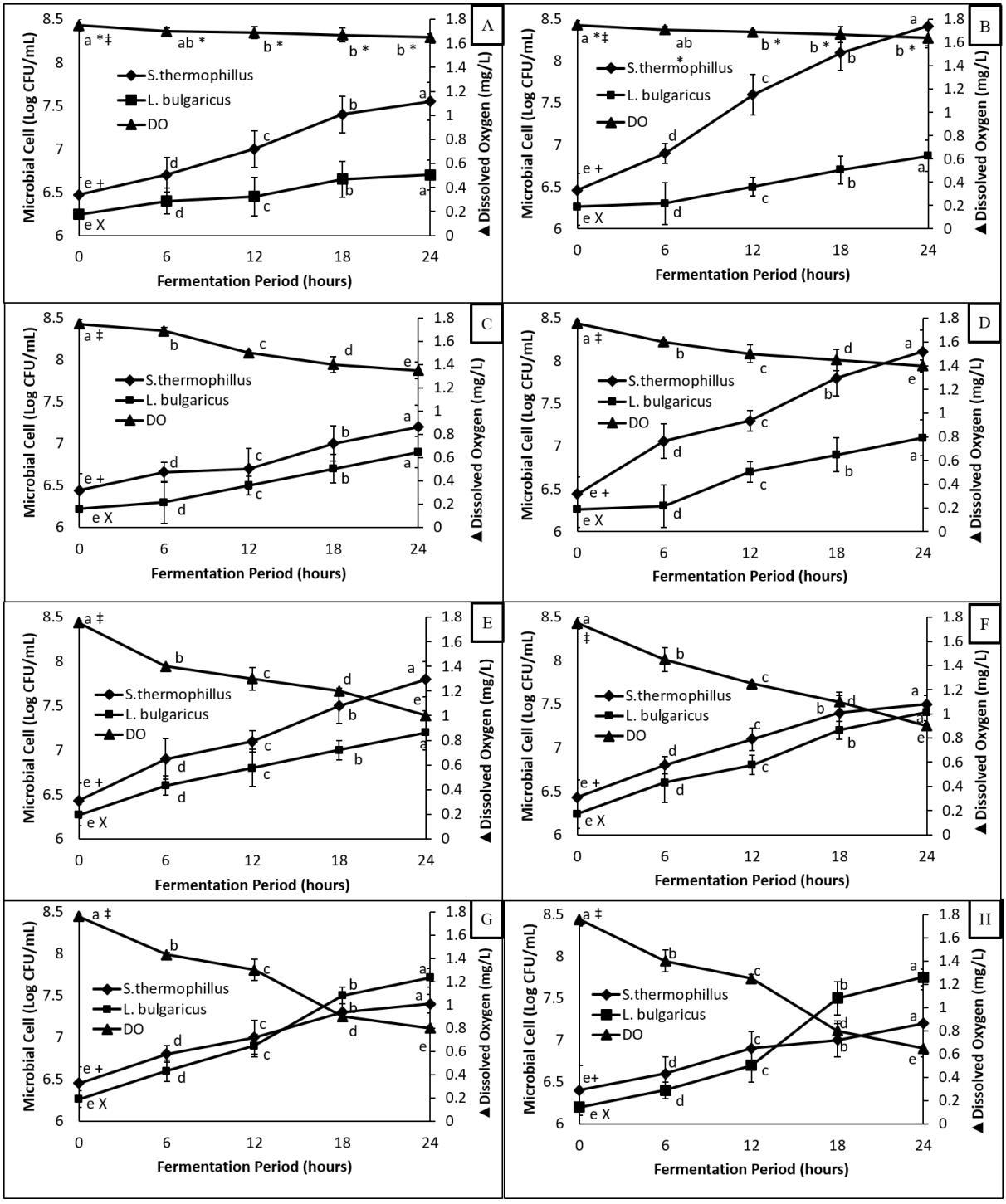

Figure 3. Growth of *S. thermophillus* and *L. bulgaricus*, as well as the dissolved oxygen (DO) during 24 h fermentation of the yoghurt supplemented with 0% **(A)**, 2% **(D)**, 4% **(E)**, 6% **(F)**, 8% **(G),** and 10% **(H)** of the CTF extracts. The control was yoghurt supplemented with glucose (2.5 g/L) and sucrose (3 g/L), with no antioxidant supplementation **(B)**; and supplemented with 2% ascorbic acid with an initial antioxidant activity of 23.18 ± 0.02% **(C)**. Values are mean ± standard error of the mean of triplicates (n = 3). Means followed by different lowercase letters in the fermentation period-wise comparison are significantly different ($p < 0.05$). Means with the same symbols in **(A)** and **(B)** comparison (*) and in the CTF extracts supplementation-wise comparison (‡, +, X) are non-significantly different ($p > 0.05$).

for bacterial cell viability in a fermented dairy product (≥ 7 log CFU/mL) as indicated in the international food standards of Codex Alimentarius (FAO and WHO, 2011). On the other hand, the concentrations of inulin and pectin did not significantly change during fermentation (Figures 2D - 2H) since *S. thermophillus* and *L. bulgaricus* did not secrete inulinase and pectinase to the medium for the metabolism of those sugars. Inulin and pectin have been long incorporated in food products (dairy products, mayonnaise, margarine, bakery products, and beverages) for health benefits since those sugars could potentially promote the growth of gut microflora to prevent digestive system disorders (Gomez *et al*., 2014; Zbikowska *et al*., 2017; Wongkaew *et al*., 2021). Furthermore, the concentration of pectin in the yoghurt could also improve and maintain the yoghurt texture (Gomez *et al.*, 2014; Millan-Linares *et al*., 2021; Wongkaew *et al*., 2021).

Microbial growth during fermentation had a consequence impact on lactic acid production. In the supplementation of CTF extract (10%), the lactic acid production increased up to 1.74 ± 0.37% during fermentation (Figure 4A). The supplementation of CTF extract (10%) increased the sugars concentration in the yoghurt (Figure 1B) which were further used by the starter cultures for growth and partially converted into lactic acid (Nurhartadi *et al*., 2017). Our results are similar to the lactic acid production published by Szołtysik *et al*. (2021), in which the addition of fruit extract into yoghurt increased the lactic acid production due to the presence of higher sugar concentration in the medium. A consequence of the lactic acid production is a decrease in pH up to 4.5 ± 0.11 during fermentation (Figure 4A). Furthermore, as the pH decreased, there was a dramatic change in the yoghurt appearance in which its colour turned from light cyan to purple (Figure 4B).

On the other hand, we also measured the colour changes in the yoghurt using a colorimeter, as a confirmation for the colour changes we observed visually. Other authors analysed the colour changes in the yoghurt using the L*a*b* colour system (Scibisz *et al*., 2019; Szołtysik *et al*., 2021). This colour system (developed by CIELAB) is quite relevant for food colour evaluation since it could analyse small differences in the colour, and is designed to represent the human visual perception (Milovanovic *et al*., 2020). In the present work, we also used L*a*b* colour system to evaluate the colour changes in the yoghurt during fermentation and storage (Table 1). The lightness values (L*) of yoghurt decreased significantly after the first 6 h of fermentation as the yoghurt colour turned to darker colours (blue grey and lavender purple) (Table 1 and Figure 4B). Meanwhile, the a* and b* values were negative in the first 12 h of fermentation, indicating the greenness and the blueness, respectively (Table 1). The combination of both (the greenness and the blueness) in different values was analysed using our colour analysis software, and resulted in various colours in the blue realm (light turquoise, light blue, and blue grey) (Table 1). Those predicted colours were close to the actual colours of yoghurt (Figure 4B). Furthermore, the a* values were positive (indicating the redness) while the b* values were still negative in the last 6 h of fermentation (Table 1). The red hue caused the yoghurt colour turned to purple (lavender purple) (Figure 4B). The prediction and the actual colours were not so different (Table 1 and Figure 4B). One of the substances in the CTF extract that is quite sensitive to pH changes is anthocyanin (Nair *et al*., 2015; Chusak *et al*., 2018; Anuyahong *et al*., 2020). The presence of lactic acid (as the donor of H^+ ions) in the fermentation medium may change the stability of conjugated systems in the anthocyanin chemical structure, thus changing the anthocyanin colour (Juhadi and Marpaung, 2021). However, the reaction did not affect the concentration of anthocyanin as it was relatively stable during fermentation (Figure 4A). In a basic condition, the colour of anthocyanin is between green to cyan, but turns from blue to red in an acidic condition (Chusak *et al*., 2018; Juhadi and Marpaung, 2021). At the beginning of fermentation (pH neutral), anthocyanin molecules were in light cyan (light turquoise) as shown by small green and blue proportions in the colour (Table 1 and Figure 4B). As the pH decreased, the colour spectrum of anthocyanin shifted to red (Juhadi and Marpaung, 2021). The changes were recorded in Table 1 as the greenness decreased (shown by an increase in the a* value), and the blueness increased (shown by a decrease in the b* value) in the first 12 h of fermentation. Later, the blueness decreased (shown by an increase in the b* value), and the redness increased (shown by an increase in the a* value) in the last 6 h of fermentation (Table 1). However, not all anthocyanin molecules turned red in the last 6 h of fermentation. There were

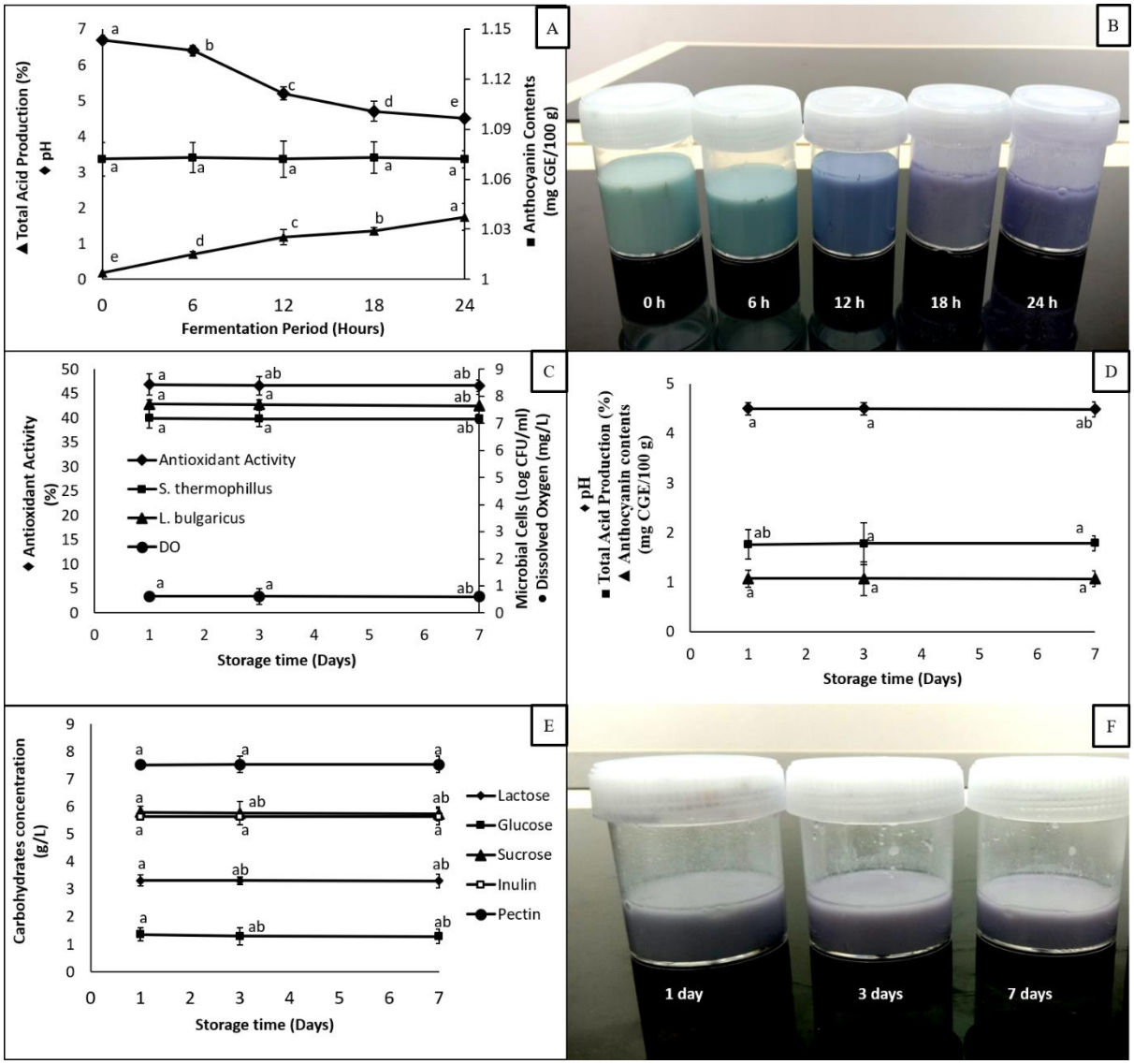

Figure 4. Total acid production, anthocyanin content, pH **(A)**, and colour changes **(B)** of the yoghurt supplemented with 10% CTF extract during fermentation. Meanwhile, the rest was the yoghurt properties supplemented with 10% CTF extract during storage were described as follows: antioxidant activity, DO, *S. thermophillus*, and *L. bulgaricus* cell growth **(C)**; anthocyanin content, pH changes, and total acid production **(D)**; carbohydrate concentration **(E)**; and colour changes **(F)**. Results are mean ± standard error of the mean of triplicates (n = 3). Means followed by different lowercase letters in the fermentation period and the storage time-wise comparison are significantly different ($p < 0.05$).

Table 1. The colour coordinates and predicted colours of yoghurt during fermentation and storage.

Time	Colour coordinate			Predicted colour∞
	L*	a*	b*	
Fermentation period (hour)				
0	90.9 ± 0.4^{a}	-10.2 ± 0.71^{e}	-10.3 ± 0.55^{a}	Light Turquoise
6	84.1 ± 0.43^{b}	-7.8 ± 0.64^{d}	-17.6 ± 0.49^{b}	Light Blue
12	60.4 ± 0.11^{d}	-0.2 ± 0.018^{c}	-31.2 ± 0.41^{e}	Blue Grey
18	69.47 ± 0.21^{c}	12.77 ± 0.76^{b}	-26.33 ± 0.77^{d}	Lavender
24	69.47 ± 0.2^{c}	14.78 ± 0.15^{a}	-21.77 ± 0.2^{c}	Lavender
Storage period (day)				
1	69.47 ± 0.32^{a}	14.78 ± 0.23^{a}	-21.776 ± 0.2^{ab}	Lavender
3	69.466 ± 0.4^{ab}	14.776 ± 0.32^{ab}	-21.776 ± 0.2^{ab}	Lavender
7	69.467 ± 0.5^{ab}	14.776 ± 0.4^{ab}	-21.78 ± 0.2^{a}	Lavender

Values are mean ± standard error of the mean of triplicates (n = 3). Means followed by different lowercase superscripts in a row are significantly different ($p < 0.05$). ∞obtained from computer based colour analysis software of the colour coordinates for the yoghurt.

anthocyanin molecules which were still in blue in the last 6 h of fermentation as indicated by the negative b* values (indicating the blueness) (Table 1). It is suggested that the amount of H^+ ions in the fermentation medium may not be adequate to react with the whole conjugated systems of anthocyanin molecules in the yoghurt, so that it may cause the colour of anthocyanin molecules only partially changed (Juhadi and Marpaung, 2021). Therefore, the combination of the colours (red and blue) resulted in the purple colour (lavender purple) in the last 6 h of fermentation (Table 1 and Figure 4B). Furthermore, the colour changes in the yoghurt (due to the CTF extract supplementation) could potentially be a low-cost natural detection for an increasing concentration of lactic acid in the fermentation system. In addition, the application of these results would hopefully help the yoghurt producers, especially in the micro- and small-scale levels, to know whether their fermentation process goes successfully or not.

One of the post-fermentation challenges is to keep the stability of nutrition and microbial viability during storage (Szołtysik *et al*., 2021). After 24 h fermentation, antioxidant activity and microbial cell growth were relatively stable during 7 d storage (Figure 4C). The results also did not show a statistically significant difference ($p > 0.05$). Other authors also confirmed that antioxidant activity and microbial growth were relatively stable in the yoghurt supplemented with plants extract during storage (Raikos *et al*., 2019; Szołtysik *et al*., 2021). Lower temperature may limit the dynamics of polyphenolic compounds which were consequently correlated to the dynamics of antioxidant activity (Juhadi and Marpaung, 2021). Hence, there was no significant changes in the oxygen concentration during storage (Figure 4C). Lactic acid production, anthocyanin content, and pH were also stable during storage (Figure 4D), in which they may have a correlation to the stability of yoghurt colour (Juhadi and Marpaung, 2021). Figure 4F shows that the final yoghurt colour (lavender purple) could be retained during the 7 d storage. The colour stability was also indicated by the stability of L*a*b* values (statistical test did not show a significant difference as indicated by $p > 0.05$) during the storage as shown in Table 1. Meanwhile, lower temperature storage may also decrease the activity of bacterial enzymes, thus limiting the metabolism of both bacteria (Raikos *et al*., 2019). Consequently, the bacteria consumed the carbohydrates slowly, thus, resulting in a relatively flat growth and stable lactic acid production during storage (Figures 4C and 4E). Furthermore, the bacteria may enter the end of stationary phase, and form spores if the temperature decreased considerably, in a longer storage time (Raikos *et al*., 2019; Szołtysik *et al*., 2021).

Conclusion

The supplementation of CTF extracts (0 - 10%) into yoghurt increased antioxidant activity and carbohydrate concentrations in the yoghurt. The presence of antioxidant in the yoghurt may contribute

to the decrease in oxygen concentration. The higher carbohydrate concentration and the more anaerobic condition enabled *L. bulgaricus* to grow drastically. In contrast, the growth of *S. thermophillus* was suppressed as the fermentation condition became more anaerobic. The presence of inulin and pectin in the extract was firstly reported which could give a contribution in the nutritional sciences. The colour of yoghurt changed as the pH decreased during the fermentation process. The colour changes could also be a natural detector for the presence of lactic acid in the yoghurt. After fermentation process, the application of a lower temperature during the storage could maintain the stability of yoghurt properties as antioxidant activity, anthocyanin content, carbohydrate concentration, DO, lactic acid concentration, pH, colour, and bacterial cell viability only showed an insignificant decrease.

References

Ahn, S. J., Cho, E. J., Kim, H. J., Park, S. N., Lim, Y. K. and Kook, J. K. 2012. The antimicrobial effects of deglycyrrhizinated licorice root extract on *Streptococcus mutans* UA159 in both planktonic and biofilm cultures. Anaerobe 18(6): 590-596.

Anuyahong, T., Chusak, C., Thilavech, T. and Adisakwattana, S. 2020. Postprandial effect of yogurt enriched with anthocyanins from riceberry rice on glycemic response and antioxidant capacity in healthy adults. Nutrients 12(10): 2930.

Carillo, P., Fusco, G. M., Del Gaudio, R. S., De Pascale, S. and Paradiso, R. 2022. Cold treatment modulates changes in primary metabolites and flowering of cut flower tulip hybrids. Horticulturae 8(5): 371.

Chusak, C., Henry, C. J., Chantarasinlapin, P., Techasukthavorn, V. and Adisakwattana, S. 2018. Influence of *Clitoria ternatea* flower extract on the *in vitro* enzymatic digestibility of starch and its application in bread. Foods 7(7): 102.

Dione, N., Khelaifia, S., La Scola, B., Lagier, J. C. and Raoult, D. 2016. A quasi-universal medium to break the aerobic/anaerobic bacterial culture dichotomy in clinical microbiology. Clinical Microbiology and Infection 22(1): 53-58.

Food and Agriculture Organization (FAO) and World Health Organization (WHO). 2011. Codex Alimentarius - Milk and milk products. Retrieved from FAO website: https://www.fao.org/3/i2085e/i2085e00.pdf

Gomez, B., Gullón, B., Remoroza, C., Schols, H. A., Parajó, J. C. and Alonso, J. L. 2014. Purification, characterization, and prebiotic properties of pectic oligosaccharides from orange peel wastes. Journal of Agricultural and Food Chemistry 62(40): 9769-9782.

Hamad, G. M., Abd Elaziz, A. I., Hassan, S. A., Shalaby, M. A. and Mohdaly, A. A. A. 2020. Chemical composition, antioxidant, antimicrobial and anticancer activities of licorice (*Glycyrrhiza glabra* L.) root and its application in functional yoghurt. Journal of Food and Nutrition Research 8(2): 707-715.

Hemu, X., Zhang, X., Bi, X., Liu, C. F. and Tam, J. P. 2019. Butelase 1-mediated ligation of peptides and proteins. In Nuijens, T. and Schmidt, M. (eds). Enzyme-mediated ligation methods, p. 83-109. New York: Humana.

Hu, C. H., He, J., Eckert, R., Wu, X. Y., Li, L. N., Tian, Y., … and Shi, W. Y. 2011. Development and evaluation of a safe and effective sugar-free herbal lollipop that kills cavity-causing bacteria. International Journal of Oral Science 3(1): 13-20.

Juhadi, W. and Marpaung, A. M. 2021. Optimization of time, temperature, and pH for the extraction of anthocyanin from Buni (*Antidesma bunius*) fruit. Food Science and Technology Journal 4(1): 8-15.

Khabibullaev, J., Zagorska, J., Galoburda, J. and Cinkmanis, J. 2019. Rheological properties of lactose-free yoghurt in relation to enzyme concentrations. In Proceeding of 13th Baltic Conference on Food Science and Technology 2019. Jelgava: Latvia University of Life Science and Technologies.

La Scola, B., Khelaifia, S., Lagier, J. C. and Raoult, D. 2014. Aerobic culture of anaerobic bacteria using antioxidants: A preliminary report. European Journal of Clinical Microbiology and Infectious Diseases 33(10): 1781-1783.

Lakshan, S. A. T., Jayanath, N. Y., Abeysekera, W. P. K. M. and Abeysekera, W. K. S. M. 2019. A commercial potential blue pea (*Clitoria ternatea* L.) flower extract incorporated

beverage having functional properties. Evidence-Based Complementary and Alternative Medicine 2019: 2916914.

Marchyshyn, S., Polonets, O., Savych, A. and Nakonechna, S. 2020. Determination of carbohydrates of *Chrysanthemum morifolium* L. leaves and flowers by GC-MS. Pharmakeftiki 32(4) : 202-212.

Millan-Linares, M. C., Montserrat-de la Paz, S. and Martin, M. E. 2021. Pectins and olive pectins: From biotechnology to human health. Biology 10(9): 860.

Milovanovic, B., Djekic, I., Miocinovic, J., Djordjevic, V., Lorenzo, J. M., Barba, F. J., … and Tomasevic, I. 2020. What is the color of milk and dairy products and how is it measured? Foods 9(11): 1629.

Nair, V., Bang, W. Y., Schreckinger, E., Andarwulan, N. and Cisneros-Zevallos, L. 2015. Protective role of ternatin anthocyanins and quercetin glycosides from butterfly pea (*Clitoria ternatea* Leguminosae) blue flower petals against lipopolysaccharide (LPS)-induced inflammation in macrophage cells. Journal Agriculture and Food Chemistry 63: 6355-6365.

Nurhartadi, E., Utami, R., Nursiwi, A., Sari, A. M., Widowati, E., Sanjaya, A. P. and Esnadewi, E. A. 2017. Effect of incubation time and sucrose addition on the characteristics of cheese whey yoghurt. IOP Proceeding of International Conference on Food Science and Engineering 2016. Surakarta: Universitas Negeri Sebelas Maret.

Oguis, G. K., Gilding, E. K., Jackson, M. A. and Craik, D. J. 2019. Butterfly pea (*Clitoria ternatea*), a cyclotide-bearing plant with applications in agriculture and medicine. Frontiers in Plant Science 10: 645.

Oliveira, F. C., Marques, T. R., Machado, G. H. A., Carvalho, T. C. L., Caetano, A. A., Batista, L. R. and Corrêa, A. D. 2018. Jabuticaba skin extracts: Phenolic compounds and antibacterial activity. Brazilian Journal of Food Technology 21: e2017108.

Oszmianski, J., Wojdyło, A. and Lachowicz, S. 2016. Effect of dried powder preparation process on polyphenolic content and antioxidant activity of blue honeysuckle berries (*Lonicera caerulea* L. var. *kamtschatica*). LWT - Food Science and Technology 67: 214-222.

Qureshi, F. 2021. COVID-19 pandemic, economic indicators and sectoral returns: Evidence from US and China. Economic Research-Ekonomska Istraživanja 35: 2142-2172.

Raikos, V., Ni, H., Hayes, H. and Ranawana, V. 2019. Antioxidant properties of a yogurt beverage enriched with salal (*Gaultheria shallon*) berries and blackcurrant (*Ribes nigrum*) pomace during cold storage. Beverages 5(1): 2.

Rupasinghe, V. H. P., Boehm, M., Sekhon-Loodu, S., Parmar, I., Bors, B. and Jamieson, A. 2015. Anti-inflammatory activity of haskap cultivars is polyphenols-dependent. Biomolecules 5(2): 1079-1098.

Scibisz, M., Ziarno, M. and Mitek, M. 2019. Color stability of fruit yogurt during storage. Journal of Food Science and Technology 56(4): 1997-2009.

Suharman, Sutakwa, A. and Nadia, L. S. 2021. Effects of sucrose addition to lactic acid concentrations and lactic acid bacteria population of butterfly pea (*Clitoria ternatea* L.) yogurt. In Proceeding of Second UPY International Conference on Applied Science and Education (2nd UPINCASE). Yogyakarta: Universitas PGRI.

Sutakwa, A., Nadia, L. S. and Suharman. 2021. Addition of blue pea flower (*Clitoria ternatea* L.) extract increase antioxidant activity in yogurt from various types of milk. Jurnal Agercolere 3(1): 31-38.

Szołtysik, M., Kucharska, A. Z., Dabrowska, A., Zieba, T., Bobak, Ł. and Chrzanowska, J. 2021. Effect of two combined functional additives on yoghurt properties. Foods 10(6): 1159.

Szołtysik, M., Kucharska, A. Z., Sokol-Letowska, A., Dabrowska, A., Bobak, L. and Chrzanowska, J. 2020. The effect of *Rosa spinosissima* fruits extract on lactic acid bacteria growth and other yoghurt parameters. Foods 9(9): 1167.

Talwalkar, A., Miller, C. W., Kailasapathy, K. and Nguyen, M. H. 2004. Effect of packaging materials and dissolved oxygen on the survival of probiotic bacteria in yoghurt. International Journal of Food Science and Technology 39(6): 605-611.

Veazie, P., Cockson, P., Henry, J., Perkins-Veazie, P. and Whipker, B. 2020. Characterization of

nutrient disorders and impacts on chlorophyll and anthocyanin concentration of *Brassica rapa* var. *Chinensis*. Agriculture 10(10): 461.

Widyaningrum, T., Prastowo, I., Parahadi, M. and Prasetyo, A. 2016. Production of bioethanol from the hydrolysate of brown seaweed (*Sargassum crassifolium*) using a naturally β-glucosidase producing yeast *Saccharomyces cerevisiae* JCM 3012. Biosciences Biotechnology Research Asia 13(3): 1333-1340.

Winarti, S., Harmayani, E. and Nurismanto, R. 2011. Characteristics and profile of inulin of various wild yams (*Dioscorea* spp.). Agritech 31(4): 378-383.

Wongkaew, M., Chaimongkol, P., Leksawasdi, N., Jantanasakulwong, K., Rachtanapun, P., Seesuriyachan, P., … and Jantrawut, P. 2021. Mango peel pectin: Recovery, functionality and sustainable uses. Polymers 13(22): 3898.

Zbikowska, A., Marciniak-Lukasiak, K., Kowalska, M. and Onacik-Gür, S. 2017. Multivariate study of inulin addition on the quality of sponge cakes. Polish Journal of Food Nutrition Science 67(3): 201-209.