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EXOPOLYSACCHARIDE PRODUCTION, CHARACTERIZATION, and ASSESSMENT of RHEOLOGICAL and ANTIOXIDANT PROPERTIES in *Saccharomyces cerevisiae* STRAINS ISOLATED from TRADITIONAL KEFIR SAMPLES

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ABSTRACT

This study aimed to investigate the production, characterization, and technofunctional properties of exopolysaccharides (EPS) produced by *Saccharomyces cerevisiae* strains isolated from traditional kefir samples. Three different *S. cerevisiae* strains, designated KY4, KY9, and KY17, were used in the study. The results showed that the highest EPS production, 65.41±4.18 mg/L, was observed in the KY4 strain. HPLC analyses revealed that the EPSs have been defined as heteropolysaccharides composed mainly of mannose. FT-IR analysis confirmed the polysaccharide structure through the presence of O-H bonds. EPSs exhibited water-holding capacity (WHC) values of approximately 103% and water solubility index (WSI) values ranging from 82.30% to 90.61%, which were not statistically significant ($P>0.05$). Rheological analysis demonstrated that all EPS samples displayed shear-thinning behavior. Moreover, the EPSs showed strong antioxidant activity ($P\leq0.05$). These findings indicate that EPSs produced by kefir-derived *S. cerevisiae* strains can be utilized in the food industry due to their technofunctional and bioactive properties.

Key words: Kefir yeast, *S. cerevisiae*, exopolysaccharide, HPLC, rheology, antioxidant activity

GELENEKSEL KEFİR ÖRNEKLERİNDEN İZOLE EDİLEN *Saccharomyces cerevisiae* SUŞLARINDA EKZOPOLİSAKARİT ÜRETİMİ, KARAKTERİZASYONU VE REOLOJİK VE ANTİOKSİDAN ÖZELLİKLERİN DEĞERLENDİRİLMESİ

ÖZ

Bu çalışmada, geleneksel kefir örneklerinden izole edilen *Saccharomyces cerevisiae* suşlarından elde edilen ekzopolisakkaritlerin (EPS), karakterizasyonu ve teknofonksiyonel özellikleri araştırılmıştır. Çalışmada KY4, KY9 ve KY17 olarak adlandırılan üç farklı *S. cerevisiae* suşu kullanılmıştır. Sonuçlar, en yüksek EPS üretiminin 65.41±4.18 mg/L ile KY4 suşunda gözlemlendiğini göstermiştir. HPLC analizi, EPS'lerin başlıca mannozdan oluşan heteropolisakkaritler olduğu ortaya çıkarmıştır. FT-IR analizi, O-H bağlarının varlığı aracılığıyla polisakkarit yapısını doğrulamıştır. EPS'ler, yaklaşık %103'lük su tutma kapasitesi (WHC) değerleri ve %82.30 ile %90.61 arasında değişen su çözünürlük indeksi (WSI) değerleri sergilemiştir ($P>0.05$). Reolojik analiz, tüm EPS örneklerinin kayma incelmesi davranışı gösterdiğini göstermiştir. Ayrıca, EPS'ler güçlü antioksidan aktivite göstermiştir. Bu bulgular, kefir kaynaklı *S. cerevisiae* suşları tarafından üretilen EPS'lerin teknofonksiyonel ve biyoaktif özellikleri nedeniyle gıda endüstrisinde kullanılabileceğini göstermektedir.

Anahtar kelimeler: Kefir mayası, *S. cerevisiae,* ekzopolisakkarit, HPLC, reoloji, antioksidan aktivite

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INTRODUCTION

Fermented dairy products, due to their rich microbial diversity and biologically active ingredients, play a significant role in nutrition and health (Gentry et al., 2023). Among these, kefir stands out as a traditional fermented dairy product from the Caucasus, enriched with probiotic microorganisms and known for its positive health effects (Xiao et al., 2021). Kefir grains develop through the symbiotic relationship of a complex microbial community that includes lactic acid bacteria, yeasts, and acetobacter species (Wang et al., 2023). This microbial community not only guides the fermentation process but also influences the nutritional and functional properties of the product (Xiao et al., 2021).

One of the key components produced during kefir fermentation is exopolysaccharides (EPSs) (Rahbar Saadat et al., 2021). EPS are high-molecular-weight polysaccharides synthesized by microorganisms that play a crucial role in maintaining the structural integrity of kefir grains. Additionally, EPS is reported to be a valuable biopolymer in functional foods because of its viscosity-enhancing, prebiotic properties, and immune-regulating effects (Xiao et al., 2021).

Among this microbial diversity, *Saccharomyces cerevisiae* is one of the most prominent yeast species in kefir fermentation (Dertli and Çon, 2017). During fermentation, *S. cerevisiae* metabolizes carbohydrates to produce ethanol and carbon dioxide, contributing to the product's characteristic aroma and texture (Saygili et al., 2023). Furthermore, through symbiotic interactions with bacteria within the kefir microbiota, it plays a role in EPS production and the formation of bioavailable metabolites (Chen et al., 2016; Dertli and Çon, 2017). Furthermore, yeast EPSs have gained significant research interest recently due to their technological properties-such as low viscosity, high stability, and ease of production-and their functional properties, including antioxidant, antitumor, and immunomodulatory effects (Ma et al., 2018). To date, various yeast genera, including *Saccharomyces, Cryptococcus, Hansenula, Lipomyces, Bullera, Aureobasidium, Sporobolomyces,* and *Rhodotorula*, have been reported to produce EPS (Ragavan and Das, 2019). Nonetheless, screening for new yeast strains that offer high EPS yields remains necessary, along with a comprehensive understanding of their technofunctional characteristics.

Studies on microbial-derived EPS production have primarily focused on lactic acid bacteria. Although lactic acid bacteria and yeasts play a joint role in the formation of kefir granules, information on the EPS production potential of yeast strains isolated from kefir samples and on the structural and functional properties of these EPSs is limited. This study aims to elucidate the relationship between the EPS yield, composition, and functional properties of *S. cerevisiae* strains derived from traditional kefir. In this study, the monosaccharide composition of the obtained EPSs was determined by HPLC, their functional groups were identified by FT-IR analysis, and their rheological behavior and antioxidant activities were evaluated. The aim is to shed light on the potential of kefir-derived yeast EPSs in functional food applications and to fill the gap in the literature regarding kefir-derived yeast EPSs.

MATERIAL AND METHODS

EPS producer *S. cerevisiae* strains and culture conditions

The *S. cerevisiae* strains used for EPS production and characterization in this study were KY4, KY9, and KY17. These strains were isolated from kefir samples in a previous study, which examined their probiotic properties (Goktas et al., 2021). YPD medium was used to pre-activate S. cerevisiae strains, which were incubated at 30°C for 48 h. The activated strains were then inoculated at 2% (v/v) into sucrose-modified EPS production medium. BHI medium was modified with 3% sucrose and used as the EPS production medium. This EPS production medium was incubated for 96 hours at 30°C.

EPS production from *S. cerevisiae* strains

EPS production was conducted following the methodology described by İspirli and Dertli (2018). After incubation, the upper phase was collected by centrifugation at 5000 rpm for 15 minutes. Three times the amount of cold ethanol was added to the upper phase and kept at 4°C overnight to precipitate EPS. The lower phase was then collected under the centrifugation conditions described above and completely dissolved in water. Cold ethanol was added again to precipitate more EPS. Pure water was added until the precipitate was completely dissolved. TCA was added, and the mixture was incubated for 3 hours. Proteins and other substances were removed via centrifugation, and the pH of the upper phase was adjusted to 7. Finally, cold ethanol was added once more to obtain pure EPS. The resulting

substance was collected by centrifugation and then lyophilized for further analysis.

Monosaccharide composition of EPSs by HPLC

To analyze the monosaccharide composition of EPSs, an HPLC system (Shimadzu, Japan) equipped with a COSMOSIL Sugar-D (4.6 mm ID x 250 mm) column and an RID-10A detector was used. The analysis involved a mobile phase of 85% acetonitrile, a flow rate of 1 mL/min, and a column temperature of 40°C. An 800 µL sample of EPS solution (10 mg/mL) was hydrolized with 218 µL of 72% perchloric acid at 90°C for 90 minutes. The mixture was then neutralized with KOH, the upper phase was separated by centrifugation, and the sample was passed through a 0.22 µm filter into HPLC vials for analysis (Özpınar et al., 2024).

FT-IR spectroscopy analysis of EPSs

The major structural groups of the EPSs were identified using FT-IR spectroscopy. The FT-IR spectrum was recorded in transmission mode over the 4000-400 cm^{-1} range, with 40 scans (FT-IR-4600, Jasco, Japan).

Determination of water holding capacity (WHC) and water solubility index (WSI) of EPSs

To determine the WHC and WSI of EPSs, 0.2 g of EPS powder was dissolved in 5 mL of pure water, vortexed for 3 minutes to create a homogeneous suspension, and incubated for 20 minutes at 40°C. The supernatant was then separated by centrifugation for 30 minutes at 5000 rpm, dried in glass petri dishes for 4 hours at 105°C (Yilmaz et al., 2022). Finally, the petri dishes were weighed, and the WSI of EPSs was calculated using the following equation (Eq. 1). Additionally, the residue remaining in the tube after centrifugation was weighed, and the WHC of EPSs was determined using the following equation (Eq. 2).

$$WSI = \frac{Weight\ of\ dry\ solids\ in\ supernatant}{Weight\ of\ dry\ sample} * 100 \quad \text{Eq. 1}$$

$$WHC = \frac{Weight_{Tube+Residue} - Weight_{Tube}}{Weight\ of\ dry\ EPS\ sample} * 100 \quad \text{Eq. 2}$$

Rheological characteristics of EPS samples

To examine the rheological characteristics of EPSs, a 1 L stock solution was prepared containing 0.1M CaCl2, 0.1M NaCl, and 11% (w/v) skim milk powder to simulate a milk medium. EPSs were then dissolved in this solution at a concentration of 2 mg/mL. The solution was homogenized by vortexing for 5 minutes. Subsequently, shear stress was measured at 25 °C across a shear rate range of 1-100 s-1, with data collected at 40 discrete shear rate points (Anton Paar MCR 302, Austria). Guar gum was used as the positive control, and measurements were performed in duplicate (Wang et al., 2015).

Antioxidant activity of EPS samples

The antioxidant activity of EPS samples was assessed using the DPPH, ABTS, and FRAP methods. EPS solutions were prepared at a concentration of 4 mg/mL in pure water, vortexed for 1 minute, and sonicated for 15 minutes to ensure complete dissolution for the antioxidant assays.

DPPH capacity of EPS samples

2 mL of the EPS solution was mixed with 2 mL of a 0.2 mM DPPH solution in ethanol to evaluate DPPH scavenging. The mixture was then incubated in the dark for 30 minutes, and absorbance was recorded at 517 nm (Liu, Xu, Na, et al., 2022). The DPPH radical scavenging activity was calculated using the following equation:

$$DPPH\ (\%) = (A_1 - A_2) / A_1 \times 100,$$

where A1 is the absorbance of DPPH solution, and A2 is the absorbance of the mixture (DPPH solution and EPS solution).

ABTS capacity of EPS samples

To assess ABTS radical scavenging, 100 µL of EPS solution was mixed with 2 mL of ABTS radical solution and incubated at room temperature for 5 minutes. Absorbance was then measured at 734 nm (Liu, Xu, Du, et al., 2022). The ABTS radical scavenging activity was calculated as:

$$ABTS\ (\%) = (A_1 - A_2) / A_1 \times 100,$$

where A1 is the absorbance of ABTS solution, and A2 is the absorbance of the mixture (DPPH solution and EPS solution)..

Statistical analysis

EPS production was performed in two replicates, and data from the EPS sample analysis are presented as the mean and standard deviation of these replicates. Statistical differences among the results were tested using one-way ANOVA and the Tukey test in JMP Pro 14.

RESULTS AND DISCUSSION

Monosaccharide composition of *S. cerevisiae* EPSs

EPS concentrations of three different *S. cerevisiae* strains ranged from 65.41±4.18 to 46.09±2.10 mg/L (Figure 1). The highest EPS concentration was observed in *S. cerevisiae* KY4, while the lowest was found in KY9. These findings indicate that different *S. cerevisiae* strains produce EPS at varying levels.

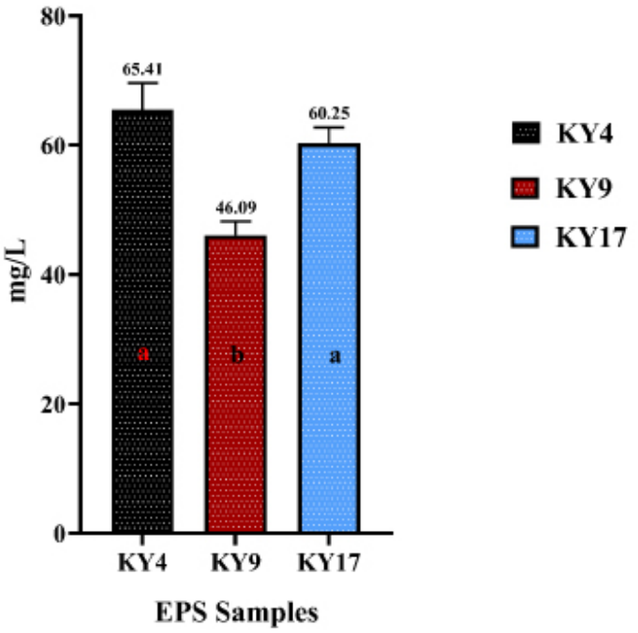

Figure 1. EPS production levels of *S. cerevisiae* strains (KY4, KY9, and KY17) in mg/L. Different letters indicate statistically significant differences among strains ($P \leq 0.05$).

Additionally, HPLC chromatograms of the monosaccharide compositions of EPSs are shown in Figure 2. EPSs mainly consisted of mannose units and had a heteropolysaccharide structure. The most abundant monosaccharide in all three EPS samples was mannose, with EPSs from KY4, KY9, and KY17 containing 226.89, 194.91, and 203.81 mg/g of mannose, respectively. Glucose was another monosaccharide commonly found in all three EPS samples. In KY9 and KY17 EPS, glucose was the second most abundant monosaccharide after mannose, whereas xylose was present in KY4 EPS. Literature reports indicate that EPSs derived from different yeast strains also have a heteropolysaccharide structure, as seen in this study. Specifically, EPSs from different *S. cerevisiae* strains (HD-01 and Y3) were reported to have a heteropolysaccharide structure composed of glucose and mannose units (Liu, Xu, Na, et al., 2022; Yang et al., 2024). Overall, the findings of this study support, in agreement with existing literature, the heteropolysaccharide structure of yeast EPSs.

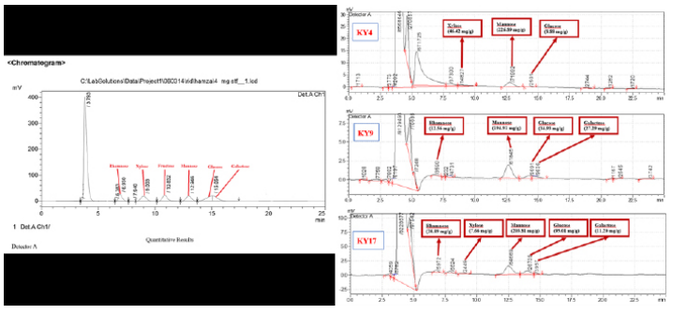

Figure 2. Monosaccharide composition of *S. cerevisiae* strains analyzed by HPLC

Functional groups of *S. cerevisiae* EPSs by FTIR

FT-IR spectroscopy is a powerful tool for identifying the functional groups of exopolysaccharides. As shown in Figure 3, the spectra of EPSs range from 3400 to 500 cm^{-1}. The presence of spectra between 3400 and 3000 cm-1 indicates O-H bonds, confirming that EPSs have a polysaccharide structure (İspirli, 2023). Peaks observed around 2300 cm-1 suggest the presence of -SH functional groups (Raj et al., 2016). The spectrum showing C=O carboxyl groups appears at 1630-1670 cm^{-1} in FTIR spectra (Soliemani et al., 2022). Peaks in the 1540 cm^{-1} region indicate methyl groups, while peaks at 1390 cm^{-1} are associated with methylene groups (Hamidi et al., 2020; Liu, Xu, Na, et al., 2022). The peaks observed at 1200-950 cm-1 constitute the fingerprint region and are linked to C-O-C and C-O stretching vibrations, indicating two monosaccharide components (Oztekin et al., 2025; Silambarasan et al., 2019). Consistent with HPLC results, these findings support the heteropolysaccharide structure. Similar FTIR spectra have been reported in the literature for EPSs obtained from different yeast strains, which agrees with this study (Hamidi et al., 2020; Liu, Xu, Na, et al., 2022; Oztekin et al., 2025; Silambarasan et al., 2019; Yang et al., 2024). Beyond structural validation, FTIR results also reveal the functional properties of EPSs.

The presence of functional groups such as -OH in the FTIR spectrum indicates the abundance of hydroxyl groups, which contribute to antioxidant activity through hydrogen donation and radical scavenging (Yang et al., 2024). Furthermore, hydrophilic functional groups such as O-H and C-O-C are closely related to water solubility and water-holding capacity, facilitating strong interactions between EPS molecules and water (Hamidi et al., 2020; Silambarasan et al., 2019). Therefore, FTIR findings not only support the polysaccharide nature of EPSs but also help elucidate their solubility, hydration behavior, and antioxidant potential.

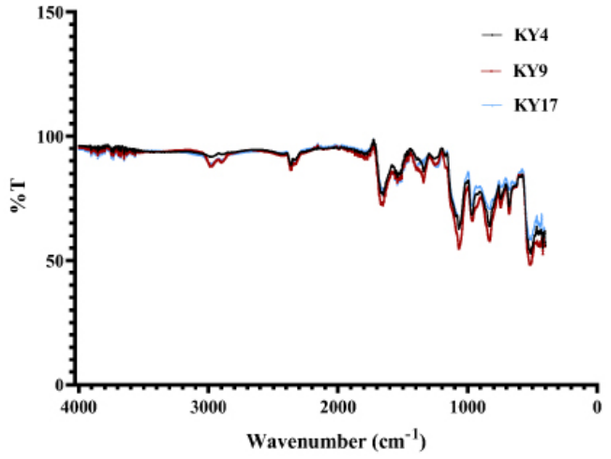

Figure 3. FT-IR spectrum of the EPSs from *S. cerevisiae* strains displays the functional groups at the wavelength of 400-4000 cm^{-1}

WHC and WSI properties of *S. cerevisiae* EPSs

The WHC and WSI values of the EPSs are shown in Table 1. The EPSs have similar WHC values, and no significant differences were found among the results (P>0.05). In this context, the WHC values of KY4, KY9, and KY17 EPSs were measured as 103.65±0.47%, 103.05±0.36%, and 102.98±0.58%, respectively. Additionally, the WHC values indicate that EPSs possess high water retention capacity. Therefore, high WHC suggests the potential of EPS to retain moisture and enhance product texture in food applications. Although there were some differences in the WSI values of EPSs, no significant difference was detected (P>0.05). The WSI values for KY4, KY9, and KY17 EPSs were recorded as 82.30±0.21, 82.92±3.61, and 90.61±0.54, respectively. The WHC and WSI values of *S. cerevisiae* Y3 EPS were reported as 229.67±10.45% and 75.23±5.83%, respectively (Liu, Xu, Du, et al., 2022). In another study, *Rhodotorula glutinis* EPS showed WHC and WSI values of 190.0±0.2% and 60.6±8.3%, respectively (Oztekin et al., 2025). In this study, the obtained WHC values were lower than the literature reports for *S. cerevisiae* and *Rhodotorula glutinis* EPSs, while the WSI values were higher. Ultimately, the high WHC and WSI values of EPSs could serve as alternatives in the food industry for extending shelf life, improving texture, and ensuring uniform distribution and desired consistency.

Table 1. WHC and WSI of *S. cerevisiae* EPSs

EPS Samples	WHC (%)	WSI (%)
KY4	103.65±0.47[a]	82.30±0.21[a]
KY9	103.05±0.36[a]	82.92±3.61[a]
KY17	102.98±0.58[a]	90.61±0.54[a]

WHC: Water holding capacity, WSI: Water solubility index. Different letters within the same column indicate statistically significant differences (P≤0.05).

Rheological properties of *S. cerevisiae* EPSs

The rheological properties of EPSs were plotted using shear rate (1/s) versus viscosity (mPa·s) (Figure 4). The rheological behavior of EPSs was compared with guar gum, which was used as a positive control. EPSs exhibited shear-thinning flow behavior because their viscosity decreased with increasing shear rate (Table 2). Shear-thinning flow behavior is important for desirable sensory properties such as mouthfeel and flavor release, as well as for improving certain processes such as mixing, pouring, pumping, and spray drying. At this point, shear-thinning flow exhibited by EPSs is important for improving the sensory and technological properties of foods. Although the EPS samples showed shear-thinning behavior, their viscosities were significantly lower than guar gum, a common viscosity-enhancing hydrocolloid. As a result, these EPSs might be better suited as stabilizers or emulsifiers rather than primary thickening agents. It has been reported that both bacterial and yeast-derived EPSs exhibit shear-thinning flow behavior. For example, (Oztekin et al., 2025) and (Ragavan

and Das, 2019) reported shear-thinning flow behavior for EPSs obtained from *R. Glutinis* and L. *starkeyi,* respectively. Additionally, EPS obtained from L. plantarum and *W. confusa* have also been reported to exhibit shear-thinning flow behavior (Wang et al., 2015; Zhao et al., 2020). The findings indicate that yeast-derived EPSs exhibit shear-thinning flow behavior, and these findings are consistent with the literature.

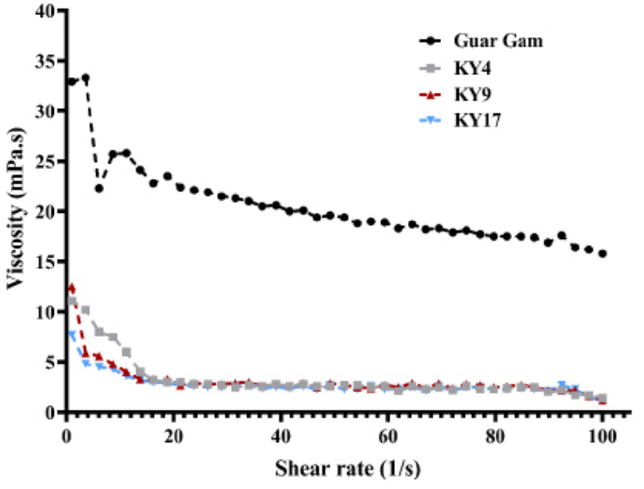

Figure 4. Shear-thinning flow behavior of EPS solutions from *S. cerevisiae* strains, measured at 25°C.

Table 2. Apparent viscosity values of EPS solutions (2 mg/mL) from *S. cerevisiae* strains, measured at different shear rates

Shear rate	Viscosity (mPa.s)			
(1/s)	Guar Gum	KY4	KY9	KY17
1.00	32.90	11.10	12.50	7.70
11.20	25.80	6.00	4.00	3.60
21.30	22.40	3.00	2.70	2.70
51.50	19.40	2.70	2.70	2.30
100.00	15.80	1.40	1.20	1.10

Antioxidant properties of *S. cerevisiae* EPSs

The antioxidant properties of EPSs were demonstrated by ABTS, DPPH, and FRAP methods (Figure 5). EPSs exhibited a high level of DPPH radical scavenging activity, ranging from 45.33% to 47.45%. There was no significant difference in the DPPH radical scavenging abilities among the three EPS samples (P>0.05). Literature reports varied results regarding the DPPH radical scavenging capacity of EPSs from different yeast strains. For example, the DPPH radical scavenging capacity of EPS from *S. cerevisiae* HD-01 strain was noted as 43.17% at 4 mg/mL (Yang et al., 2024). It was also reported that *S. cerevisiae* Y3 EPS had a DPPH scavenging capacity of 35% at 3 mg/mL (Liu, Xu, Na, et al., 2022). EPS from *R. mucilaginosa* CICC 33013 and *R. mucilaginosa* sp. GUMS16 strains were reported to have DPPH radical scavenging capacities of 25.05% and 28.7% at concentrations of 1 mg/mL and 8 mg/mL, respectively (Hamidi et al., 2020; Ma et al., 2018). This study found that the DPPH radical scavenging capacities of *S. cerevisiae* strains isolated from kefir samples were higher than those previously reported in the literature.

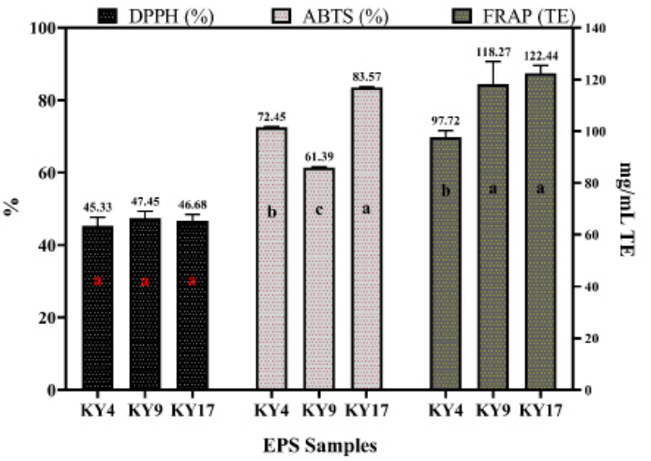

Figure 5. Antioxidant activities of EPSs obtained from *S. cerevisiae* strains were evaluated using DPPH, ABTS, and FRAP assays. Different letters indicate statistically significant differences among EPS samples within the same assay (P≤0.05).

The ABTS radical scavenging capacities of EPSs obtained from KY4, KY9, and KY17 strains were 72.45%, 61.39%, and 83.57%, respectively. In contrast to the DPPH radical scavenging capacity, significant differences were observed in the ABTS radical scavenging capacities of EPSs (P≤0.05). At this point, EPS obtained from the KY17 strain demonstrated the highest ABTS radical scavenging capacity. Literature reports the ABTS radical scavenging capacities of EPSs from *S. cerevisiae* HD-01, *R. mucilaginosa* CICC 33013, and *S. cerevisiae* Y3 strains

as 35.65%, 50%, and 24.8%, respectively, at concentrations of 4 mg/mL, 8 mg/mL, and 3 mg/mL (Liu, Xu, Na, et al., 2022; Ma et al., 2018; Yang et al., 2024). The results from this study indicate that the DPPH radical scavenging capacity, as well as the ABTS radical scavenging capacity, were higher than the values reported in the literature. Additionally, the FRAP activities of the EPSs ranged from 97.72 to 122.44 mg/mL. EPSs from KY9 and KY17 showed high FRAP activity, while KY4 EPS demonstrated a lower level of FRAP activity ($P \leq 0.05$). Differences in antioxidant activity among EPS samples are likely influenced by variations in their monosaccharide composition. In this study, all EPSs were mainly composed of mannose, but varying proportions of mannose, glucose, and other monosaccharides across strains could have contributed to differences in antioxidant activity. In this context, previous studies have linked differences in antioxidant activity to differences in monosaccharide composition, molecular weight, conformation, and the presence of functional groups (Liu, Xu, Na, et al., 2022; Yang et al., 2024). These differences among strains and antioxidant methods can be explained by the radical scavenging abilities of EPS and the influence of -OH groups in the polysaccharide structure, which may reduce free radicals to stable forms or break free radical chains by donating electrons (Zaghloul et al., 2024). Because the antioxidant activity of polysaccharides is largely determined by structural properties such as molecular weight, monosaccharide composition, and glycosidic bond configuration, it's affected by a mix of multiple factors rather than just one (Zheng et al., 2014).

CONCLUSION

In this study, the structural groups and technofunctional properties of EPSs obtained from three different *S. cerevisiae* strains (KY4, KY9, and KY17) isolated from traditional kefir samples were examined. EPSs have a heteropolysaccharide structure mainly composed of mannose units. The EPSs, with WHC values of approximately 103% and relatively high WSI values, indicate good solubility but moderate water-holding capacity compared with previously reported yeast-derived EPSs. Furthermore, EPSs have shown shear-thinning rheological behavior. Additionally, the EPSs exhibited strong antioxidant properties, with their antioxidant activities varying depending on the method used. Variations in the structural and functional characteristics of these EPSs highlight that each strain exhibited intraspecific variation in EPS production capacity and provide a foundation for further research on using these natural polymers as additives in functional food formulations. Overall, given the technofunctional properties exhibited by EPSs produced by kefir-derived *S. cerevisiae* strains, these EPSs may find potential applications in the food industry as stabilizers or dispersion-promoting agents, or as natural antioxidant components in functional food formulations. Therefore, these yeast-derived EPSs can serve as multifunctional natural additives that contribute to both technological performance and bioactive value in food systems.

CONFLICT OF INTEREST

The author(s) declare no conflict of interest.

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