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Stability and flavor of set yogurt fortified with *Tremella fuciformis* polysaccharide during cold storage

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ABSTRACT

Tremella fuciformis is an edible and medicinal fungus containing excellent nutritional value. *T. fuciformis* polysaccharide (TFP) is the important bioactive ingredients of *T. fuciformis*, which has gained great attention. The aim of this study was to investigate the effect of TFP on the stability and flavor of set yogurt. Our results revealed that the addition of 0.1% TFP had a positive effect on improving the stability of set yogurt including the water holding capacity, texture, rheological properties and microstructure at the cold storage period of 1, 7, 14 and 21 days. It is remarkable that the hardness, gumminess and chewiness of the set yogurt were significantly improved by the addition of TFP during the cold storage. Moreover, the set yogurt containing TFP was able to maintain better stability in the three intervals thixotropy test. In particular, the addition of 0.1% TFP had no adverse effects on the flavor of set yogurt, including sourness, sweetness, umami, bitterness, richness and saltiness. These data suggested that TFP can be used as a natural potential stabilizer for the set yogurt.

1. Introduction

Tremella fuciformis belongs to the medicinal and edible fungus, which is well-known as “Crown of Fungi” (Wu et al., 2022; Xiao et al., 2021). *T. fuciformis* has been demonstrated to be widely distributed in the world, including Brazil, China and other East Asian countries (Wu et al., 2019; Zhang et al., 2023). There are two major production regions of *T. fuciformis* in China, Gutian in Fujian Province and Tongjiang in Sichuan Province, respectively (Huang et al., 2021). In particular, the production of *T. fuciformis* from Fujian province accounts for 90% of the total production in China. *T. fuciformis* normally enriched a variety of nutrients, including polysaccharides, proteins, vitamins and mineral elements (H. Li et al., 2022).

T. fuciformis polysaccharide (TFP) has gained much attention owing to the stable physicochemical properties, especially improve the food quality and flavor. Recently, Zhang et al. found that TFP improved noodle quality, enriched the flavor and reduced starch digestion (Zhang et al., 2022). The stability of tteok with TFP addition was significantly enhanced, meanwhile the short-term retrogradation and the hardness were alleviated during storage (Fan et al., 2023). In addition, it is remarkable that TFP interacted with myofibrillar protein to significantly enhance the gel properties of low-fat pork (Zhang et al., 2017).

Yogurt is the dairy product with high nutritional value, which is obtained by the raw cow (goat) milk or milk powder with a bacterial culture in which *Lactobacillus bulgaricus* and *Streptococcus thermophilus* species (Atik et al., 2023). Indeed, yogurt has become the most widely-consumed product around the world due to unique flavor and high nutritional value (Wu et al., 2023). There is also evidence that the quality parameters of yogurt were changed during production, transportation and storage, including texture, flavor and nutritional value (Deshwal et al., 2021; Fan et al., 2022). In the past few decades, increasing attention has also been focused on improving the stability and flavor of yogurt. Wang et al. reported that apple pomace improved the viscosity of yogurt and reduced whey release during cold storage (Wang et al., 2020). The carrot soluble dietary fiber might be a valuable ingredient to increase the quality of yogurt (Dong et al., 2022). It is remarkable that few studies have investigated the effect of TFP on the stability and flavor of set yogurt during cold storage.

In the present study, we evaluated the effect of 0.1% TFP addition on the stability and flavor of set yogurt during cold storage. Here, stability

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and flavor of set yogurt were investigated by TPA, rheometer, SEM, electronic tongue, and electronic nose at the cold storage period of 1, 7, 14 and 21 days. To the best of our knowledge, this is the first report to study the effect of TFP on the stability and flavor of set yogurt during cold storage, which provides a new insight on a potential stabilizer in set yogurt.

2. Material and methods

2.1. Extraction of TFP

T. fuciformis polysaccharide (TFP) was extracted according to the previously reported method with several modifications (Xiao et al., 2021). Briefly, *T. fuciformis* (Gutian, Fujian) was dried at 70 °C to constant weight and crushed into powder (200 meshes). The *T. fuciformis* powders were mixed with deionized water (1: 50, w/v) in water bath at 90 °C for 3 h to extract the polysaccharide. Then, the obtained extract was centrifuged at 4000 r/min for 20 min by centrifuge (L550, CENCE, China). Three times the volume of pre-cooled absolute ethanol was added to the supernatant obtained above and incubated overnight at 4 °C to obtain polysaccharides. Finally, it was lyophilized to obtain TFP.

2.2. Preparation of set yogurt

Commercial milk (MENGNIU, China) was heated to 70 °C in water bath. TFP was added to milk for 0 and 0.1% respectively. The sugar (7%, w/v) was added to the milk and dissolved. Then, the mixed-milk was pasteurized at 90 °C for 5 min. The milk was cooled to 43 °C, inoculated with 0.01% lactic acid bacteria (YO-PROX BA986, BIOPROX, France), and transferred to sterile containers with lids for incubation at 43 °C for 5 h. Finally, all samples were stored at 4 °C for 1, 7, 14 and 21 days.

2.3. Water holding capacity

The WHC was conducted by the method as previously described (Almusallam et al., 2021). The set yogurt sample (10 g, W1) was centrifuged at 3000 r/min for 30 min, and the supernatant was weighed (W2). The experiments were repeated in triplicates. The water holding capacity was calculated as follows:

$$\mathrm{WHC} = \frac{\mathrm{W1} - \mathrm{W2}}{\mathrm{W1}} \times 100\%$$

2.4. Texture profile analysis

The texture profile analysis (TPA) of set yogurt sample was performed according to the method of Pan et al. with minor modifications (Pan et al., 2022) using a texture analyzer (TA-XT plus, Stable Micro Systems, UK) equipped with a P/36R probe. The return distance was set at 50 mm with 20 mm/s return speed. The set yogurt was tested at the probe speed of 1 mm/s. The trigger force was 5.0 g and the compression ratio was 30%.

2.5. Rheological properties analysis

The rheological properties of set yogurt sample were measured by rheometer (Physica MCR 301, Anton Paar, Germany) equipped with a PP50 flat plate (diameter: 49.959 mm) following the method of Zhao et al. with slight modifications (Zhao et al., 2020). Viscosity was determined by a shear rate scan from 0 to 100 s^{-1}. Oscillation strain tests were performed at frequency of 1 Hz from 0.1 to 100% to determine the linear viscoelastic region. The results indicated that the 0.5% strain was suitable for all set yogurts. In the three intervals thixotropy test (3ITT), set yogurt was treated under constant strain of 0.5% at 1 Hz (~300 s). Then, the set yogurt was loaded with a high instantaneous shear rate (1000 s^{-1} × 0.5 s). Subsequently, the shear rate was withdrawn until steady state recovered (~600 s). Finally, the storage modulus (G′), loss modulus (G″), and loss tangent (tan δ) were monitored at the cold storage period of 1, 7, 14 and 21 days.

2.6. Scanning electron microscopy

Microstructure observation of set yogurt was performed with scanning electron microscopy (SEM, TM3030Plus, HITACHI, Japan) according to the previous method with modification (Khubber et al., 2021). Set yogurt sample was diluted in deionized water (1: 6) and frozen. The frozen sample was lyophilized and then subjected to gold sputtering for 120 s, and the microstructure of set yogurt was observed at 1.0 k.

2.7. Electronic tongue analysis

The taste of set yogurt was determined by Taste Sensing System (SA402B, Insent, Japan) according to the method of Li et al. with slight modifications (Ding et al., 2022). The set yogurt sample (40 g) was diluted with distilled water (1: 2), which was filtered by sterile gauze (four layers) to obtain the supernatant. Nine basic tastes were measured, including astringency, sourness, sweetness, umami, bitterness, saltiness, richness, aftertaste-A and aftertaste-B.

2.8. Electronic nose analysis

The flavor of set yogurt was determined by electronic nose device (PEN 3, AIRSENSE Analytics, Germany) according to the method of Qiu et al. with slight modifications (Qiu et al., 2021). The set yogurt sample (3.5 g) was taken into 50 mL headspace bottle and let stand for 30 min. The testing time and cleaning time both were set as 120 s.

2.9. Statistical analysis

Results were expressed as means ± SEM, which was obtained by testing in triplicate. The statistical differences between groups were evaluated by *t*-test using Microsoft Excel 2021. Statistically significant difference was labeled as $^*p < 0.05$ or $^{**}p < 0.01$.

3. Results and discussion

3.1. Water holding capacity of set yogurt

Water holding capacity (WHC), an important index affecting the stability and flavor of yogurt, was used to evaluate the acceptability of yogurt (Du et al., 2021; Korkmaz et al., 2021). As shown in Fig. 1, the WHC of the set yogurt showed a decreasing trend during the cold storage. Notably, the WHC of set yogurt containing 0.1% TFP was significantly higher ($p < 0.05$) than the controls at the cold storage period of 14 and 21 days. This phenomenon was occurred due to the electrostatic interactions between proteins and polysaccharides (Gentile, 2020). In addition, Wang et al. found that *Auricularia cornea* var. Li polysaccharides improved the WHC of set yogurt during the storage period (Wang et al., 2022). Accordingly, TFP exhibited the ability to improve the WHC of set yogurt during storage time.

3.2. Texture profile analysis of set yogurt

The texture of yogurt was described by hardness, gumminess, chewiness and springiness (Feng et al., 2019). Hardness is the most important determinant of yogurt texture, which directly reflected the strength of yogurt gel (H. Li et al., 2022; Y. Li et al., 2022). The hardness of set yogurt with 0.1% TFP was significantly higher ($p < 0.05$) than that of the controls during the storage period (Table 1). There has been reported that the gumminess of yogurt is related to the hardness (Mohsin et al., 2022). The gumminess and chewiness of set yogurt after adding of

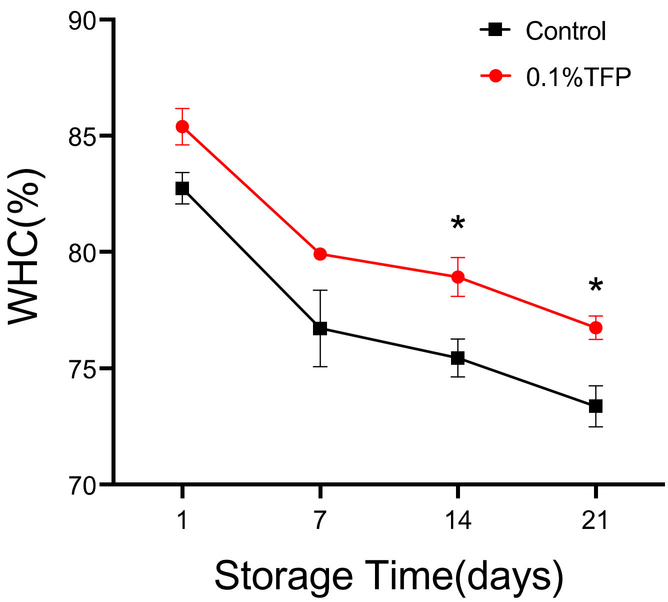

Fig. 1. Water holding capacity of set yogurt at the cold storage period of 1, 7, 14 and 21 days.

Table 1
Texture profile analysis of set yogurt at the cold storage period of 1, 7, 14 and 21 days.

Texture	Storage period (Day)	Control	TFP
Hardness/g	1	144.41 ± 1.72	181.91 ± 1.83**
	7	131.57 ± 2.87	156.23 ± 1.43**
	14	112.45 ± 1.28	141.13 ± 0.86**
	21	175.19 ± 4.96	198.32 ± 1.21*
Gumminess	1	51.92 ± 0.49	67.70 ± 1.48**
	7	48.91 ± 0.87	59.84 ± 0.93**
	14	42.89 ± 1.09	58.85 ± 1.25**
	21	63.32 ± 2.40	71.35 ± 3.13
Chewiness	1	50.37 ± 0.82	65.02 ± 1.19**
	7	45.62 ± 0.78	58.00 ± 0.85**
	14	41.74 ± 1.16	57.07 ± 1.02**
	21	59.93 ± 1.56	63.97 ± 3.31
Springiness	1	0.97 ± 0.00	0.96 ± 0.00
	7	0.93 ± 0.03	0.97 ± 0.00
	14	0.97 ± 0.01	0.97 ± 0.00
	21	0.95 ± 0.01	0.84 ± 0.05

TFP were significantly higher than that of the controls at the cold storage period of 1, 7, and 14 days. In particular, the hardness, gumminess and chewiness of set yogurt were significantly improved by 13.2–26.0%, 12.7–37.2% and 6.7–36.7%, respectively. Additionally, springiness slightly fluctuated during storage period, while there was no significant difference between two groups. The improvement of set yogurt texture might be the result of the interaction between TFP and proteins. Likewise, a similar result was also found that the stability of set yogurt was significantly enhanced by the addition of okra polysaccharide (Xu et al., 2019). Previously, Li et al. also found that the improvement of yogurt texture was associated with the interaction between *Lactobacillus paracasei* polysaccharide and casein (Li et al., 2020). Moreover, the stability of yogurt was increased after incorporating pineapple pomace powder (Meena et al., 2022). In conclusion, the results of texture profile analysis indicated that TFP had a positive effect on the stability of set yogurt.

3.3. Rheological properties analysis of set yogurt

The rheological properties of the set yogurt with the addition of TFP were shown in Fig. 2. The viscosity of set yogurt decreased with the increasing the shear rate, which indicated that the set yogurt showed shear thinning behavior (Fig. 2a, d, g and j). In particular, the viscosity of set yogurt was enhanced slightly after adding TFP at the cold storage period of 14 and 21 days, which was mainly due that TFP could interact with the opposite charges in proteins to form a stronger structure (Wijaya et al., 2017). The shear stress affecting yogurt stability was usually presented during production, transportation and consumption (Devnani et al., 2022). Therefore, 3ITT was conducted to evaluate the stability of the set yogurt under applied transient shear stress. In addition, the G′ and G″ of set yogurt with TFP were higher than that of the controls (Fig. 2b, e, h and k). G′ and G″ of the set yogurt dropped instantly under a high-frequency shear stress, which suggested that the structure of all set yogurts were irreversibly destroyed under high shear stress (Lu et al., 2021). The tan δ (tan δ = G″/G′) of all set yogurts were shown in Fig. 2c, f, i and l. The tan δ values of all set yogurts were less than 1, representing that the set yogurts had the solid behavior (Ayyash et al., 2022). In particular, the tan δ of set yogurt with TFP was slightly lower than the controls. It has been reported that the yogurt with a lower tan δ value hard to be dehydrated, which was consistent with the results of set yogurt added the exopolysaccharides from *Lactobacillus helveticus* MB2-1 (Ge et al., 2022). Consequently, the results of 3ITT revealed that the addition of TFP improved the stability of set yogurt and reduced the threat of external collisions to set yogurt.

3.4. Microstructure of set yogurt

Scanning electron microscopy (SEM) is a powerful technique to observe the microstructure and surface morphology micrographs of sample. The microstructure of set yogurt adding 0.1% TFP was presented in Fig. 3. Here, more compact structures have been observed in the TFP group at the same cold storage period. The results showed that the addition of TFP improved the stability of set yogurt, which was consistent with the results of WHC, texture and rheological properties (Gilbert and Turgeon, 2021). It has been reported that the compact and uniform microstructure played a certain positive role in the stability and quality of yogurt. Previously, Pan et al. found that the interaction of phenols from pomegranate juice powder with proteins formed a compacter structure, which made the set yogurt more stable during storage (Pan et al., 2019). In addition, Tian et al. suggested that the WHC of yogurt was improved due to the presence of denser protein clusters in microstructure of yogurt (Tian et al., 2022). Furthermore, the anti-shear performance of yogurt was enhanced because of Salecan (a linear β-glucan) improving the microstructure (Fu et al., 2018). Taken together, we speculated that TFP contributed to the formation of a compact and dense microstructure of set yogurt, which improved the physicochemical properties and enhanced the stability of set yogurt.

3.5. Flavor of set yogurt

The e-tongue and e-nose are the analysis tools for reflecting the gustation and olfaction (Lu et al., 2022). E-tongue and e-nose were performed to investigate the effects of 0.1% TFP addition on the flavor of set yogurt. According to the e-tongue analysis results, the set yogurts were no significant difference at the same storage day (Fig. 4a). As shown in Fig. 4b, the W5S (nitrogen oxides), W1W (sulfur compounds) and W2W (organic sulfides) showed high responding values in the set yogurts. It indicated that the corresponding substances of W5S, W1W and W2W were the main contributions of the flavor of set yogurt. Among them, W5S was significantly increased ($p < 0.05$) after adding TFP at the cold storage period of 7 and 14 days. We concluded that collectively, TFP increased the amount of nitrogen oxides that is one of the main contributors to the flavor of set yogurt. The reason was speculated that the increase of these sensor signals were related to the aroma produced during the fermentation (Yang et al., 2023). Principal component analysis (PCA) demonstrated the similarities and differences of set yogurt, which was used to analyze the flavor characteristics. The PCA of e-tongue (Fig. 5a) showed that the PC1 and PC2 accounted for 53.4%

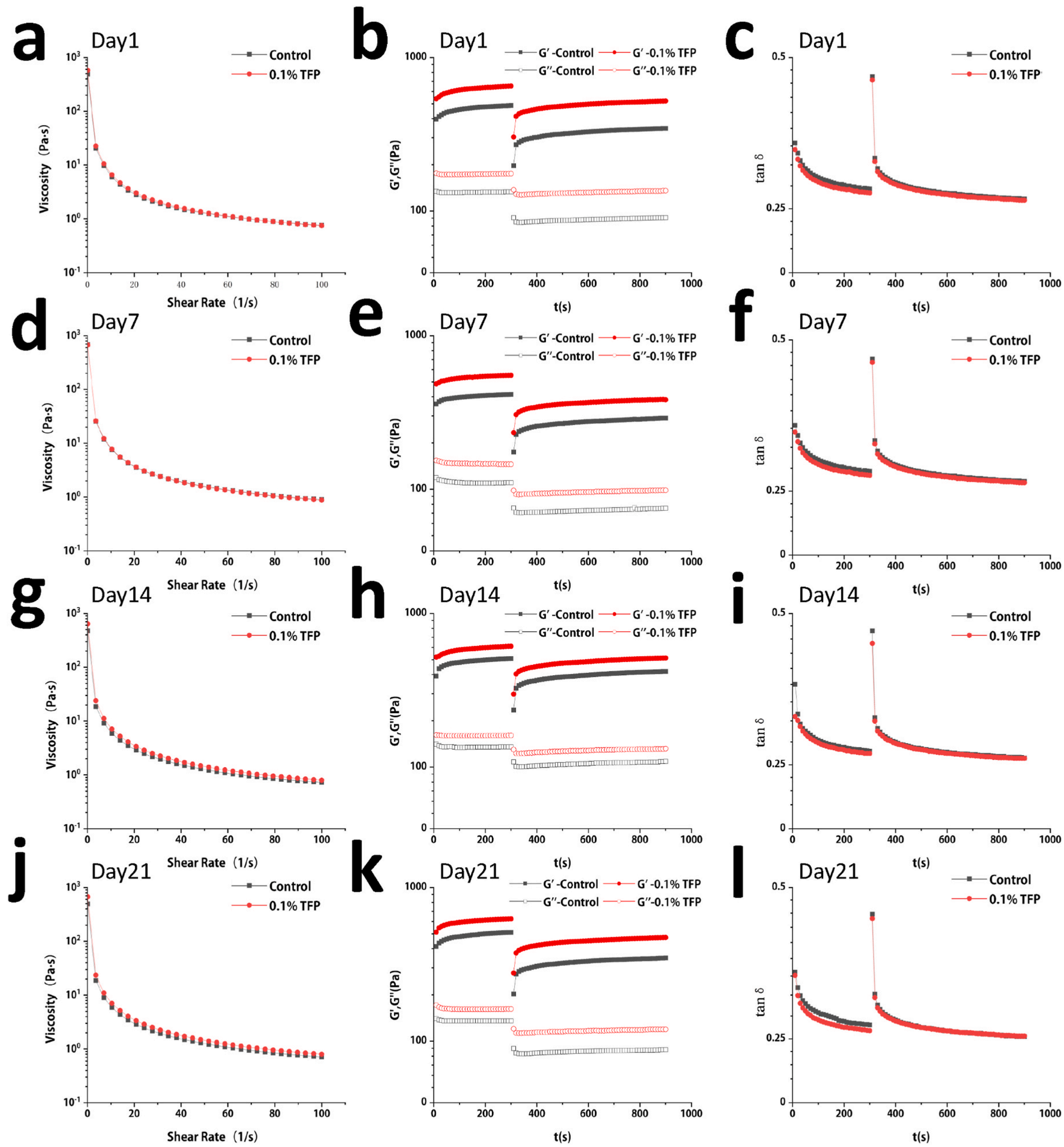

Fig. 2. Rheological properties of set yogurt at the cold storage period of 1, 7, 14 and 21 days. Viscosity of set yogurt with or without 0.1% TFP at different shear rates (a, d, g and j). The G′, G″ (b, e, h and k) and tan δ (c, f, i and l) value of set yogurt with 0.1% TFP in the 3ITT.

and 21.4% of the variation, respectively. The PCA of e-nose was shown in Fig. 5b, PC1 and PC2 contributed 84.7% and 10.2% of the variation, respectively. This indicated that PC1 and PC2 could explain the overall flavor characteristics of set yogurt. The distance between the set yogurt with TFP and the controls was short at the same cold storage, which showed that there were almost identical flavor profiles (Dan et al., 2022). Therefore, we concluded that the addition of TFP had little effect on the flavor of set yogurt and not brought adverse flavor.

4. Conclusion

In conclusion, the addition of TFP enhanced the WHC, texture, rheological properties of set yogurt. Moreover, the microstructure of set yogurt with TFP was more compact compared with the controls. These results showed that TFP improved the stability of set yogurt. Encouragingly, the addition of 0.1% TFP had no unfavorable effects on the flavor of set yogurt. It was concluded that TFP existed the potential role in increasing the stability and maintaining the flavor of set yogurt, which contributed to the application of TFP in set yogurt. Consequently, this work provided a new insight on a potential stabilizer in set yogurt production for desired stability and flavor.

CRediT authorship contribution statement

Lin Wang: Investigation, Data curation, Formal analysis, Writing – original draft. **Fan Zhang:** Investigation, Writing – review & editing. **Baodong Zheng:** Conceptualization, Project administration. **Yi Zhang:** Conceptualization, Funding acquisition. **Lei Pan:** Investigation, Writing – review & editing, Project administration, Funding acquisition.

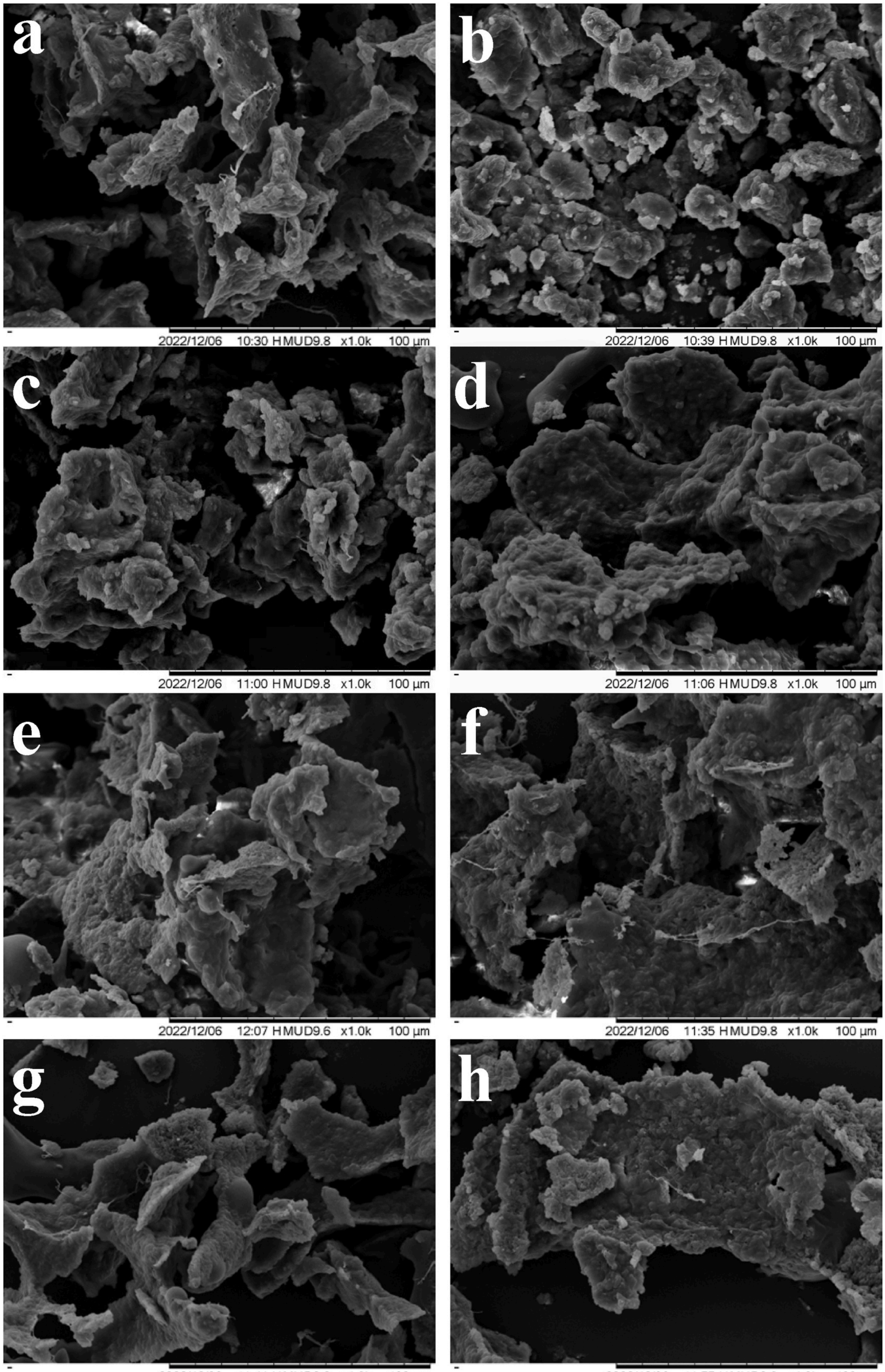

Fig. 3. Microstructure of set yogurt containing 0.1% TFP with magnification of 1.0 k at the cold storage period of 1, 7, 14 and 21 days. Control (a, Day1; c, Day7; e, Day14; g, Day21), TFP (b, Day1; d, Day7; f, Day14; h, Day21).

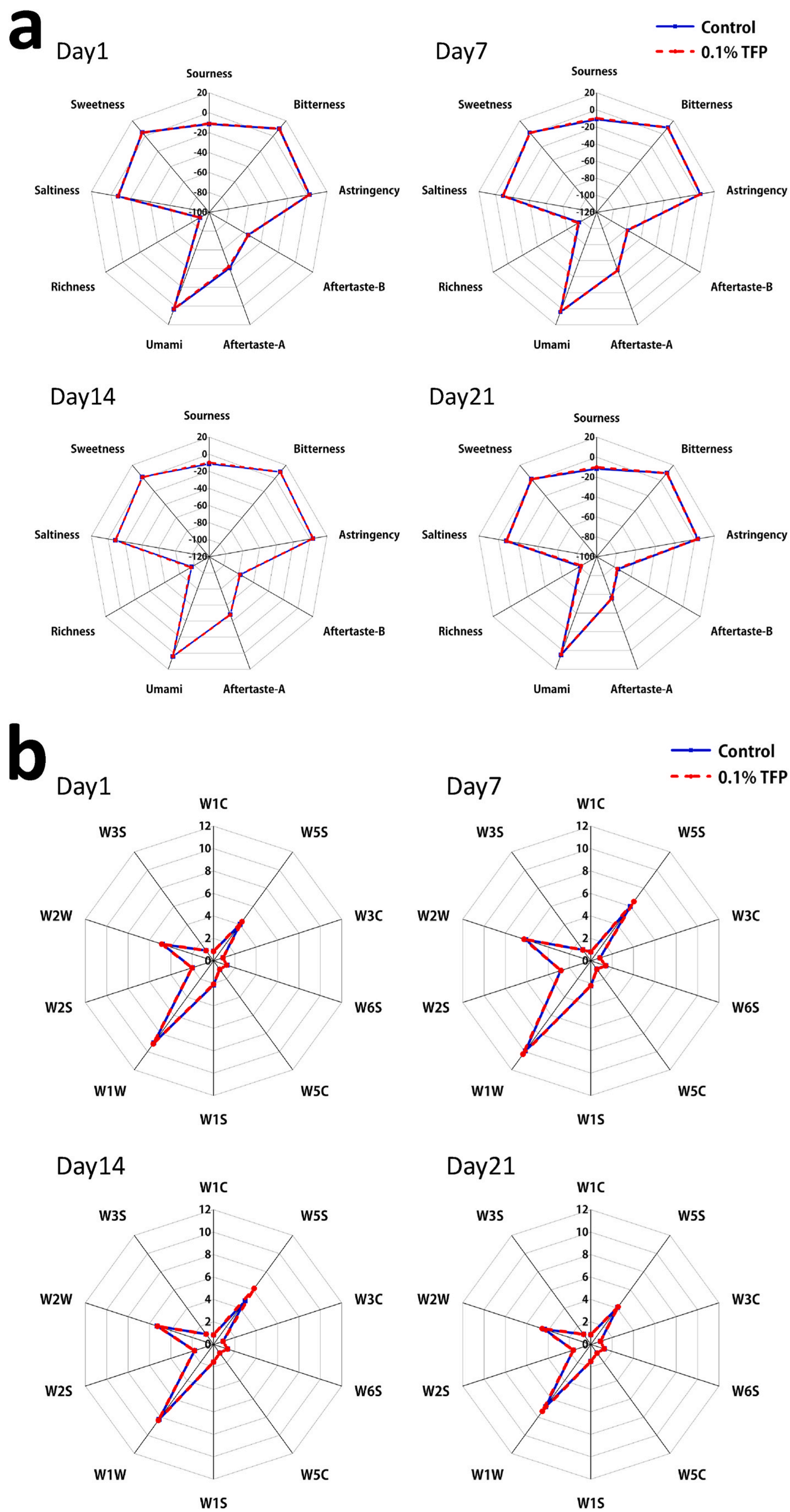

Fig. 4. The e-tongue (a) and e-nose (b) analysis of set yogurt containing 0.1% TFP at the cold storage period of 1, 7, 14 and 21days.

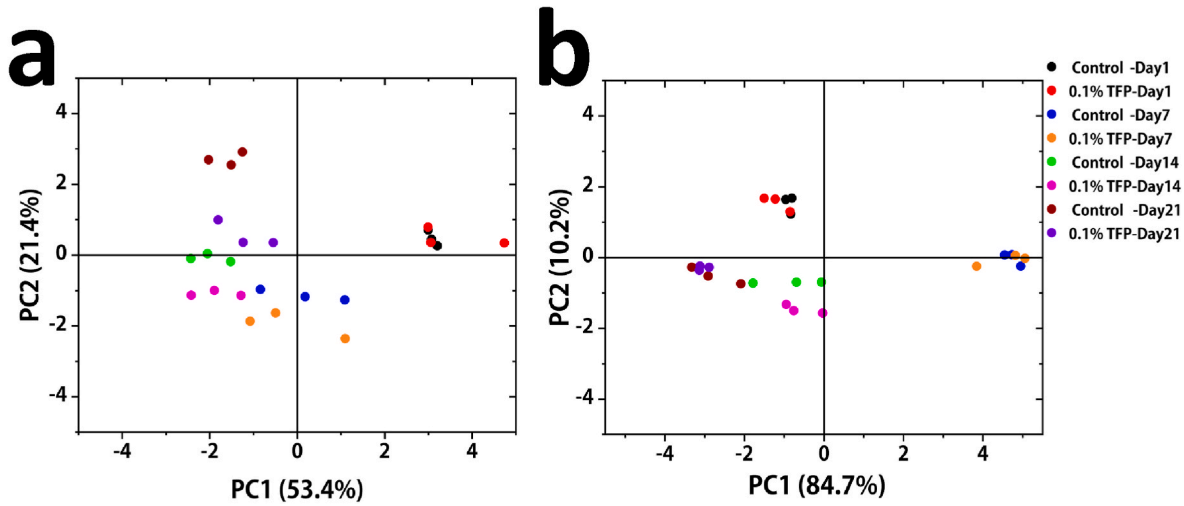

Fig. 5. Principal component analysis of e-tongue (a) and e-nose (b) for set yogurt at the cold storage period of 1, 7, 14and 21 days.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgements

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