



## Development of Cheese as an Antioxidant Functional Food with the Addition of Orthodox Black Tea

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### ABSTRACT

COVID-19 and the increase in degenerative diseases are the reasons for the high consumption of functional foods. This study investigated the physicochemical, sensory, and functional characteristics of cheese enhanced with orthodox black tea. The primary research materials were 40 liters of fresh cow's milk, orthodox black tea (OBT), mesophilic bacteria (*Lactococcus lactis*), animal calf rennet, and CaCl<sub>2</sub>. An experimental completely randomized design (CRD) was employed in the study to four treatments, namely control (T<sub>0</sub>), and the addition of 0.5% OBT (T<sub>1</sub>), 1% OBT (T<sub>2</sub>), 1.5% OBT (T<sub>3</sub>), and 2% OBT (T<sub>4</sub>). Each treatment was repeated four times and performed in duplicate. The observed variables were physical properties (colors and textures), chemical properties (moisture content, total solids, pH, and total titratable acidity), antioxidant activity, and sensory properties of cheese. The results showed that adding OBT up to 2% produced significantly different levels of pH, total titratable acidity, values (L\*, a\*, b\*), hardness, antioxidant activity, and sensory properties of cheese. No significant difference was observed in the moisture content, total solids, and stickiness of cheese. Conclusively, incorporating OBT up to 2% in cheese making tends to increase the functional properties of cheese that include the a\* value, total titratable acidity, and antioxidant activity, but it also decreases the L\* and b\* values, hardness, and pH value. The panelist's preference was the highest for cow's milk cheese with the addition of 0.5% OBT.

**Keywords:** cheese; functional food; orthodox black tea; physicochemical; sensory

### INTRODUCTION

The increasing incidence of degenerative diseases and the COVID-19 outbreak have inspired many people to have more awareness of the importance of health. The 2018 data from Basic Health Research shows an escalating incidence of heart and blood vessel diseases. At least 15 out of 1,000 people (equals to 4.2 million people today) suffer from cardiovascular disease, and 2,784,064 have heart disease. This high prevalence has changed people's lifestyles and food consumption; for example, Indonesian people have now adopted healthier food preferences like functional food. Functional food, according to Beltrán-Barrientos *et al.* (2016), contains nutritional values, provides benefits for body health, and wards off some diseases. Al-Hamdani *et al.* (2021) stated that functional food could be made by incorporating probiotic bacteria into food processing to increase immunity and anti-cancer properties and prevent heart disease, diarrhea, and lactose intolerance. One of the functional foods derived from livestock dairy products is cheese. Cheese contains bioactive compounds, such as calcium, polyunsaturated fatty acids (PUFA), lactose, and probiotic bacteria, used as starters in production. People in Indonesia have developed an interest in con-

suming cheese and processing milk into cheese because cheese has been identified to contain high nutrients and is effective against specific pathogens (Tomazou *et al.*, 2019). Probiotic bacteria and bioactive compounds in cheese are considered effective for improving the digestive and immune systems.

Cheese is a processed animal product obtained from the clumping and fermentation process of milk using lactic acid bacteria, namely *Lactobacillus lactis*, and can be added with spices or BPOM-approved food additives (Nzekoue *et al.*, 2021). Incorporating spices and lactic acid bacteria into cheese-making can increase its nutritional values, including 19.4% protein, 21.6% fat, and 2.20% carbohydrates. To improve these nutritional values and functional properties in cheese, adding other ingredients that contain high antioxidants, such as black tea, is possible. According to Fadhlurrohman *et al.* (2023), black tea contains the most extensive flavonoids as antioxidants in the form of theaflavin (10%) and 50%-60% thearubigin. The antioxidant content, especially the theaflavin and thearubigin in black tea, can prevent cell damage due to free radicals (Yılmaz *et al.*, 2020). Therefore, one alternative to improve the functional properties of cheese is by incorporating orthodox black tea (OBT) into the cheese-making process.

Orthodox black tea (OBT) is a type of tea that exhibits good properties for human health, has high nutritional value, and acts as a natural dye. Black tea powder contains 7.27% moisture content, and a measure using Trolox equivalent antioxidant capacity (TEAC) indicates that black tea powder also contains 488 mg/g antioxidant activity consisting of total polyphenols 35.2g/100g GAE, 29.8 g/100g thearubigins, and other ingredients (Perera *et al.*, 2015). The high contents of polyphenols, thearubigins, and theaflavin in OBT are potential to improve the functional properties of cheese, mainly phenols. According to research by Giroux *et al.* (2013), adding green tea extract at a concentration of 1 or 2 g/kg milk can increase the anti-radical activity of cheese by 25% and 44%, respectively, and increase the hardness texture of the cheese. More specifically, 2 g/kg green tea extract can decrease cheese moisture by 1.9%. Therefore, the enrichment of tea extract in cheese making can produce new cheese with the increased health benefits. Meanwhile, the natural dye properties of black tea potentially affect the cheese product, but Fadhlurrohman *et al.* (2023) stated that as long as the OBT addition does not exceed 2%, it will not result in darker-colored cheese. This study adopts this percentage in cheese making.

There have been little to no studies on the addition of tea, especially OBT, into cheese making that could enrich the quality of the produced cheese. Previous research utilized green tea in several food products from dairy, such as ice cream (Sanguigni *et al.*, 2017), skimmed set-type yogurt (Liu, 2018; Mortazavian *et al.*, 2018), yogurt (Chaikham, 2015), milk tea beverage (Qie *et al.*, 2021), UHT milk (Jansson *et al.*, 2019), brown fermented milk (Yang *et al.*, 2022), and processed cheese (Giroux *et al.*, 2013; Rashidinejad *et al.*, 2014; Lamothe *et al.*, 2016; Rashidinejad *et al.*, 2016a; Rashidinejad *et al.*, 2016b; Rashidinejad *et al.*, 2017; Robalo *et al.*, 2022). However, to the best of our knowledge, there are no research results utilizing orthodox black tea (OBT) in making cow's milk cheese. This research is expected to improve the functional properties of cheese, which is already highly nutritious, by adding OBT as a source of antioxidants. Adding black tea is expected to improve the antioxidant activity, texture hardness, total solids, and total titratable acidity of cheese and produce distinctive color and desired taste. Based on the above review, research needs to be done to examine the effect of orthodox black tea on the sensory, physicochemical properties, and antioxidant activity of cheese.

## MATERIALS AND METHODS

### Cheese Making

The process of making cheese was inspired by Fadhlurrohman *et al.* (2023). It began with pasteurizing the milk and the addition of four concentrations of orthodox black tea (OBT), namely 0.5% w/w (T<sub>1</sub>), 1% w/w (T<sub>2</sub>), 1.5% w/w (T<sub>3</sub>), and 2% w/w (T<sub>4</sub>). Each treatment was added with 0.01 g/L milk of mesophilic bacteria (*Lactococcus lactis*). Then, into the milk was incorporated 0.2 g/L milk CaCl<sub>2</sub> and 1 g/L milk animal calf rennet,

and the milk was let sit for ± 60 minutes to allow the coagulation process (clumping of casein). After that, the curd was separated from the whey using a cheesecloth for ± 12 hours at refrigerator temperature. Then, the curd was pressed using a 2 kg weight for 1 hour to obtain the cheese yield. Each treatment was repeated four times in duplicates.

### Determination of Physicochemical Properties

The AOAC International (1990) thermogravimetric method was applied in this study to analyze the moisture content, total solids, and total titratable acidity. Total titratable acidity was based on titration of NaOH (Merck, Darmstadt, Germany) and phenolphthalein indicator (Merck, Darmstadt, Germany). Cheese pH was measured by inserting a pH meter (Hanna Instrument Pty) probe into the cheese and reading the pH value on the monitor screen (Setyawardani *et al.*, 2019). Cheese color was measured using a colorimeter (Konica Minolta CR-10) based on coordinates L\* (dark to light values), a\* (red to green values), and b\* (yellow to blue values) (Fadhlurrohman *et al.*, 2023). The cheese texture profile measured included hardness and stickiness assessed using texture profile analysis (Setyawardani *et al.*, 2018) from the TA-XT21 apparatus (Stable Micro Systems, Haslemere, U.K.). Antioxidant activity was measured with DPPH (2,2-diphenyl-1-picrylhydrazyl) method using a spectrophotometer (Mapada V-1100D) (Samadi & Fard, 2020). Samples were taken 1g and dissolved with 5 mL of methanol PA (Merck, Darmstadt, Germany). After dissolving, the sample was centrifuged using a centrifuge (Corona 80-2) at 4000 rpm for 10 minutes. The supernatant was taken 1 mL and added with 1 mL of 0.20 M DPPH reagent. Then, the sample was incubated at room temperature for 30 minutes. The absorbance of the sample was measured using a spectrophotometer at a wavelength of 517 nm. The blank solution used was 1 mL of methanol with 1 mL of DPPH, which was measured at the same wavelength.

### Sensory Properties Measurement

Sensory properties measurements were conducted based on a previous study (Setyawardani *et al.*, 2017b) to test the panelists' preference for samples presented in a sensory way. The test was conducted with 30 semi-trained panelists who had agreed to sensory testing and were not allergic to the test product. The sample was scored based on likes and dislikes of the attributes of color, taste, aroma, texture, and overall preference. The assessment criteria used a hedonic scale, namely immensely dislike (scale 1), dislike (scale 2), somewhat like (scale 3), like (scale 4), and really like (scale 5).

### Statistical Analysis

The data were examined using Analysis of Variance, followed by the orthogonal polynomials and Honest Significant Differences (HSD) test using SPSS version 22.

## RESULTS

## Cheese Physicochemical Properties

**Cheese color.** The results showed that adding OBTe affected the cheese color significantly. The results of the statistical analysis demonstrated that the very significant effects ( $p < 0.01$ ) were observed on the lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ) values of cheese. The average colors of cheese with the addition of OBTe are presented in Table 1. The color appearances of the cheese with the addition of OBTe are presented in Figure 1.

**Cheese texture.** Based on the statistical analysis, the addition of OBTe had a significant effect ( $p < 0.05$ ) on cheese hardness. Meanwhile, the stickiness of the cheese was not significantly affected by the addition of OBTe. The average values of the cheese texture characteristics with the addition of OBTe are presented in Table 1.

**Cheese moisture content and total solids.** Making cow's milk cheese with the addition of OBTe up to 2% produced cheese with moisture contents between 50.42% and 54.84%. It showed that the moisture contents and total solids of cow's milk cheese were not significantly affected by the addition of OBTe (see Table 1).

**Cheese pH and total titratable acidity.** Statistical analysis showed that the pH of the cheese decreased with the addition of OBTe. It was proven from the milk pH measured before and after adding OBTe. Prior to treatments, milk pHs were 6.60–7.02, while each treatment resulted in different milk pHs, namely 6.57 ( $T_0$ ), 6.41 ( $T_1$ ), 6.35 ( $T_2$ ), 6.29 ( $T_3$ ), and 6.25 ( $T_4$ ).

## Cheese Antioxidant Activity

The results of measuring the antioxidant activity showed that the more OBTe was incorporated, the higher the antioxidant activity of the cheese. The main

Table 1. Physicochemical properties of cheese made with the addition of orthodox black tea

Variables	The addition of orthodox black tea (%)					Significancy
	0	0.5	1	1.5	2	
$L^*$	55.01±1.64 <sup>d</sup>	43.62±0.58 <sup>c</sup>	39.14±2.76 <sup>bc</sup>	34.58±2.64 <sup>ab</sup>	31.30±2.36 <sup>a</sup>	**
$a^*$	1.76±0.41 <sup>a</sup>	8.65±0.85 <sup>b</sup>	10.83±1.39 <sup>c</sup>	13.03±0.94 <sup>d</sup>	14.45±1.10 <sup>d</sup>	**
$b^*$	25.70±0.40 <sup>b</sup>	26.32±1.23 <sup>b</sup>	25.72±1.37 <sup>b</sup>	23.98±1.30 <sup>ab</sup>	22.39±1.41 <sup>a</sup>	**
H (g/mm <sup>2</sup> )	256.58±31.62 <sup>b</sup>	207.80±48.88 <sup>ab</sup>	177.74±73.63 <sup>ab</sup>	167.69±36.79 <sup>ab</sup>	117.05±39.01 <sup>a</sup>	*
S (g/mm <sup>2</sup> )	-67.62±12.73	-51.90±25.82	-42.81±20.05	-52.42±21.66	-31.09±23.25	ns
MC (%)	50.42±2.99	51.96±4.00	54.00±3.50	54.46±0.39	54.84±0.84	ns
TS (%)	49.59±2.99	48.05±4.00	46.00±3.50	45.54±0.39	45.17±0.84	ns
pH	5.83±0.13 <sup>c</sup>	5.34±0.11 <sup>b</sup>	5.19±0.11 <sup>ab</sup>	5.12±0.12 <sup>ab</sup>	5.03±0.16 <sup>a</sup>	**
TTA (%)	0.08±0.01 <sup>a</sup>	0.09±0.01 <sup>ab</sup>	0.10±0.01 <sup>bc</sup>	0.12±0.01 <sup>cd</sup>	0.13±0.01 <sup>e</sup>	**

Note: values are presented as mean±SD. Means in the same row with different superscripts differ significantly ( $p < 0.05$ ).  $L^*$ : lightness value,  $a^*$ : redness value,  $b^*$ : yellowness value, H: hardness value, S: stickiness value, MC: moisture content, TS: total solids, TTA: total titratable acidity. \*: significant, \*\*: very significant, ns: non-significant ( $p > 0.05$ ).

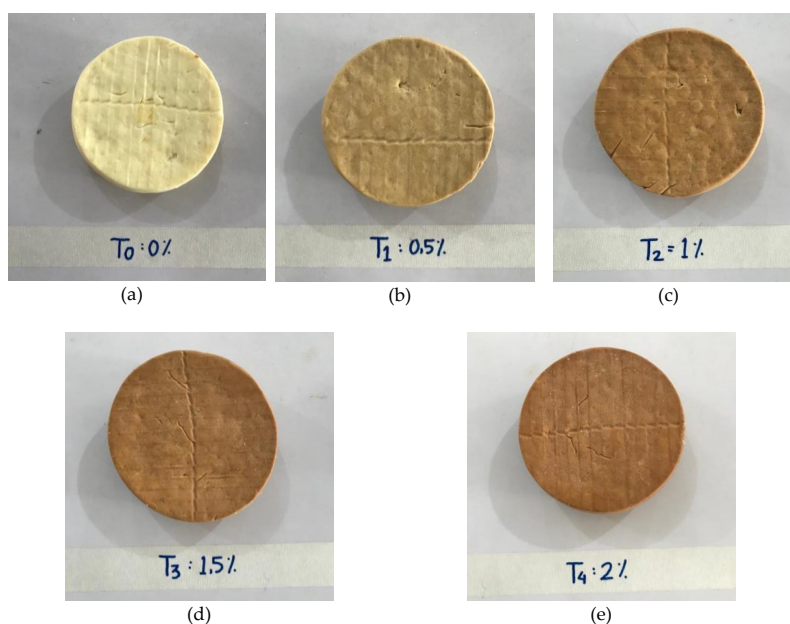


Figure 1. Color appearance of cheese with the addition of orthodox black tea 0% (a), 0.5% (b), 1% (c), 1.5% (d), 2% (e)

contributors to this increase were flavonoids and tannins (theaflavins and thearubigins) and the antioxidants contained in OB. High tannin content was evident from the browning of cheese color as a higher concentration of OB was incorporated into cheese making. The antioxidant activity of cheese with OB could increase up to 33.49% (Figure 2). The results of the antioxidant activity properties of cheese added with OB are presented in Figure 2.

**Cheese Sensory Properties**

The results of sensory property analysis by panelists of cow’s milk cheese added with 2% OB are presented in Table 2. The panelists’ preference scores of cheeses added with 2% OB were given for cheese color (2.97-4.33), flavor (2.90-3.70), aroma (3.23-3.73), and texture (2.43-3.83). Overall, the panelists’ cheeses made with the addition of OB tended to be somewhat liked by the panelists, with a score of 2.93-3.77. On average, panelists assigned a score range between 3 and 4 based on the criteria of “rather like” to “like” for the cheese produced.

**DISCUSSION**

**Cheese Physicochemical Properties**

**Cheese color.** The higher concentration of OB incorporated in the production of cow’s milk cheese has decreased the lightness (L\*) and yellowness (b\*) values (Table 1), but increased the redness (a\*) value. Thearubigin and theaflavin contents in OB play a role

in generating yellow-brown color. Polyphenol oxidases would oxidize catechins in the black tea fermentation process. Most of the catechins would be oxidized into theaflavins and thearubigins in OB, 10% and 50%-60% of the total flavonoids (Rohdiana, 2015; Fadhlorrohman *et al.*, 2023).

Making cow milk cheese with the addition of up to 2% OB made the cheese’s lightness (L\*) values decrease from 55.01 to 31.30. It was because the control cheese (without OB) had a yellowish-white color, while cheese added with up to 2% OB had a brownish color. BSN (2016) states that the brewing quality of OB is brown to reddish. Research by Giroux *et al.* (2013) revealed that cheese enriched with green tea extract had significantly lesser brightness (L\* value), higher a\* value (redness) and the b\* value (yellow).

Based on the color appearance of the cheese (Figure 1), the higher the percentage of OB added to the cheese making, the browner the color of the cheese produced will be. It is due to the presence of theaflavins and thearubigins in OB. These ingredients can give the product a distinctive taste and natural color (Fadhlorrohman *et al.*, 2023). Theaflavins are believed to give an astringent taste and quickly create a yellowish-gold color, while thearubigins contribute to giving a reddish-brown color (Wong *et al.*, 2022). Moreover, during the processing of OB, theaflavin and thearubigins compounds will be produced by the oxidation of catechins through polyphenol oxidases. It causes the number of theaflavins and thearubigins in OB to increase. In addition, several factors that affect the color of the cheese are the food additives used in its

Table 2. Sensory properties of cheese made with the addition of orthodox black tea

Variables	The addition of orthodox black tea (%)					Significancy
	0	0.5	1	1.5	2	
Color	4.33±0.92 <sup>b</sup>	3.37±0.96 <sup>a</sup>	3.20±0.85 <sup>a</sup>	3.03±1.07 <sup>a</sup>	2.97±1.30 <sup>a</sup>	**
Aroma	3.73±1.17	3.37±0.96	3.23±0.77	3.27±0.91	3.27±0.98	ns
Texture	3.63±1.13 <sup>c</sup>	3.83±1.12 <sup>c</sup>	3.27±0.94 <sup>bc</sup>	2.87±1.04 <sup>ab</sup>	2.43±0.97 <sup>a</sup>	**
Flavor	3.70±0.99 <sup>b</sup>	3.27±0.94 <sup>ab</sup>	3.07±1.05 <sup>ab</sup>	2.93±1.14 <sup>a</sup>	2.90±1.16 <sup>a</sup>	*
Overall preference	3.77±1.04 <sup>b</sup>	3.50±0.86 <sup>ab</sup>	3.30±0.92 <sup>ab</sup>	2.93±0.94 <sup>a</sup>	2.97±1.07 <sup>a</sup>	**

Note: values are presented as mean±SD. Means in the same row with different superscripts differ significantly (p<0.05). \*: significant, \*\*: very significant, ns: non-significant (p>0.05).

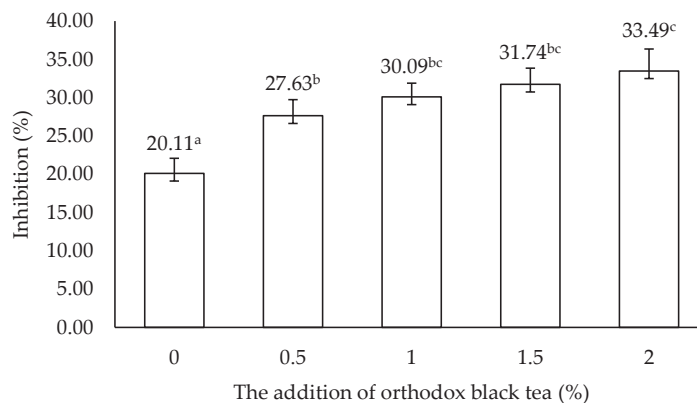


Figure 2. Antioxidant activity of cheese with the addition of orthodox black tea. The data with different superscripts differ significantly (p<0.05).

manufacture, the type of milk, and also the length of storage of the cheese (Setyawardani *et al.*, 2017b; Astuti *et al.*, 2021; Fadhlorrohman *et al.*, 2023).

**Cheese texture.** Adding OBT to make cow milk cheese has reduced cheese hardness but remained within the average range. Setyawardani *et al.* (2018) found that the hardness of cheese made from fresh goat milk without any addition (control) was 227.40-275.95 gf. In this study, the higher the OBT addition, the softer and stickier the cheese. It was confirmed by Giroux *et al.* (2013) that adding tea extract to cheese-making would increase the loss of cohesiveness and elasticity due to changes in the para-casein matrix. Néstor *et al.* (2013) explained that the contributing factors to the texture of cheese are the composition of raw materials (milk composition, lactic acid starter, type of coagulant, and other additives), cheese-making procedures, ripening conditions, and chemical composition of cheese (moisture content, protein, and fat). Moreover, reducing fat content can increase cheese hardness because milk fat makes cheese softer by evenly distributing the fat globules in the casein matrix, so when fat is removed, casein plays a significant role in cheese texture. According to Setyawardani *et al.* (2018), the presence of fat breaks down the protein matrix and acts as a lubricant so that the texture of the cheese becomes softer and smoother.

This study showed that the stickiness of cow milk cheese was not significantly affected by adding up to 2% OBT. Fat and casein affect cheese's stickiness, which, in turn, affects the cheese matrix's formation. Setyawardani *et al.* (2016) suggested that the higher the fat content, the stickier the cheese. Cheese stickiness is one of the texture profiles of cheese produced by the casein matrix by binding to fat granules. Several other factors can affect the texture of cheese, the chemical composition of cheese (moisture, protein, and fat content), lactic acid bacteria used, the types of coagulant, cutting size, and biochemical activity during cheese storage (Néstor *et al.*, 2013; Zheng *et al.*, 2016; Mezo-Solís *et al.*, 2020).

**Cheese moisture content and total solids.** The moisture content of cow milk cheese in this study tended to be lower than that of previous studies using the same raw material, namely 54.92% (Néstor *et al.*, 2013) and 63.22% (Hamad, 2015). Total cheese solids in this study ranged from 45.17% to 49.59%, which can be classified as semi-soft cheese. This range was within the required standards of BSN (2018), which is above 40%. The total solids of cheese in this study were similar to those reported in previous studies, namely 38.55%-41.25% (Al-Hamdani *et al.*, 2021) and 55.75% (Vacca *et al.*, 2020). Factors influencing the total cheese solids of cheese are the number of lactic acid bacteria, fat content, milk casein, curd-cutting time, and long storage time (Vacca *et al.*, 2018; Vacca *et al.*, 2020; Al-Hamdani *et al.*, 2021). Reale *et al.* (2022) explained that the increase of lactic acid bacteria would affect the total solids produced. The higher the milk acidity produced by lactic acid bacteria, the higher the ability to bind water during coagulation (Vacca *et al.*, 2020; Al-Hamdani *et al.*, 2021). In the coagulation process with acid, the lower the curdling pH, the curd's ability to hold water will increase, thus resulting in

higher moisture content and lower total solids. The acidic condition makes it easier for the whey to come out of the curd, so cheese with the highest pH will hold more water when filtering is finished. Conversely, cheese with the lowest pH value or in more acidic conditions will lose the most water and have the lowest moisture content. Qi *et al.* (2023) state that tea polyphenols are hygroscopic and readily absorb water. It can increase the moisture content and water activity of food substances incorporated with black tea. The higher moisture content is because black tea will bind more water during the manufacturing or storage of the product.

**Cheese pH and total titratable acidity.** The level of acidity is one of the determinants in the cheese-making process. In general, cheese-making can be done in two ways: direct acidification and acidification with the help of lactic acid bacteria (Casado-coterillo *et al.*, 2023; Mangione *et al.*, 2023). In this study, cow milk cheese was produced with the help of mesophilic bacteria in the form of *Lactobacillus lactis* and rennet enzymes. These additions significantly affected the sustainability of cheese-making process and the pH value of the cheese product. According to Setyawardani *et al.* (2016), the decrease in pH was due to lactic acid production and other organic acids produced by starters and probiotic cultures. The main factor affecting the pH level of cheese is the addition of lactic acid bacteria. *Lactobacillus lactis*, as a mesophilic bacteria starter, will convert sugar in milk into lactic acid. The addition of higher lactic acid bacteria (LAB) will cause the pH value to decrease. However, this study used the same amount of mesophilic bacteria and rennet enzymes, so cheese pH across treatments could not be distinguished. Also, we observed that the addition of acidic substances accelerated the pH decrease because black tea tends to be acidic, so the higher the OBT level, the lower the cheese pH. Patil *et al.* (2016) stated that theaflavin content in black tea is weakly acidic, while thearubigin is strongly acidic. The number of lactic acids produced by microorganisms influences the decrease of cheese pH. The higher the lactic acid, the lower the cheese pH due to bacterial activity in cheese (Setyawardani *et al.*, 2017a). In this study, the pH values of cheese ranged from 5.03 to 5.83, which is not considerably different from the other studies, namely 5.23–5.40 in cheese added with green tea extract (Rashidinejad *et al.*, 2016), 4.61–5.31 (Los *et al.*, 2021) and 4.34–5.29 (Vacca *et al.*, 2018).

Total titratable acidity is the determination of the total acid concentration that can affect the taste and aroma of a food product. Based on this study, total titratable acidity in cheese increased by adding OBT up to 2%, and the averages of total titratable acidity ranged from 0.08% to 0.13%. These results were not much different from a previous study, namely 0.06% (Setyawardani *et al.*, 2017a). The lowest total titratable acidity was shown in the control cheese (T<sub>0</sub>), and the highest was in the 2% OBT treatment (T<sub>4</sub>). It is inversely proportional that the more OBT added, the lower the pH value. This statement was emphasized by Sumarmono *et al.* (2019) that a decrease in pH would mark an increase in the total titratable acidity because more sugar was hydrolyzed into acid, the higher the lactose content, the higher the total acid produced. Factors that affect the total

titratable acidity are the activity of lactic acid bacteria, temperature, and storage time (Mamo, 2017).

**Cheese antioxidant activity.** The lowest antioxidant activity of cheese was obtained from the control treatment, namely 20.11%. Research by Giroux *et al.* (2013) stated that several components of milk, such as casein and whey protein, have been shown to have antioxidant properties. In this study, incorporating OBT up to 2% in cheese making resulted in the highest antioxidant activity, namely 33.49%. The higher black tea was added to the cheese, the more antioxidant activity of the cheese would increase. It is because the main components in black tea leaves are polyphenols and flavonoids, which act as antioxidants and protect biomolecules from free radicals like reactive oxygen and nitrogen species (Giroux *et al.*, 2013). The polyphenol contents found in black tea range from 3%-10%. Polyphenol antioxidants provide health benefits by fighting harmful free radicals, mitigating the risk of heart disease, and inhibiting skin cancer cells (Elgailani, 2015; Wong *et al.*, 2022). In contrast, polyphenol contents in black tea range from 3% to 10%. Fadhlorrohman *et al.* (2023) stated that the most extensive flavonoids in black tea are theaflavins (10%) and thearubigin (50%-60%). Theaflavin and thearubigin compounds in black tea have the potential to be potent antioxidants (Fernando & Soysa, 2015). Moreover, the most active flavonoid component with anti-inflammatory and antioxidant properties is epigallocatechin-3-gallate (EGCG). It has been reported that EGCG can protect cells from damage by inhibiting DNA damage and LDL (Low-Density Lipoprotein) oxidation (Wong *et al.*, 2022).

Antioxidants can act as inhibitors (blockers) of oxidation reactions by reactive free radicals (Muniandy *et al.*, 2016). Based on this study, the range of averages antioxidant activity of cheese based on the percentage of inhibition was 20.11%-33.49% (Figure 2). Research by Giroux *et al.* (2013) stated that the antioxidant activity of cheese after 29 days of storage decreased by 25% in control cheese and by 18% in cheese containing green tea extract (1%/L of milk). However, after 29 days, in green tea cheese, the antioxidant activity persisted at a 2%/kg milk concentration, and the cheese product contained a 44% level of antioxidant activity. It proved that the antioxidant activity of cheese could be affected by the length of storage, in addition to additional ingredients in the form of tea, which contains relatively high antioxidants. Additionally, green tea has slightly higher antioxidant properties than black tea. According to Samadi & Fard (2020), the antioxidant activity of black tea tested using the DPPH method was 71.052 (% inhibition). While green tea has higher antioxidant activity, namely 81.052 (% inhibition), black tea has potent antioxidant activity (although moderate) and provides some benefits, including the source of alkaloids, phenolics, flavonoids, saponins, and cardiac glycosides (Elgailani, 2015; Fernando & Soysa, 2015; Patil *et al.*, 2016).

**Cheese sensory properties.** The more OBT added to the production of cow milk cheese, the lower the panelists' preference for cheese color, which became darker. It may be because the panelists were unfamiliar with brown cheese and preferred the regular yellowish-white cheese.

The contributing factor to differences in cheese color is the raw material (type of milk), beta-carotene content in milk (Sharma *et al.*, 2020), and food additives in cheese making. Sant'Ana *et al.* (2013) added that cheese made of cow milk without additional food ingredients containing natural dyes would produce yellowish-white cheese because the carotene pigments dissolve in milk fat. In this study, cow milk cheese was produced by adding up to 2% OBT, resulting in a browner cheese.

According to the panelists, this study demonstrated that the more OBT added to cow milk cheese production, the less preferred the cheese taste because it was relatively more bitter due to OBT addition. According to Wong *et al.* (2022), catechin compounds strongly influence the taste and aroma of tea, and Qi *et al.* (2023) revealed that polyphenols compounds in tea could affect the bitter taste of tea, so black tea that undergoes an enzymatic oxidation process will produce the most potent aroma with a lighter taste. This enzymatic oxidation process catalyzes catechin compounds in tea by polyphenol oxidase enzymes to produce theaflavins and thearubigins (Rohdiana, 2015).

The panelists' assessment of cheese aroma was relatively the same across treatments because the percentage of OBT in this study was not much different (only 0.5%), thus emitting a distinctive aroma of tea. According to Sant'Ana *et al.* (2013), most of the cheese aroma comes from the type of milk used as the raw material. The aroma and taste of fermented milk products are contributed by metabolites produced by several lactic acid bacteria that are produced by several mechanisms. Another significant contributor to cheese aroma and taste is lactic acid (Setyawardani *et al.*, 2018). According to Los *et al.* (2021), incorporating a starter can cause a distinctive flavor or taste in cheese because the starter will break down lactose into lactic acid which has a distinctive taste. Besides acting as a coagulant for primary proteolysis or the breakdown of proteins into peptides, the rennet enzyme plays a role in developing cheese flavor. Regarding aroma, protein decomposition emits the aroma in black tea. Wong *et al.* (2022) assert that the oxidation of polyphenolic compounds is always directly or indirectly connected to the development of aroma in black tea. Wong *et al.* (2022) explained that the polyphenolic part contained in black tea would be oxidized into theaflavins and thearubigins, as well as unstable compounds that give complexity to the aroma of black tea. Black tea has 650 complex aroma compounds compared to green tea, while green tea has only 250 complex aroma compounds.

The score of panelists' preference for the texture of cow milk cheese in this study was 2 to 4, ranging from dislike to rather like and like. It is because cow milk cheese added with 2% OBT concentrates produced the highest moisture content, making the cheese softer. Meanwhile, 0.5% OBT ( $T_1$ ) produced neither hard nor soft cheese, so the panelists liked it the most. Some researchers agree that one of the contributing factors to cheese texture is the moisture content (Zheng *et al.*, 2016; Mezo-Solis *et al.*, 2020; Wang *et al.*, 2023). Néstor *et al.* (2013) emphasized that cheese texture is related to chemical composition at the micro and macrostructural levels, such as protein and fat contents. Generally, cheese texture is influenced by several factors, including moisture content, fat, and protein content.

This study demonstrated that the more OBT added to cow milk cheese production, the less preferred the cheese by the panelists because the OBT produced cheese with darker color, more softness, and more sour and bitter taste. According to Setyawardani *et al.* (2018), cheese preference is the overall consumer acceptance of cheese based on its taste, aroma, color, and texture.

## CONCLUSION

Cow milk cheese added with OBT is a potential functional food item with a high antioxidant. Adding up to 2% OBT in cheese-making increases the antioxidant activity of cheese to 33.49% and improves the color  $a^*$  and total titratable acidity. However, it tends to decrease cheese color  $L^*$  and  $b^*$ , hardness, and pH value, and panelists' preference for cheese. The most preferred cow milk cheese is added with 0.5% OBT.

## CONFLICT OF INTEREST

We certify that there are no financial, interpersonal, or other connections with individuals or groups related to the information discussed in the manuscript that might create a conflict of interest.

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