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Effect of *Clitoria ternatea* Flower Extract Addition on Yoghurt Properties during Production and Storage

Abstract

The aim of this research was to analyze yoghurt incorporated with *Clitoria ternatea* flower (CTF) extract (0 – 10%) during the production and the storage. Antioxidant activity, microbial growth, carbohydrates constituent, dissolved oxygen (DO), lactic acid concentration, anthocyanin content, pH and color changes were observed during the 24 hours fermentation process. Meanwhile, the stability of the product properties (antioxidant activity, lactic acid concentration, anthocyanin content, carbohydrates concentration, color, DO, pH and cell viability) was also observed during the 7 days storage.

The incorporation of CTF extracts (0 – 10%) increased carbohydrates concentration and antioxidant activity in the medium during the fermentation process. As the antioxidant activity increased up to 44.43±4.1%, the DO decreased up to 0.65±0.023 mg/L. The higher carbohydrates concentration and the 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 concentration increased. However, the viability of both bacteria still met the international standards (≥ 7 Log CFU/mL). Surprisingly, prebiotic sugars of inulin and pectin were discovered in the CTF extract. The yoghurt color turned from light turqoise to purple (L* = 64.47±0.2; a* = 14.77±0.15; b* = - 21.2±0.2) as the pH droped to 4.5±0.11 and the lactic acid concentration increased up to 1.74±0.37 %. Furthermore, the yoghurt properties were stable during the storage.

Keywords: yoghurt, *Clitoria ternatea* flower extract, microbial growth, antioxidant activity, stability.

Introduction

Yoghurt is a probiotic food made by bacterial fermentation of milk which has a long history in some cuisines as dips, dressings, drinks, etc (Hamad *et al*., 2020; Szołtysik *et al*., 2021). The consumption of yoghurt shows positive benefits since it helps the work of the digestive system and also 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 addition of natural ingredients into yoghurt. Researchers reported that the addition of plants extract into yoghurt has shown significant impacts on the yoghurt nutritions (Hamad *et al*., 2020; Szołtysik *et al*., 2021). Szołtysik *et al*., (2021) reported that the addition of fruit extract into yoghurt increased polyphenols concentration which is related to antioxidant activity. Meanwhile, the addition of plants extract into yoghurt also increased the concentration of sugars which furthermore play an important role in microbial growth in the yoghurt (Hamad *et al*., 2020).

Another plants extract that could potentially enrich the yoghurt nutritional value is flower extract. The flower extract of *Clitoria ternatea* has been used in many purposes, ranging from medicines to foods (Oguis *et al*., 2019). It is reported that the *Clitoria ternatea* flower (CTF) has substances (triterpenoids, anthocyanin, flavonols, cliotides, etc) that potentially have good benefits for the health (Oguis *et al*., 2019). The CTF extract was reported to show a high antioxidant activity that potentially inhibits the growth of cancer cells (Oguis *et al*., 2019). Hence, the incorporation of CTF extract into yoghurt would potentially enhance the functional value of yoghurt (not only for the digestive system but also for a cancer medication). However, reports on the use of CTF extract in the yoghurt production are very limited, even never reported yet. On the other hand, holticultural products naturally contain a wide variety of carbohydrates (glucose, sucrose, cellulose, pectin, etc) which potentially improve the growth of microflora (Gomez *et al*., 2014; Hamad *et al*., 2020; Szołtysik *et al*., 2021). However, the information related to the carbohydrates constituent in the CFT extract is also very limited.

In this research, we elucidated the production process of yoghurt in the addition of CTF extract; including the relationship between carbohydrates constituent, antioxidant activity and the growth of *Streptococcus thermophillus* and *Lactobacillus delbrueckii subsp. bulgaricus* in the yoghurt which have not been reported by other authors. The color changes

in yoghurt were observed during the fermentation process as the pH decreased. In food science, the color could be an indicator for a particular chemical reaction in foods and also could attract the customer attention. The stability of yoghurt properties (antioxidant activity, anthocyanin contents, color, DO, pH, lactic acid concentration, carbohydrates concentration and microbial growth) was also observed during the storage since it determines the quality of the product.

Materials and Methods

Materials

Clitoria ternatea flowers were obtained from a local farm in Bantul, the Special Region of Yogyakarta, Indonesia. Pasteurized milk was obtained from PT Ultrajaya Milk Industry (Bandung, Indonesia). Starter (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, ascorbic acid were purchased from Merck KgaA (Darmstadt, Germany).

C*litoria ternatea* extract preparation

Clitoria ternatea flowers (glucose 9.22 – 9.5%, sucrose 7.8 – 8%, inulin 5.7 – 6%, and pectin 7.5 – 8.2%) were firstly dried and then pulverized using an electric pulverizer (Mitochiba CH 100, Japan). The powder was later sifted using an 85 mesh-electrical vibrating sifter (Kencana MS 3Mn, Indonesia). The powder was sterillized at 125 ^{o}C for 10 minutes. The powder (0, 2, 4, 6, 8, and 10 g) was subsequently macerated in a sterillized

water (100 mL) at a room temperature for 5 minutes. The mixtures were filtered and later stored at 5 ^{o}C until used.

Yoghurt Production

The yoghurt starter (20 mL) was added into 200 mL of pasturized 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 ^{o}C, 60 rpm for 24 h. Polyphenolic compunds, antioxidant activity, microbial growth (*S. thermophillus* and *L. bulgaricus*), carbohydrates constituent, dissolved oxygen (DO), total acid production, pH, anthocyanin contents and color changes were later determined every 6 hours of the 24 hours yoghurt production. The control was a yoghurt added with glucose (2.1 g/L) and sucrose (3 g/L), with no antioxidant addition and a yoghurt added with 2% ascorbic acid.

Stability during Storage

After 24 hours of the fermentation process, the yoghurts were stored in 4 ^{o}C for 7 days. Antioxidant activity, lactic acid concentration, carbohydrates concentration, anthocyanin content, DO, the growth of *S. thermophillus* and *L. bulgaricus*, as well as color and pH changes were subsequently evaluated at 1, 3, and 7 days.

Analysis

Determination of Total Polyphenolic Compounds

The total polyphenolic compounds (TPC) in yoghurt were 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 Contents Measurement

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

Antioxidant Activity Assay

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

Microbial Growth Analysis

The microbial growth was analyzed according to Szołtysik *et al*., (2020). Samples were dilluted in a serial dillutions and then poured into M-17 and MRS bacterial medium for *S. thermophillus* and *L. bulgaricus*, respectively. Bacterial cells of *S. thermophillus* and *L.*

bulgaricus were incubated in an anaerobic condition at pH 5.4, 37 ^{o}C; for 48 h and 72 h, respectively. The bacterial colonies were later counted.

Carbohydrate Constituents Analysis

Glucose and sucrose were determined according to Widyaningrum *et at*., (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 according to Winarti *et al*., (2011) using carbazol as the reagent.

pH Changes and Total Acid Production Analysis

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

Color Changes Measurement

The color changes were measured using a colorimeter (Konica Minolta CR-10 Plus, Japan) which was connected to a personal computer (PC). The colors were analyzed using the Color Research Lab Tools software developed by Frepik and the Leizer Color Analysis

software version 4 developed by Leizer Soft. The colors were expresed in L* (the lightness); a* (a positive value [+] indicates the redness; a negative value [-] indicates the greeness); and b* (a positive value [+] indicates the yellowness; a negative value [-] indicates the blueness). The color measurement of the samples was carried out using a white plate background.

Dissolved Oxygen Measurement

The dissolved oxygen (DO) in yoghurt was monitored during fermentation process using a DO meter (Xylem Analytics Lab 745, Germany).

Statistical Analysis

The whole experiments were conducted in triplicate. Graphical figures, tables, means and standard errors were made using the Microsoft Excell version 2016. Data were statistically analyzed by one-way ANOVA using the SPSS software version 23. The differences between means were analyzed by the Duncan's test ($p < 0.05$).

Results and Discussions

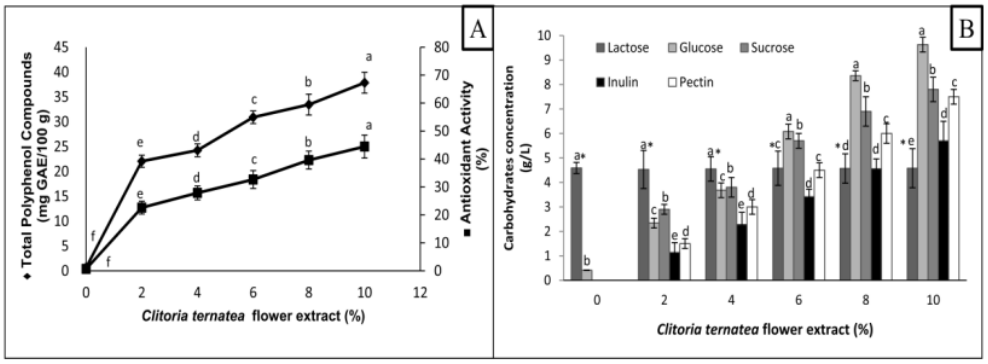

Fig. 1. Total Polyphenolic Compound (TPC) and antioxidant activity (A); as well as Carbohydrates concentration at the begining of fermentation process (B) in the yoghurt production supplemented with the CTF extract (0 – 10%). The research results were expressed as mean ± standard error of the mean, with n = 3. Means with the same letters (a, b, c, etc) in the CTF extract-wise comparison (A) and in the carbohydrates group-wise comparison (B) indicated a statistically significant similarity. Meanwhile, means with the same symbol (*) in the CTF extract-wise comparison (B) denoted a statistically significant similarity. The experimental data were statistically analyzed at $p < 0.05$.

The addition of CTF extracts (0 – 10%) into yoghurt increased Total Polyphenolic Compounds (TPC) (up to 37.75±2.1 mg GAE/100 g) and antioxidant activity (up to 44.43±4.1%) as described in Fig. 1A. Other authors also reported an increased level of the TPC and the antioxidant activity in the yoghurt due to the addition of plants extract (Oszmianski *et al*., 2016; Raikos *et al*., 2019; Hamad *et al*., 2020; Szołtysik *et al*., 2021). Even, the TPC of the yoghurt with 10% CTF extract (37.75±2.1 mg GAE/100 g) was higher than the TPC obtained by Rupasinghe *et al*., (2015) and Szołtysik *et al*., (2021). Meanwhile, the antioxidant activity of the yoghurt with 10% CTF extract (44.43±4.1%) was also higher than the antioxidant activity obtained by Hamad *et al*., (2020).

On the other hand, the incorporation 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) (Fig. 1B). One of the attractions of this research was the first discovery of inulin and pectin in *C. ternatea* flower. The addition of CTF extracts (up to 10%) into yoghurt increased inulin and pectin concentrations up to 5.7±0.8 g/L and 7.5±0.3 g/L, respectively (Fig. 1B). Inulin is a long chain carbohydrate comprising of the fructose molecules which is produced in some

flowers, tubers, and fruits in wide range of concentrations (Zbikowska *et al*., 2017). Meanwhile, pectin is a complex heteropolysaccharides consisting of galactose, arabinose, rhamnose, fucose, xylose, apiose molecules linked with an α-1,4-D-galacturonic acid backbone which is 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. Furthermore, this finding would be advantageous for those working in the nutritional science area.

Fig. 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 microbes (*S. thermophillus* and *L. bulgaricus*) during the fermentation process. During the fermentation process, *S. thermophillus* secretes lactase to facilitate the degradation of lactose into glucose and galactose (Nurhartadi *et al*., 2016). The carbohydrates consumption increased the bacterial cell growth (*S. thermophillus* and *L. bulgaricus*) up to 7.55±0.22 and 6.7±0.17 Log CFU/mL, respectively (Fig. 3A). Meanwhile, the dissolved oxygen (DO) did not show any significant decrease (Fig. 3A). The antioxidant activities in the yoghurt with no extracts addition and the yoghurt with no ascorbic acid addition (but with the sugars addition) were also relatively low (Fig. 4A). The antioxidant activities were suggested from the milk's peptides activity (Szołtysik *et al*., 2021).

On the other hand, the addition of a higher carbohydrates 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 (Fig. 2B and 3B).

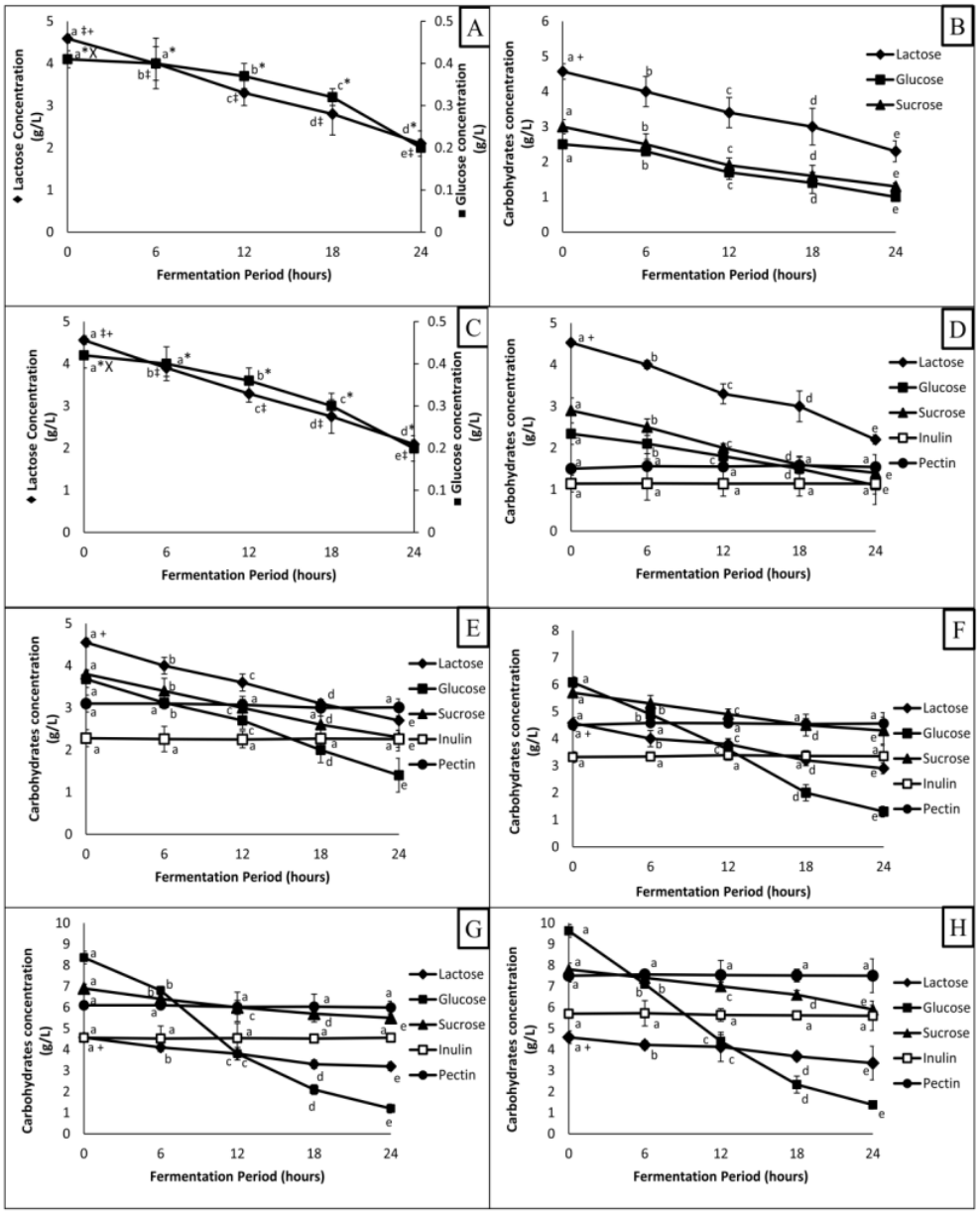

Fig. 2. Carbohydrates concentration during a 24 hours fermentation process of the yoghurt supplemented with 0% (A); 2% (D); 4% (E), 6% (F), 8% (G) and 10% of the CTF extract (H). The control was a yoghurt in the addition of glucose (2.5 g/L) and sucrose (3 g/L), with no antioxidant addition (B) and in the addition of 2 % asorbic acid with an initial antioxidant activity of 23.18±0.02% (C). The research results were expressed as mean ± standard error of the mean, with n = 3. Means with the same letters (a, b, c, etc) in the fermentation period-wise comparison indicated a statistically significant similarity. Meanwhile, means with the same symbols in A and C comparison (*, ‡) and in the CTF extract addition-wise comparison (+, X) denoted a statistically significant similarity. The experimental data were statistically analyzed at $p < 0.05$.

Meanwhile, the carbohydrates addition into yoghurt did not affect the DO, in which the DO was statistically similar to the DO of the yoghurt without the CTF extract addition ($p > 0.05$) (Fig. 3A and 3B). Carbohydrates supplementation provides energy for bacteria to build and maintain their cells (Nurhartadi *et al*., 2016). A higher concentration of glucose is an important element in a fermentation process which promotes the growth of microbes (Hamad *et al*., 2020). Meanwhile, an addition of sucrose into yoghurt also impacts the growth of microbes (Nurhartadi *et al*., 2016). Some authors reported that some *Streptococcus* species could metabolize 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* we employed, produced invertase with the activity of 5.78±0.02 U/g. Thus, this bacteria could metabolize sucrose during the fermentation process.

In the addition of 2% antioxidant (ascorbic acid) into yoghurt, the carbohydrates consumption was at the same level as the carbohydrates consumption in the yoghurt with no addition (Fig. 2A and 2C). The experimental data of both treatments were also statistically similar ($p > 0.05$). On the other hand, the DO decreased up to 1.35±0.07 mg/L while the antioxidant activity increased up to 24.98±0.5% at the end of fermentation process (Fig. 3C and Fig. 4B). This final DO (1.35±0.07 mg/L) was also 1.25 times lower than the final DO of the yoghurt with no addition (Fig. 3A and 3C). Both microbes (*S. thermophillus* and *L. bulgaricus*) grew up to 7.2±0.26 and 6.9±0.19 Log CFU/mL, respectively. However, the final cell concentration of *S. thermophillus* was 2.2 times lower than the final cell concentration of that bacteria in the yoghurt with no addition (Fig. 3A and 3C). In contrast, the final cell concentration of *L. bulgaricus* was 1.6 times higher than the final cell concentration of that microbe in the yoghurt with no addition (Fig. 3A and 3C).

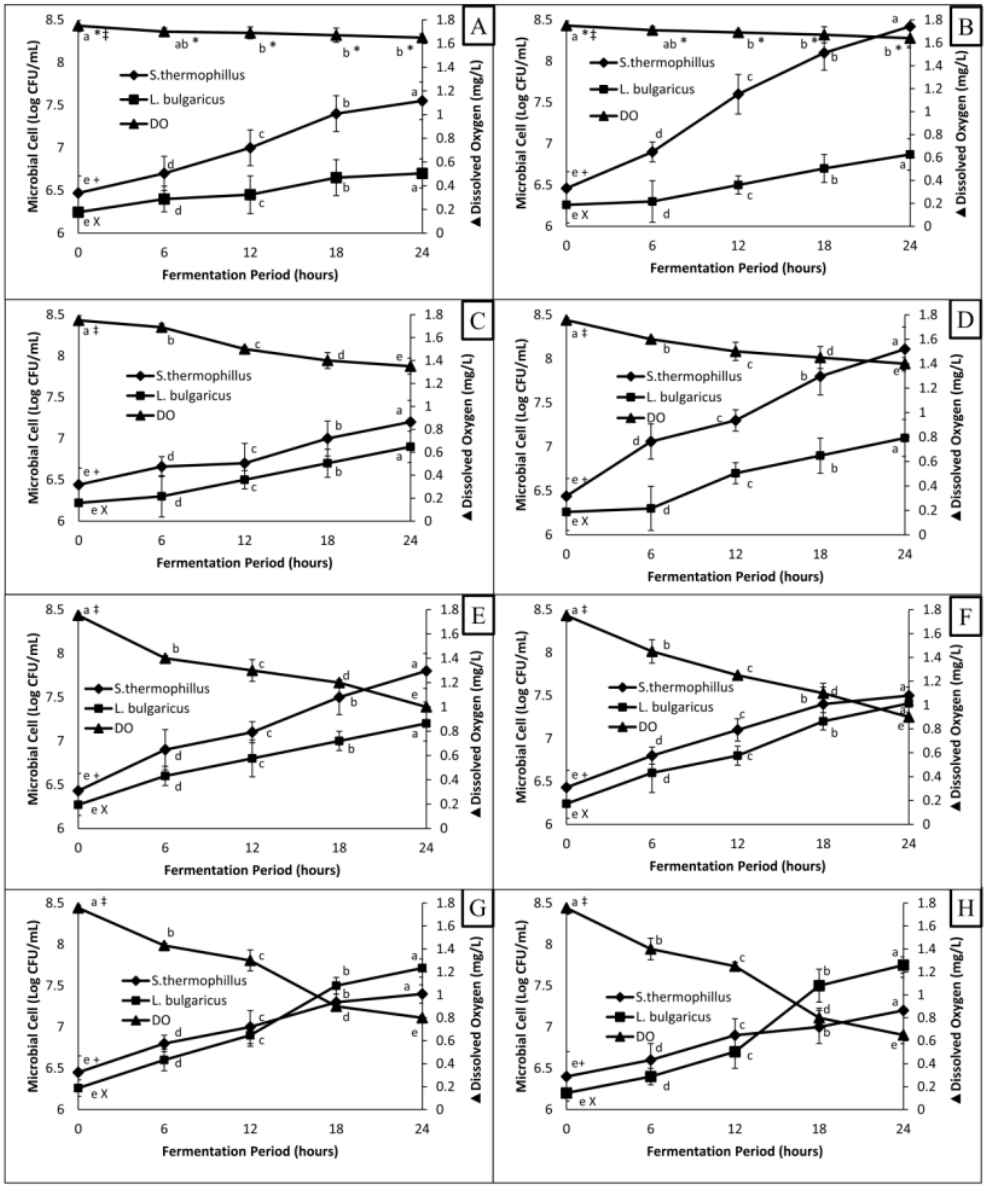

Fig. 3. The growth of *S. thermophillus* and *L. bulgaricus*, as well as the dissolved oxygen (DO) during a 24 hours fermentation process of the yoghurt supplemented with 0% (A); 2% (D); 4% (E), 6% (F), 8% (G) and 10% of the CTF extract (H). The control was a yoghurt in the addition of glucose (2.5 g/L) and sucrose (3 g/L), with no antioxidant addition (B) and in the addition of 2% asorbic acid with an initial antioxidant activity of 23.18±0.02% (C). The research results were expressed as mean ± standard error of the mean, with n = 3. Means with the same letters (a, b, c, etc) in the fermentation period-wise comparison indicated a statistically significant similarity. Meanwhile, means with the same symbols in A and B comparison (*) and in the CTF extract addition-wise comparison (‡, +, X) denoted a statistically significant similarity. The experimental data were statistically analyzed at $p < 0.05$.

These phenomena were also found in yoghurts added with the CTF extracts (2 – 10%) (Fig. 2D - 2H and 3D - 3H). In general, there was a higher carbohydrates concentration in the yoghurt medium at the beginning of fermentation process as the extracts were added (2 – 10%) (Fig. 1B). Furthermore, as both bacteria (*S. thermophillus* and *L. bulgaricus*) metabolized glucose, lactose and sucrose at different levels (Fig. 2D - 2H), the cell concentrations increased considerably (Fig. 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 added into yoghurt (Fig. 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) (Fig. 3D - 3H). It is suggested that the presence of antioxidant in a fermentation system may reduce oxygen which turns the fermentation condition to be more anaerobic (La Scola *et al*., 2014; and Dione *et al*., 2016). Fig. 3D to 3H show that the DO of the yoghurt decreased up to 0.65±0.023 mg/L as the extracts were added (2 – 10%). Meanwhile, antioxidant activities increased during the fermentation process, possibly indicating the capture of oxygen species (Fig 4B - 4D). Facultative anaerobic bacteria, like *S. thermophillus*, naturally grow in both aerobic and anaerobic conditions (Dione *et al*., 2016). In a more aerobic condition, facultative anaerobic bacteria metabolize 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). Thus, the reduction of 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* (Fig. 3D - 3H) decreased as the extracts increased (2 – 10%). However, in general, the bacterial density still increased during the fermentation process due to the consumption of higher carbohydrates concentration (Fig. 3D - 3H). In contrast, obligate anaerobic bacteria, like *L.*

bulgaricus, grow rapidly in a more anaerobic condition. Thus, *L. bulgaricus* increased drastically up to 7.74±0.1 Log CFU/mL as the extracts were added (2 – 10%) due to, not only the presence of a higher carbohydrates concentration but also the more anaerobic condition (Fig. 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 (Fig. 3). However, in this research, the final microbial cell concentrations of both bacteria were still higher than the minimum level for bacterial cell viability in a fermented dairy product (≥ 7 Log CFU/mL) as indicated in international food standards of Codex Alimentarius (FAO, 2011).

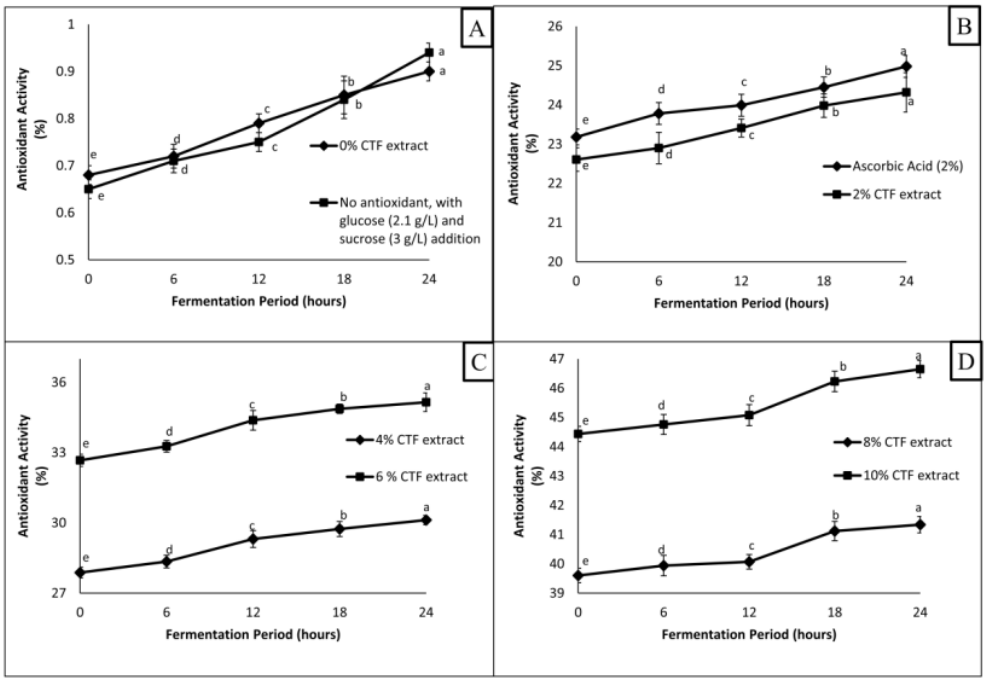

Fig. 4. Antioxidant activities in the yoghurt during 24 hours fermentation process. The research results were expressed as mean ± standard error of the mean, with n = 3. Means with the same letters (a, b, c, etc) in the fermentation period-wise comparison indicated a statistically significant similarity. The experimental data were statistically analyzed at $p < 0.05$.

On the other hand, the concentrations of inulin and pectin did not significantly change during the fermentation process (Fig. 2D - 2H) since *S. thermophillus* and *L. bulgaricus* did not secrete inulinase and pectinase to the medium, to metabolize both sugars (data not shown). Inulin and pectin have been long incorporated in food products (dairy products, mayonnaise, margarine, bakery products, and beverages) for health benefits in which those sugars could potentially promotes 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 improve and maintain its texture (Gomez et al., 2014; Millan-Linares *et al*., 2021; Wongkaew *et al*., 2021).

The growth of microbes during the fermentation process consequently increased the acid production. In the addition of CTF extract (10 %), the lactic acid production increased up to 1.74±0.37% during the fermentation process (Fig. 5A). The addition of CTF extract (10 %) increased the sugars concentration in the yoghurt (Fig 1B) which were further used by the microbes for growth and partially converted into lactic acid (Nurhartadi *et al*., 2016). 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 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 the fermentation process (Fig. 5A). Furthermore, as the pH decreased, there was a dramatic change in the yoghurt apppearance in which its color turned from light cyan to purple (Fig. 5B). On the other hand, we also measured the color changes in the yoghurt using a colorimeter, as a confirmation for the color changes we observed visually. Other authors analyzed the color changes in the yoghurt using the L*a*b* color system (Scibisz *et al*., 2019; Szołtysik *et al*., 2021).

Table 1. The color coordinates and the predicted colors of yoghurt during the fermentation process and the storage.

Fermentation Periods (Hours)	Color Coordinates			Predicted Colors ∞
	L*	a*	b*	
0	90.9±0.4^{a}	- 10.2±0.71^{e}	- 10.3±0.55^{a}	Light Turqoise
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	69.4±0.79^{c}	12.77±0.76^{b}	- 26.33±0.77^{d}	Lavender
24	69.47±0.2^{c}	14.77±0.15^{a}	- 21.2±0.2^{c}	Lavender
Storage Periods (Days)	Color Coordinates			Predicted Colors ∞
	L*	a*	b*	
1	69.66±0.32^{a}	14.78±0.23^{a}	- 21.76±0.2^{ab}	Lavender
3	69.31±0.4^{ab}	14.77±0.32^{ab}	- 21.76±0.2^{ab}	Lavender
7	69.32±0.5^{ab}	14.77±0.4^{ab}	- 21.78±0.2^{a}	Lavender

The research results were expressed as mean ± standard error of the mean, with n = 3. Means with the same letters (a, b, c, etc) in the same row indicated a statistically significant similarity. The experimental data were statistically analyzed at $p < 0.05$. ∞ denoted the predicted colors as obtained from the computer based-color analysis softwares from color coordinates of the yoghurt (L*, a* and b* values).

Recently, this color system (developed by CIELAB) is quite relevant for food color evaluation since it could analyze small differences in the color and is designed to represent the human visual perception (Milovanovic *et al.*, 2020). In this research, we used L*a*b* color system to evaluate the color changes in the yoghurt during the fermentation and the storage, and the results were tabulated in table 1. The lightness values (L*) of the yoghurt decreased after 6 hours of the fermentation process as the yoghurt color turned to darker colors (blue grey and lavender purple) (table 1). Meanwhile, the a* and b* values were negative in the first 12 hours of fermentation process, indicating the greeness and the blueness, respectively (table 1). The combination of both (the greeness and the blueness) in

different values were analyzed using our color analysis softwares and resulted in various colors in the blue realm (light turqoise, light blue, and blue grey) (table 1). Those predicted colors were close to the actual colors of yoghurt (Fig. 5B). Furthermore, the a* values were positive (indicating the redness) while the b* values were still negative in the last 6 h of fermentation process (table 1). The red hue caused the yoghurt color turned to purple (lavender purple). The prediction and the actual colors were not so different (table 1; Fig. 5B).

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). 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 color (Juhaidi and Marpaung., 2021). However, the reaction did not affect the concentration of anthocyanin as the anthocyanin concentration was relatively stable during the fermentation process ($p > 0.05$) (Fig. 5A). In a basic condition, the color of anthocyanin is between green to cyan, but turns from blue to red in an acidic condition (Chusak *et al*., 2018; Juhaidi and Marpaung., 2021). At the beginning of fermentation process (pH neutral), anthocyanin of the CTF extract was in light cyan (light turqoise) as shown by small green and blue proportions in the color (table 1 and Fig. 5B). As the pH dropped, the color spectrum of anthocyanin shifted to red (Juhaidi and Marpaung., 2021). The changes were recorded in table 1 as the greeness 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 hours 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 anthocyanins turned red in the last 6 hours of fermentation process.

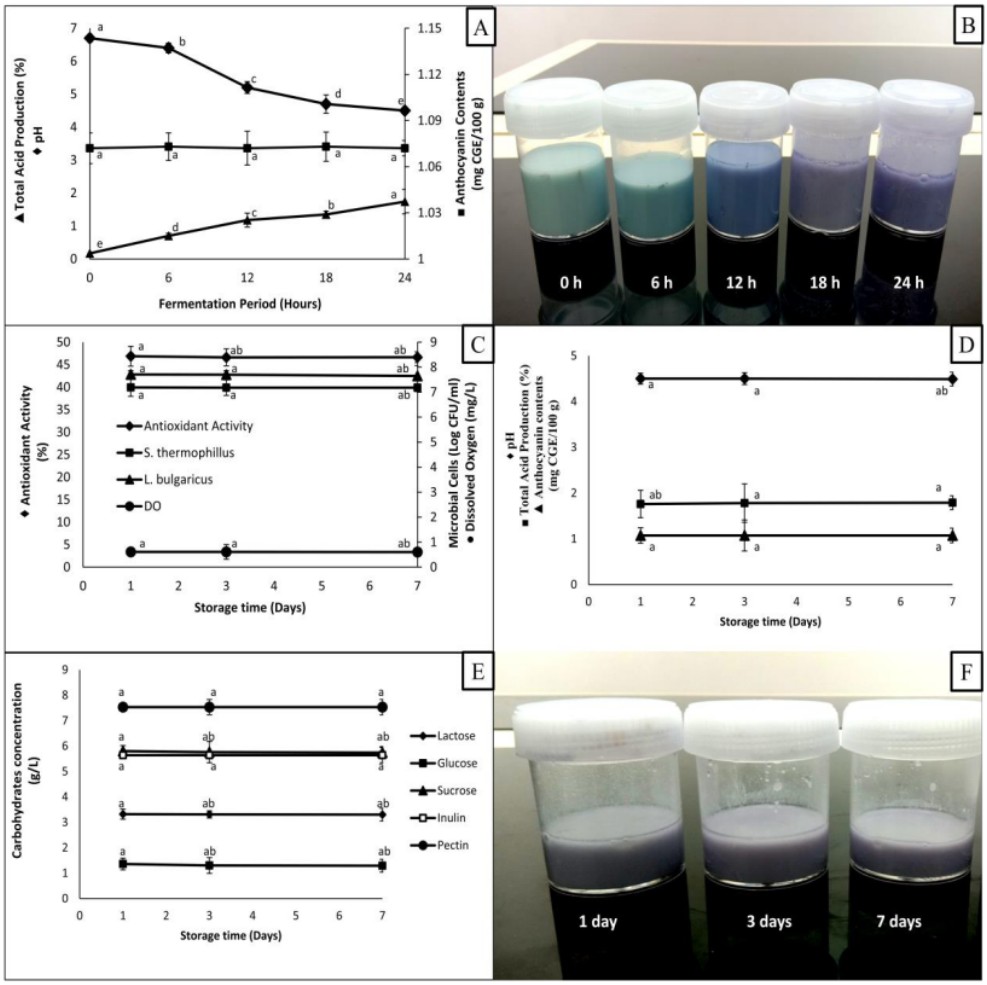

Fig. 5. The total acid production, anthocyanin content, pH (A) and color changes (B) of the yoghurt in the addition of 10% of CTF extract during the fermentation process. Meanwhile, the rest was the yoghurt properties in the addition of 10% CTF extract during the storage, as described as follow : antioxidant activity, DO, *S. thermophillus* and *L. bulgaricus* cell growth (C), anthocyanin content, pH changes, total acid production (D), carbohydrates concentration (E), as well as the color changes (F). The research results were expressed as mean ± standard error of the mean, with n = 3. Means with the same letters (a, b, c, etc) in the fermentation period and the storage time-wise comparison indicated a statistically significant similarity. The experimental data were statistically analyzed at $p < 0.05$.

There were anthocyanins which were still in blue in the last 6 hours of fermentation process 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 in the yoghurt, so that it may cause the anthocyanin color only partially changed (Anuyahong *et al*., 2020; Juhaidi and Marpaung., 2021). Thus, the combination of the colors (red and blue) resulted in the purple color (lavender purple) in the last 6 hours of fermentation process (table 1; Fig. 5B). Furthermore, the color changes in the yoghurt (due to the CTF extract addition) 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 level, to know wheather their fermentation process goes succesfully or not.

One of the post-fermentation challenges is to keep the stability of nutritions and microbial viability during storage. After 24 hours fermentation, antioxidant activity and microbial cell growth were relatively stable during the 7 days storage (Fig. 5C). The results also did not show a statistically significant difference ($p > 0.05$). Other authors also confirmed the stability of antioxidant activity and microbial growth in yoghurt enriched by plants extract during the storage (Raikos *et al*., 2019; Szołtysik *et al*., 2021). Lower temperature may limit the dynamics of polyphenolic compounds which was consequently correlated to the dynamics of antioxidant activity (Juhadi and Marpaung., 2021). Hence, there was no any significant changes in the oxygen concentration during the storage ($p > 0.05$) (Fig. 5C). As information, the antioxidant activity of the extracts decreased as the temperature dropped (data not shown). On the other hand, lactic acid production, anthocyanin content and pH were stable during the storage ($p > 0.05$) (Fig. 5D), in which they may have a correlation to the stability of yoghurt color (Juhaidi and Marpaung., 2021). Figure 5F shows that the final yoghurt color (lavender purple) could be retained during the 7 days storage. The color stability was also indicated by the stability of L*a*b* values ($p > 0.05$) during the storage as shown in table 1. Meanwhile, the 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, and thus, resulting in a relatively flat growth during the storage ($p > 0.05$) (Fig. 5C and 5E). Furthermore, the bacteria may enter the end of stationary phase and form the spores if the temperature decreased considerably, in a longer storage time (Raikos *et al*., 2019; Szołtysik *et al*., 2021).

Conclusions

The addition of CTF extract into yoghurt increased antioxidant acitvity and carbohydrates concentration in the yoghurt. The presence of antioxidant in the yoghurt may contribute to the reduction of oxygen concentration. The higher carbohydrates concentration and the more anaerobic condition enabled *L. bulgaricus* to grow drastically. In contrast, the growth of *S. thermophillus* was surpressed 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 addition of CTF extract into yoghurt also improved the attractiveness of the product due to its unique color. The color changes could also be a natural detector for the presence of lactic acid in the yoghurt. After fermentation process, the fortified yoghurt had a stable antioxidant activity, anthocyanin content, carbohydrates concentration, DO, lactic acid concentration, pH, color and bacterial cell viability during the storage.

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