International Journal of Biological Macromolecules 264 (2024) 130507

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Physicochemical and structural characterisation of a branched dextran type exopolysaccharide (EPS) from *Weissella confusa* S6 isolated from fermented sausage (Sucuk)

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ARTICLE INFO

Keywords:
Fermented sausage
Exopolysaccharides (EPS)
Dextran
Weissella confusa
Physicochemical characterisation

ABSTRACT

Exopolysaccharide (EPS) producing Lactic Acid Bacteria (LAB) species can be presented in distinct environments. In this study, Turkish fermented sausage (sucuk) was tested for the presence of EPS producer LAB strains and slimy-mucoid colonies were selected for further tests. Among the isolates, *Weissella confusa* strain S6 was identified and tested for the physicochemical characterisation of its EPS. This strain was found to produce 0.74 g L^{-1} of EPS in modified BHI medium conditions. Structural characterisation of EPS S6 by ^{1}H and ^{13}C NMR demonstrated that EPS S6 was a highly branched dextran type glucan formed by mainly (1 → 2)-linked α-D-glucose units together with low levels of (1 → 3)-linked α-D-glucose units as branching points. This structure was further confirmed by methylation analysis detected by GC–MS. An average molecular weight of 8×10^{6} Da was detected for dextran S6. The FTIR analysis supported the dextran structure and revealed the presence of distinct functional groups within dextran S6 structure. A strong thermal profile was observed for dextran S6 detected by DSC and TGA analysis and dextran S6 revealed a degradation temperature of 289 °C. In terms of physical status, dextran S6 showed amorphous nature detected by XRD analysis. SEM analysis of dextran S6 demonstrated its rough, compact and porous morphology whereas AFM analysis of dextran S6 detected in its water solution showed the irregularity with no clear cross-link within the dextran chains. These technological features of dextran S6 suggests its potential to be used for *in situ* or *ex situ* application during meat fermentations.

1. Introduction

Turkish fermented sausage locally known as sucuk is a fermented dry-cured meat product that is produced by using generally beef, tail fat, garlic salt, sugar, nitrite, and nitrate together with some spices including red pepper, black pepper, pimento, and cumin then the mixture is filled with natural or artificial casings [1–3]. Sucuk can be produced in both industrial methods using heat treatment processes by manufacturers and traditional methods including spontaneous or commercial starter cultures. Ripening of sucuk is performed to obtain a certain pH in the commercial methods which can take shorter periods in comparison to the traditional methods and effective staterter cultures are needed to obtain the desired taste, aroma, color and textural properties [2,4]. Lactic acid bacteria (LAB) are the main microbial group triggering the fermentation process during sucuk production process [3]. The most common LAB genera isolated from sucuk are *Lactobacillus*, *Weissella*, *Leuconostoc*, *Pediococcus*, and *Lactococcus* [5,6]. Besides, coagulase-negative cocci (CNC) such as *Staphylococcus xylosus*, *S. carnosus*, and *Kocuria varians*, which are responsible for biochemical reactions like color development, proteolysis, lipolysis, and decomposition of free amino acids and peroxides could be found in fermented sausages including sucuk [7]. Together with the role of LAB species for the microbial safety of fermented sausages originating from the pH decrement [8], LAB species produces important metabolites such as bacteriocins and organic acids, which exhibit antibacterial and antifungal activity, as well as exopolysaccharides (EPS) that play crucial roles for the

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https://doi.org/10.1016/j.ijbiomac.2024.130507
Received 1 October 2023; Received in revised form 24 January 2024; Accepted 26 February 2024
Available online 28 February 2024

physicochemical properties of fermented food products [9–12].

One of the fermented sausage associated LAB species is *Weissella* species and importantly *Weissella spp.*, was primarily isolated from fermented sausage as previously reported [13]. So far, several *Weissella* species belonging to *W. confusa*, *W. paramesenteroides*, *W. halotolerans*, *W. ciberia*, *W. hellinica*, *W. viridescens*, *W. minor*, and *W. thailandensis* were reported to present in various fermented sausages [14–18]. In terms of sucuk, few studies were conducted and the presence of *W. viridescens*, *W. hellinica*, and *W. cibaria* were reported [2,19,20]. The role of *Weissella* species as a member of fermented sausage microbial consortia can be linked with the function of these species to produce specific metabolites such as EPS and more studies are required to unveil both the microbial flora of traditional fermented sausages as well as to characterise these metabolites.

EPS are among the main techno-functional metabolites of LAB species and in terms of structural features, LAB species can produce two types of EPS as heteropolysaccharides and homopolysaccharides which are formed by two or more types of sugar monomers and only one type of sugar monomer, respectively [21]. For *Weissella* species EPS production is a common feature and the main EPS type produced by these species is homopolymeric glucans [22–29] although one study reported the galactan type EPS production by a *W. confusa* strain [30]. So far four distinct glucan structures were determined produced by LAB species as dextran, mutan, alternan and reuteran which alters depending on the level of linkages connecting the glucose units [23]. For instance, the main structural unit in the dextran structure is the (1 → 6)-linked α-D-glucose units and small proportion of the structure can be formed by (1 → 2), (1 → 3) or (1 → 4)-linked α-D-glucose units [22]. Identification of new LAB species with EPS production characteristics from distinct environments such as sucuk can be important to unveil new glucan structures as the technological and functional roles of glucan structures were mainly associated with the core structure of the glucans [28]. So far, limited number of studies tested the role of EPS during fermented sausages production and it was suggested that the role fermentation conditions, meat matrix, and strain specific conditions were important parameters for the function of EPS [31]. In an another study tested the role of EPS producer strain utilised for the fermentation process, *in situ* EPS production positively affected the physicochemical properties of sucuk [32]. These studies suggested the importance of obtaining an EPS producer LAB strain from sucuk environment in order to fulfill both starter functions as well as technological advancements originating from EPS formation.

From these perspectives, this study aimed to isolate an EPS producer potential starter culture from sucuk environment and to characterise this EPS in terms of structure and physicochemical functions. For this at first, traditional sucuk samples were collected from four distinct regions of Turkey and LAB strains with potential EPS production characteristics were isolated. The LAB isolates with slimy-mucoid phenotypic profiles were selected and isolates were genotypically discriminated and distinct isolates were then identified. An EPS producer strain *W. confusa* S6 were further selected for EPS extraction and the monomer composition of EPS S6 was detected by High Liquid Pressure Chromatography (HPLC) analysis whereas Nuclear Magnetic Resonance (NMR) spectroscopy and Gas Chromatography–Mass Spectrometry (GC/MS) analysis were applied to characterise the structure of EPS S6. Gel Permeation Chromatography (GPC) analysis was applied to determine the molecular weight of EPS S6. For the determination of EPS S6 functional groups, Fourier-Transform Infrared Spectroscopy (FTIR) analysis was applied whereas for thermal characteristics Differential Scanning Calorimetry (DSC) and Thermogravimetric (TGA) analysis were performed. The crystallographic properties of EPS S6 were determined by X-Ray diffraction (XRD) analysis and in terms of morphological features, Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM) analysis were performed. This study demonstrates the potential of sucuk isolate *W. confusa* S6 as a technologically important starter strain for future applications in meat industry as EPS S6 reveals crucial techno-functional roles.

2. Material and methods

2.1. Isolation of LAB strains from fermented sausage (sucuk)

In this study, four different sucuk samples produced by traditional methods were obtained from Çanakkale, Afyon, Giresun and Kayseri provinces of Türkiye. To isolate LAB strains, 10 g of sucuk sample was homogenized with 90 mL physiological saline (0.85 % *w/v*, NaCl). Afterward, serial dilutions were prepared up to 10^{-6} dilution factor with physiological saline and an aliquot of 100 μL was inoculated in Petri dishes containing MRS agar including 10 g L^{-1} peptone, 5 g L^{-1} yeast extract, 10 g L^{-1} meat extract, 20 g L^{-1} glucose, 2.0 g L^{-1} potassium phosphate, 5 g L^{-1} sodium acetate, 0.2 L^{-1} magnesium sulphate, 0.05 g L^{-1} manganese sulphate, 1.08 g L^{-1} Tween 80, 2.0 g L^{-1} ammonium citrate, and 12 g L^{-1} agar .The plates were incubated at 30 °C for 48 h anaerobically. At the end of the incubation, different colonies regarding morphological properties were picked from the plates and transferred to MRS broth, and incubated under anaerobic conditions at 30 °C for 24 h for testing of Gram stain and catalase reaction [33]. Then, 32 LAB isolates were selected, then all strains were incubated at 30 °C for 48 h anaerobically. Stock solutions of isolates were prepared in 20 % (*v*/v) glycerol and stored at −80 °C for further analyses.

2.2. Genotypic characterisation by $(GTG)_5$-PCR

The DNA extraction procedure was carried out using phenol:chloroform:isoamyl alcohol methodology. The extracted genomic DNAs were used as templates for PCR reactions. Following the extraction of DNA samples, $(GTG)_5$-PCR was applied for genotypic discrimination of the isolates. The PCR reaction was set with 10 × PCR buffer, dNTPs, primer (5′-GTG GTG GTG GTG GTG-3′), 5 U polymerase (Dreamtaq DNA Polymerase, Thermo) and extracted DNA as template in ultra-sterile H_2O. PCR condition was set as 95 °C for 10 min, 35 cycles of 94 °C for 1 min, 40 °C for 1 min, and 65 °C for 8 min and 65 °C for 16 min final extension. Afterward, the amplification was confirmed by using agarose gel electrophoresis. Distinct isolates were selected for 16S rRNA gene sequencing. For this, the PCR mixture contained ultra-sterile H_2O, 10 × PCR buffer, dNTPs, primers AMP_F (5′-GAG AGT TTG ATY CTG GCT CAG-3′) and AMP_R (5′-AAG GAG GTG ATC CAE CCG CA-3′), 5 U DNA polymerase, template DNA. The PCR program was set as 95 °C for 2 min, 20 cycles of 95 °C for 30 s, 55 °C for 1 min, and 72 °C for 30 s and 72 °C for 5 min final extension. After agarose gel electrophoresis confirmation, the 16S rRNA gene samples were sequenced by Medsantek (Istanbul, Türkiye). To determine the similarity of the sequence, the BLAST algorithm was used *via* the NCBI database with a similarity criterion of 98–100 % [34].

2.3. Detection of EPS production in selected LABs

A modified BHI media consisting of 37 g L^{-1} BHI broth (17.5 g L^{-1} Brain-Hearth Infusion Solids (porcine), 10.0 g L^{-1} Typtose, 2.0 g L^{-1} Glucose, 5 g L^{-1} Sodium Chloride, 2.5 g L^{-1} Disodium Hydrogen Phosphate), 5 g L^{-1} of meat/beef extract, 5 g L^{-1} of peptone casein, 5 g L^{-1} of sodium acetate, 1 g L^{-1} of Tween 80, 0.2 g L^{-1} of $MgSO_4.7H_2O$, 15 g L^{-1} of agar, 30 g L^{-1} of sucrose was prepared to investigate the EPS production capacities of the selected isolates as previously discussed [34]. Briefly, morphologically strong slimy colonies were chosen for EPS extraction and characterisation.

2.4. Extraction and purification of the EPS S6

The selected LAB cultures were grown in modified BHI broth at 30 °C for 48 h in anaerobic conditions. A prior defined protocol was implied for the isolation of extracellular EPS [21]. Shortly, after the incubation

period, cultures were centrifuged at 8000 rpm at 4 °C for 20 min and supernatants obtained. The 2 vol of cooled ethanol was added to the supernatant. Then the supernatants were left at 4 °C overnight for precipitating of the EPS. The pellet was obtained by centrifugation at 6000 rpm at 4 °C for 20 min, then the pellet was dissolved in the distilled water which was followed by the TCA precipitation of the proteins [21]. Following the removal of the proteins, the pH of the supernatant was set to pH 7 and 3 vol of cooled ethanol was added to the supernatant (6000 rpm at 4 °C for 20 min) and the mixture was left at 4 °C for 24 h. Partially purified EPS was obtained by centrifugation and a final step of dialysis using 12,000–14,000-Da membrane (Medicell International, UK) was performed and the membrane contents were obtained by lyophilisation (Christ, Beta 1–8LSCplus, Germany). The lyophilized EPS was stored at 4 °C for further analysis.

2.5. Determination of monosaccharide composition and molecular weight of EPS S6

To determine the monosaccharide composition of EPS S6, HPLC (Shimadzu, Tokyo, Japan) analysis was performed. Firstly, a 10 mg/mL solution was prepared from lyophilized EPS samples. Then 800 μL was taken, and following this step, 72 % perchloric acid was added and the solution was processed at 95 °C for 2 h. Afterward, the final neutralization step was carried out with 5 M KOH. Samples were then centrifuged at 12000 rpm at 4 °C for 5 min to precipitate composed salt, filtrated a 0.22 μm filter, and 20 μL was treated for HPLC analysis to specify the monosaccharide composition of EPSs. RID-10 A refractive index detector together with a Concise Carbosep CHO 87C column was used as the mobile phase of H_2O with a flow rate of 0.6 mL/min and the temperature of the column was 85 °C. Glucose, rhamnose, galactose, and fructose were used as standard sugars. To calculate the level of each sugar in the EPS structure, standard curves were constituted for each sugar. The yield of EPSs was quantified through these curves [34].

The Molecular weight (MW) of EPS S6 sample was determined using the GPC. Viscotek TDA302 GPC system (Malvern, UK) with refractive index (RI) and right-angle light scattering (RALS) detectors was used to record GPC chromatograms. The GPC column was the Tosoh G3000PWXL and the flow rate was 0.8 mL/min. GPC analysis was carried out at room temperature. For the calibration of detectors, PEO ((Mw = 21 kDa, Đ = 1.15) was used as single standard [35].

2.6. NMR and GC–MS analysis of the EPS S6

To investigate the chemical structure of EPS S6, NMR analysis (^1H) was applied at 25 °C using an Agilent 400 NMR instrument (California, USA). In this context, about 20 mg of lyophilized EPS was dissolved in 500 μL of D_2O, after 300 μL of the acquired solution was transferred to NMR tubes and processed. ^1H spectra of EPS-S6 were analyzed by MestreNova software [34]. The glycosyl linkage profiles of EPS S6 was also determined through partially methylated alditol acetate analysis using GC/MS (6890 A, Agilent Technologies Inc., California, USA) coupled with a capillary column (RTX-2330, Restek Corp., Bellefonte, PA, USA). Briely, EPS S6 samples were partially methylated followed by hydrolysis, reduction, and acetylation, as previously described by [36]. Details of the derivatization process as well as GC/MS conditions applied were given in our previous manuscript [36,37].

2.7. FTIR analysis of the EPS S6

Functional groups within the EPS S6 structure were analyzed by PerkinElmer Spectrum 65 FTIR Spectrometer (İstanbul, Turkey). Accordingly, the FTIR spectra of the lyophilized EPS was scanned in the transmission mode in the region of 4000–400 cm^{-1}. The resolution applied was set to the value of 4 cm^{-1} with 32 scans in an attempt to obtain the FTIR spectra [22,34].

2.8. Thermal analyses of the EPS S6

2.8.1. TGA analysis of the EPS S6

A PerkinElmer Simultaneous Thermal Analyzer STA 8000 (İstanbul, Turkey) was used for the TGA analysis of EPS S6. In this direction, EPS S6 was placed into an Al_2O_3 crucible and the heat was arranged between 25 °C and 1000 °C with increasing heating rate of 10 °C min^{-1}. The analysis was performed in an air atmosphere at a flow rate of 100 mL min^{-1}. Derivatization of the TGA data was constructed for DTG analysis [22,34].

2.8.2. DSC analysis of the EPS S6

DSC analysis of EPS S6 was carried out using a PerkinElmer DSC 8000 instrument (İstanbul, Turkey) in a temperature range of 10 to 500 °C with a heating rate of 10 °C min^{-1}. For this approximately 5 mg EPS sample was deposited in a sealed Aluminium pan and heat was applied and to monitor the melting point and enthalpy change, an empty pan was used as a reference [22, 34].

2.9. XRD analysis for determination of crystallographic properties of EPS S6

XRD method was applied to determine the physical properties of the EPS S6. For this, Bruker D8 Discover XRD (Massachusetts, USA) was used by loading EPS S6 on the instance plate. Accordingly, the diffractogram was achieved with Breg Brentano θ:2θ geometry by the Cu Kσ radiation (Ni filter, generator settings: 40 kV, 40 mA) application. The diffraction scan was implied in the 2θ range of 5-90° with a step size of 0.03° [38].

2.10. Morphological analyses of the EPS S6

2.10.1. SEM analysis of the EPS S6

SEM analysis was applied to morphologically characterise EPS S6 and for this, a FEI Nova Nano SEM 450 Instrument (Hillsboro, Oregon, United States) was used. For the analysis, 5 mg of EPS sample was fixed to the SEM stubs with double-sided tape, then coated with a layer of gold, ~10 nm thick. The EPS sample was tested at magnifications ranging from 1000 to 50,000 × with a voltage of 5.0 kV adjusted as in an acceleration mode [39].

2.10.2. AFM analysis of the EPS S6

Surface characteristics of EPS S6 was determined by AFM analysis. For this EPS S6 sample was dissolved in UP H_2O at a concentration of 1 mg/ mL, and about 5 μL of diluted EPS S6 solution was dropped onto the surface of a mica sample carrier and allowed to dry at room temperature. Then, AFM images of the EPS S6 were recorded by utilizing an AFM PLUS+, Nanomagnetic Instrument (Oxford, UK). The images were taken by using the tapping mode of AFM with commercial tapping silicon (Si) and the expected tip Radius was less than 10 nm. The images were obtained at 256 × 256 point resolution within the range of 5 μm/s scan rate. Finally, all images were subjected to first-order flattening [40].

2.11. Nucleotide accession numbers

The 16S rRNA sequences of the identified sucuk strains were deposited to the Genbank (NCBI) with the accession numbers of OR492663 - OR492668.

3. Results and discussion

3.1. Identification and isolation of W. confusa strains from sucuk as EPS producers

There is an increasing trend to isolate and transfer the autochthonous LAB strains found in traditional fermented products demonstrating

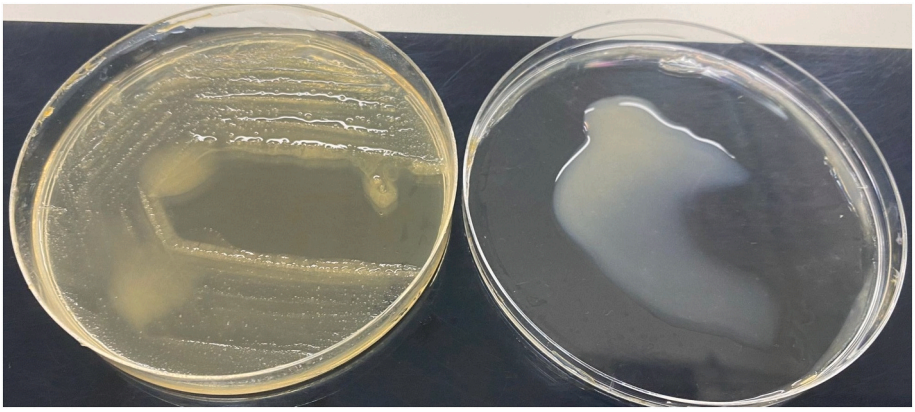

Fig. 1. Colony morphology of *Weissella confusa* S6 in sucrose containing modified BHI agar demonstrating its highly slimy profile.

technological relevance to industrial scale and applications. EPS production is one of the main technological characteristics of the LAB strains affecting mainly the physicochemical properties of food products and in this regard, this study was aimed to isolate an EPS producer sucuk strain and characterise its EPS for future applications. Sucuk samples were collected from four distinct regions of Türkiye to isolate EPS producer LAB strains and at first LAB strains were detected by catalase reaction followed by Gram staining (Fig. S1). LAB isolates were then subjected to genotypic discrimination followed by identification and this characterisation revealed the presence of six distinct EPS producer *W. confusa* strains in sucuk samples. Among the distinct strains, *W. confusa* S6 demonstrated strong slimy morphological features (Fig. 1) and this strain was selected for EPS characterisation studies. Previously several studies reported the presence of *Weissella confusa* in fermented sausages together with *W. cibaria* [17,41]. In terms of sucuk microflora distinct LAB strains including *Lactobacillus plantarum*, *Lactobacillus pentosus*, *Lactobacillus sakei*, *Weissella hellenica*, *Pediococcus acidilactici*, *Weissella cibaria*, *Leuconostoc mesenteroides* as well as Enterococci [3,20] were reported to be presented and to the best of our knowledge this is the first study reporting the presence of *W. confusa* strains in sucuk samples collected from distinct regions of Türkiye. Isolation of only *W. confusa* strains from sucuk microflora could potentially be related with the isolation strategy of current work to find EPS producer strains. Nevertheless, studies are underway to test the starter culture potential of *W. confusa* strains isolated in this study.

3.2. EPS production capability of W. confusa S6

So far one of the main capability of *Weissella* strains isolated from various fermented food products were reported to be EPS production [24,39,42] but there is a very limited information on the EPS production characteristics of this Genera originated from fermented meat products. In this regard, a modified BHI agar containing sucrose was used in this study to isolate EPS producer strains from traditional sucuk samples. EPS was extracted from all sucuk isolate *W. confusa* strains and EPSs were subjected to monomer profile analysis using HPLC system. The monomer composition of all six EPSs were comprised by only glucose suggesting that all six *W. confusa* strains produced homopolymeric glucan type EPS. Among the strains, *W. confusa* S6 was selected for further characterisation as the highest glucan production level with 0.74 g L^{-1} in the modified BHI media was observed for this strain which was in consistent with its slimy morphology (Fig. S2). This data was in line with the literature as one of the main EPS type for *Weissella* strains was homopolymeric glucan [23,39,55] although galactan and fructan type EPS production were also reported for other *Weissella* strains but this was not the case for the sucuk isolate strain S6 as a glucan producer strain [24,30]. In terms of glucan production level of strain S6, similar to our finding previous studies reported 0.8–8 g kg^{-1} EPS production by

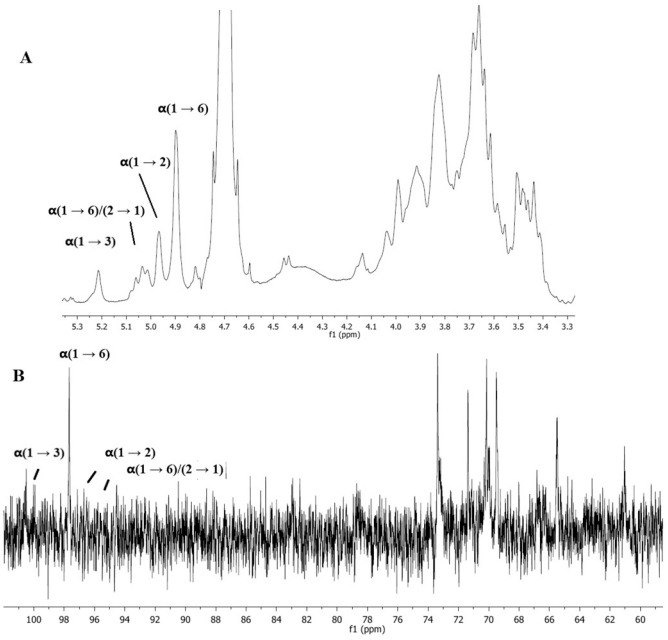

Fig. 2. (A) ^{1}H NMR spectrum of dextran S6 produced by *W. confusa* S6 recorded in D2O at 25 °C showing the presence of (1 → 2)-linked α-D-glucose units, (1 → 3)-linked α-D-glucose units and (1 → 6)-linked α-D-glucose units in the dextran structure. **(B)** ^{13}C NMR spectrum of dextran S6 demonstrating the specific resonances for the (1 → 2)−/ (1 → 3)−/(1 → 6)-linked α-D-glucose units in the dextran structure.

Weissella strains but significantly higher up to 50 g L^{-1} EPS production was also reported [39,43,44] suggesting the strain specific conditions affecting the EPS formation in which origin of *Weissella* strain might play role. Nevertheless, it should be noted that *in situ* production of glucans even at very low levels can play crucial roles for the development of the physicochemical properties of fermented foods as discussed earlier [46]. This was also the case for sucuk as up to 18.9 mg kg^{-1} EPS production during fermentation process of sucuk demonstrated to affect the physicochemical characteristics of sucuk [32]. In the aforementioned study, formation of EPS during fermentation process was shown to increase the hardness of the sucuk samples and this was associated with the role of EPS production to form harder gels as well as to increase the water holding capacity in sucuk samples due to the presence of EPS [32]. Another important role of EPS for physicochemical characteristics of sucuk was decrement of the adhesiveness of sucuk samples that was resulted in the increment of the sliceable characteristics of sucuk. Importantly, EPS formation was shown to be presented as a web-like

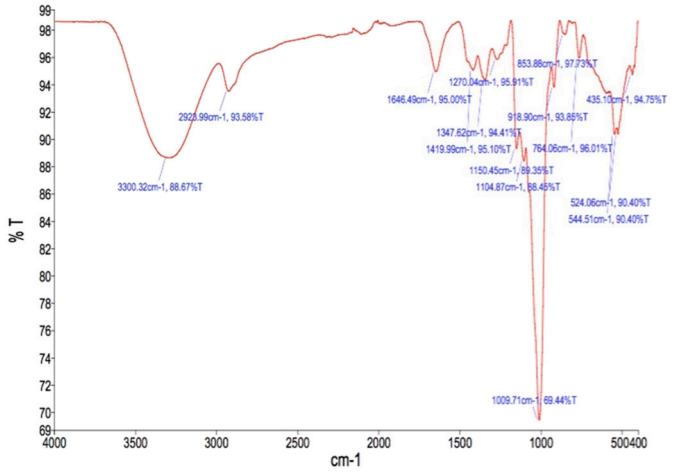

Fig. 3. FTIR spectra of the dextran S6 detected in range 400–4000 cm^{-1} revealing presence of functional groups within the dextran S6 structure.

structure in sucuk samples which was suggested to be one of the main factors for the positive roles of EPS production in sucuk environment [32]. Overall, these findings suggest that utilisation of *Weissella confusa* S6 as a glucan producer strain as a sucuk starter culture might play important roles for the technological properties of sucuk which will be tested in future studies.

3.3. Structural characterisation of glucan S6

To unveil the structure of glucan S6, ^{1}H and ^{13}C NMR analysis were applied and Fig. 2A demonstrates the ^{1}H spectra of glucan S6 in which specific resonances were observed between 3.2 and 5.3 ppm. In terms of anomeric signs characteristics to the glucosyl residues in glucan S6 structure, three specific peaks at 5.22, 4.96 and 4.88 ppm, which correspond to α-(1 → 3)-linked, α-(1 → 2)-linked and α-(1 → 6)-linked units, respectively were observed [45]. Additionally, spectral resonances for the other protons in the glucan S6 structure were observed in the region of 3.3 to 4.2 ppm [23] (Fig. 2A). In terms of ^{13}C NMR spectra, the presence of three anomeric signals at ~ 96.4, 97.9 and 100.07 ppm (Fig. 2B), which were previously assigned to the C1 of α-(1 → 2) glucosyl residues, α-(1 → 6) glucosyl residues and α-(1 → 3) glucosyl residues, respectively [45]. Furthermore, the molar ratios of glycosidic linkages presented in dextran S6 were determined through partially methylated alditol acetate analysis, and the results revealed that dextran S6 consisted of terminal (1→), (1 → 2), (1 → 3), (1 → 6), (1 → 2,6) and (1 → 3,6) glucosyl units in a molar ratio of 19.93 ± 0.29 %, 18,09 ± 1.57 %, 0.38 ± 0.07 %, 43.90 ± 3.97 %, 13.64 ± 0.73 %, and 4.06 ± 1.33 %, respectively. The branching ratio of the dextran S6 was found to be 0.29 ± 0.04 by dividing the sum of the molar ratios of monosubstituted glycosyl units ((1 → 2), (1 → 3), (1 → 6)) to that of disubstituted glycosyl units ((1 → 2,6) and (1 → 3,6)). This further confirms the highly branched structure of dextran S6. Previous studies reported the dextran type glucan production with (1 → 3)-linked α-D-glucose units as branches by other *Weissella* strains from different origins and in these studies the branching level for (1 → 3)-linked α-D-glucose units were observed to be between 2.4 % to 6.6 % [23,39,43] demonstrating the relatively novel structure of dextran S6 as a highly branched dextran with α-(1 → 2) glucosyl residues and α-(1 → 3) glucosyl residues produced by *Weissella confusa* S6. The high level of branching characteristics of dextran S6 can be important for its technological role within complex sucuk environment.

3.4. FTIR analysis of dextran S6

FTIR analysis was applied to expose the functional groups within dextran S6 structure (Fig. 3). The peaks ranging in 3000–3300 cm^{-1} region detected that the hydroxyl groups (O—H) were presented [30,34]. Signals in the 1646–2923 cm^{-1} region area indicated the stretching of vibrations of the C—H bond. These intense peaks also revealed the existence of C═O and carboxylic group in the sugar ring [34,47]. Tensile vibrations between 1419 cm^{-1} and 1347 cm^{-1} were

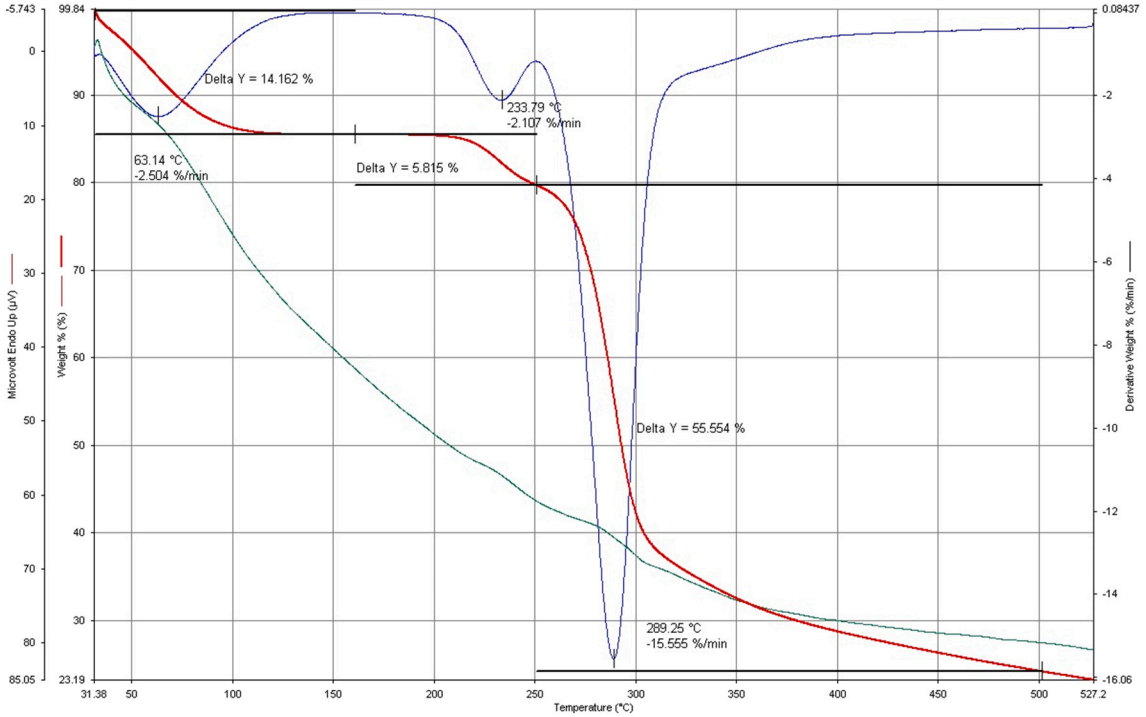

Fig. 4. TGA spectrum together with the DTG and DTA curves of dextran S6 produced by *W. confusa* S6 demonstrating the degredation temperature of dextran S6 as 289.25 °C.

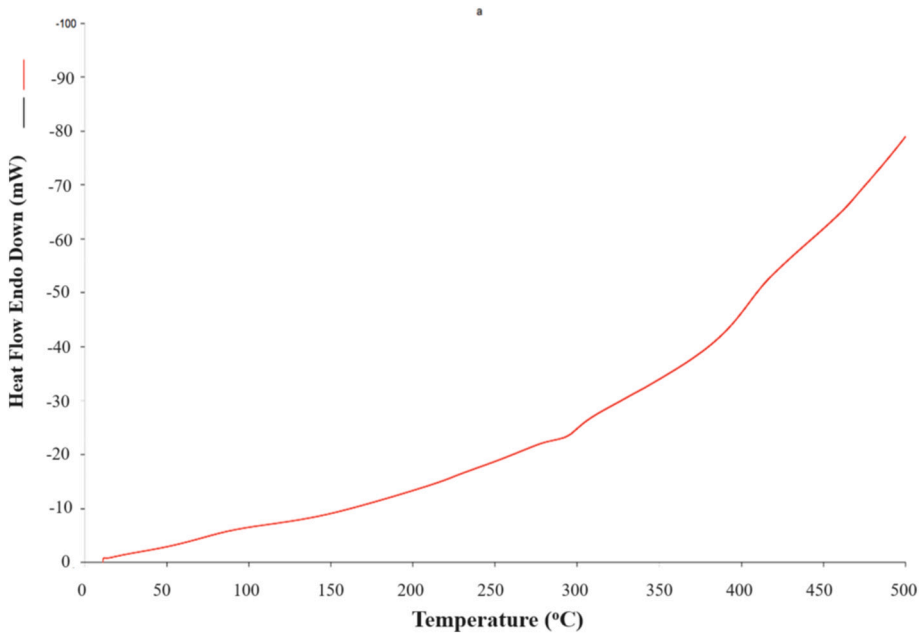

Fig. 5. DSC thermogram of dextran S6 from *W. confusa* S6 recorded between 30 and 500 °C.

revealed as the specific peaks for the carboxylic group or carboxylates, meaning that dextran S6 was a polysaccharide with acidic characteristics [30]. Two significant areas for the characterisation of polysaccharide structures were the 1200–950 cm^{-1} fingerprint region or sugar area and the 950–750 cm-1 anomeric region [34,47]. Additionally, the peak at the 1270 cm^{-1} region indicates the presence of the *o*-acetyl esters [30]. Specific glucan peaks occurred at the 1000–1200 cm^{-1} region. The peak at 1150 cm^{-1} explained that the glycosidic bridge (COC bonds) was present [48]. The pointed peak at the 1009 cm^{-1} area due to C-O-C and C—O tensions was associated with the existence of (1 → 6) - linked α-D-glucose units [22,34]. Moreover, the band that occurred at 918 cm^{-1} exhibited the pyranose form of the glycosyl residue [30]. Overall, FTIR spectra of dextran S6 demonstrated the presence of specific functional groups within its structure and verified the dextran structure.

3.5. Thermal characteristics and molecular weight of dextran S6

The TGA analysis of dextran S6 was dynamically carried out to determine the weight loss against the temperature (Fig. 4) in order to

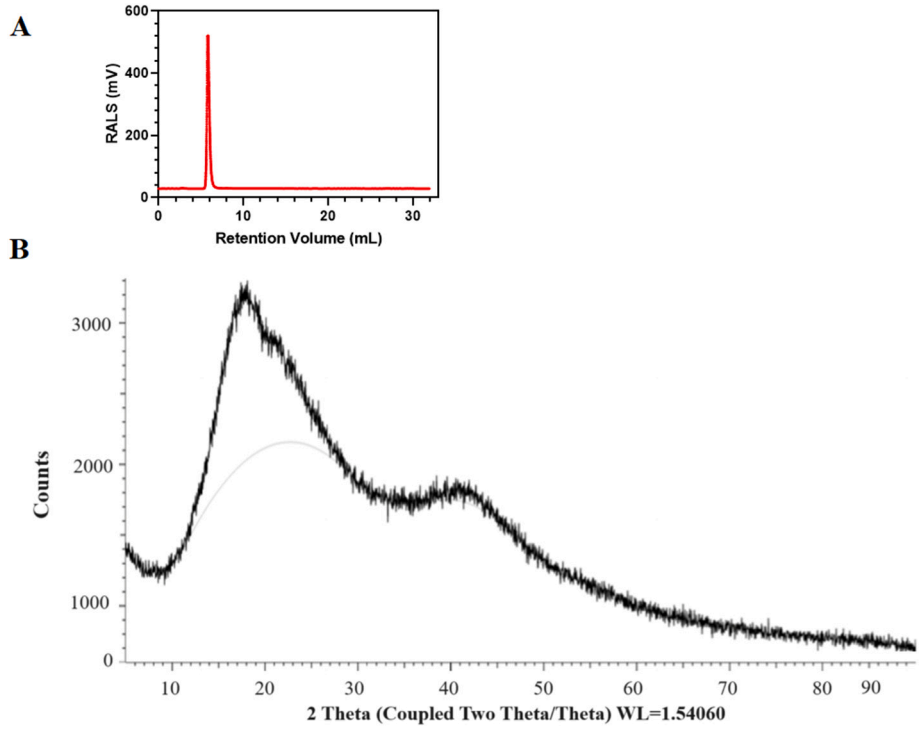

Fig. 6. (A) GPC chromatogram of dextran S6 which revealed the average molecular weight of dextran S6 as ~8 × 10^6 Da. **(B)** XRD chromatogram of dextran S6 demonstrating mainly the amorphous nature of dextran S6.

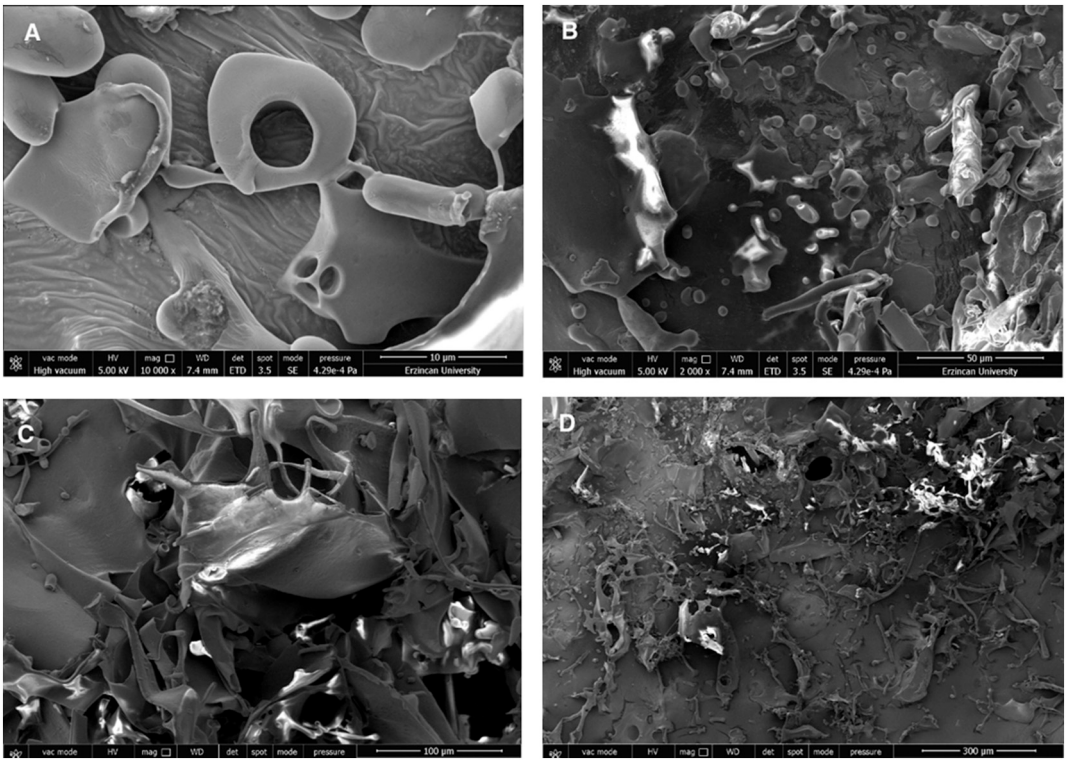

Fig. 7. SEM images of dextran S6 obtained with 10,000 × (A), 2000 × (B), 1000 × (C) and 300 × (D) magnifications showing the sheet and web-like compact morphology of dextran S6.

understand final thermal characteristics of dextran S6 to be used in food applications [39]. Fig. 4 demonstrates the TGA spectrum along with DTG and DTA curves of dextran S6. An initial weight loss around 15 % was observed between 31 °C to 125 °C demonstrating the loss in moisture originating from the interaction of carboxyl groups in the dextran structure with water molecules [49]. This was followed by a 6 % rate weight loss observed between 125 °C and 250 °C due to interlayers in the water molecule and loss in moisture content (He et al., 2020). Therefore, the dextran S6 was thought to be stable below 250 °C [49]. A significant mass loss occurred from 250 to 300 °C during the depolymerization phase with a degradation temperature observed to be 289.25 °C (T_d). Following the degradation phase a gradual weight loss was observed up to 500 °C and this reduction was not observed a sharp phase in contrast to a dextran reported earlier from *Weissella cibaria* PDER21 [39] suggesting the thermal stability of dextran S6 even at later temperature phases. Additionally, in comparison to the other observations the T_d value of dextran S6 was higher than that of dextran from *Lactobacillus sakei* L3 (272 °C) and was similar to the T_d values of L2 EPS from *Leuconostoc lactis* as well as dextran PDER21 from *Weissella cibaria* [49,50]. Another analysis that was used to test the thermal characteristics of dextran S6 was DSC analysis and Fig. 5 demonstrates the DSC curve of dextran S6. Similar to the TGA data, DSC curve of dextran S6 showed its high thermal stability up to ~300 °C. A smooth final degradation profile was observed following the main degradation phase up to 500 °C (Fig. 5). This observation was in consistent from previous observations where dextrans from distinct sources revealed high thermal stability [34,39]. Overall, these findings revealed the strong thermal stability profile of dextran S6 which can be important during sucuk production process as a final heat treatment can be applied during the production of sucuk.

The average molecular weight of dextran S6 was detected as ~8 × 10^6 Da (Fig. 6A) which was slightly higher than previous observations for other dextrans from other LAB strains [49,51] although dextrans with higher molecular weight in comparison to the dextran S6 were also reported [52]. In terms of molecular weight for *Weissella spp.*, strains, glucans showing lower and higher molecular weights in comparison to dextran S6 were reported and importantly the molecular weight of 4 × 10^6 Da was demonstrated as the required molecular weight for the physicochemical performance of dextrans in bread environment [53]. These findings reveals that the molecular weight of glucan S6 might be within the acceptable range for its physicochemical functions in sucuk environment.

3.6. XRD analysis of EPS S6

XRD is an effective analytical instrument to identify the structural and chemical properties of materials [58]. In this work, X-ray diffraction analysis was performed to determine whether the dextran S6 is a polymer having an amorphous or crystallized form. Fig. 6B demonstrates the XRD chromatogram of dextran S6 and according to these patterns, glucan S6 exhibited amorphous or weak crystalline characteristics. Similar results were reported by Yalmanci et al. [34]. Du et al. [54], and Yilmaz et al. [39], who exhibited that various dextran samples from *Weissella* strains have amorphous properties with diffraction peaks about 2θ 20^o. This amorphous nature of dextran S6 might be important in terms of biological and technological features due to the potential high level of solubility characteristics previously associated with amorphous nature of glucans [22,34].

3.7. Morphological properties of EPS S6 detected by SEM and AFM analysis

Microscopic analysis of polymers allowing the detailed characterisation of their microstructural properties is an important task to evaluate their potential utilisation for distinct applications. From this perspective, SEM and AFM analysis were applied to morphologically characterise the microstructural properties of dextran S6. Fig. 7 reveals the SEM images of dextran S6 detected at different resolutions showing the

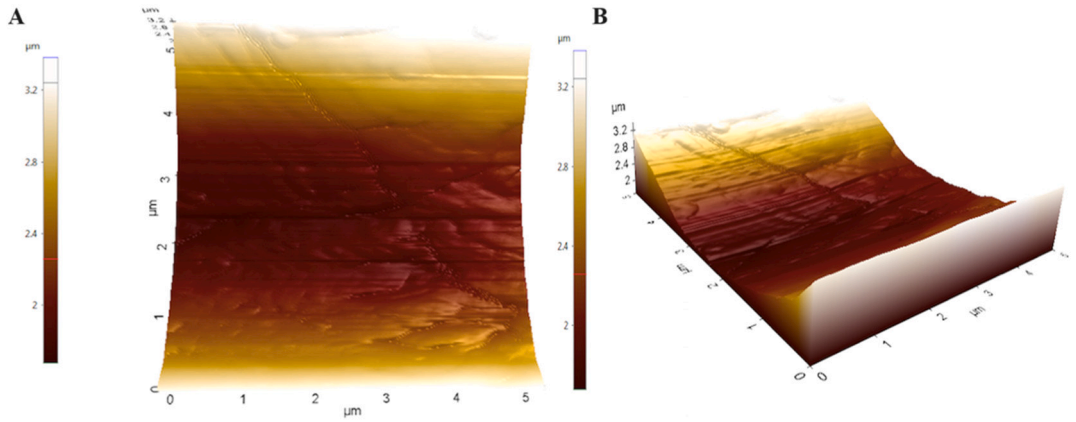

Fig. 8. AFM images of dextran S6 obtained from *W. confusa* S6 at planar (A) and cubic (B) level demonstrating dextran S6 as irregular particles with no clear cross-link within the structure.

formation of mainly sheet-like compact morphology with low levels of web-like branched as well as sphere like units. Previous studies suggested the sheet and web-like compact morphology of glucans as a positive factor in terms of the enhancement of the physicochemical characteristics of the food products [22,39,40]. Importantly, the SEM morphology of dextran S6 was similar to that of dextran obtained from fermented sausage isolate *Lactobacillus sakei* L3 [49] and this morphological features were also discussed as a desirable characteristics for physical properties of food products in the aforementioned study. In fact previously the formation of web-like morphology for EPSs in sucuk mixture during the fermentation process demonstrated and it was revealed that formation of EPS in sucuk environment resulted in enhancement of the textural properties of sucuk suggesting the potential role of this morphological features [32]. Overall, SEM analysis clearly demonstrated the microstructural properties of dextran S6 potentially as an important factor for the physicochemical properties of this dextran.

The morphological features of dextran S6 was also detected by AFM analysis and Fig. 8 demonstrates planar (Fig. 8A) and cubic (Fig. 8B) AFM images of dextran S6. As can be seen in Fig. 8A, dextran S6 appeared as irregular particles with no clear cross-link within the polymer. This fact was also the case for the dextran AK1 produced by another LAB strain and the low level of branching point in the dextran structure was suggested to be the reason for this irregular particle like structure [55]. The low level of branching in dextran S6 also supports this observation as no clear cross-linking phenomenon among the EPS molecular chains was observed. Previously AFM image of glucan E81 demonstrated roundness lump and chain like structures [40,56] although this glucan showed high level of branching (suggesting the potential role of branching level for the topographical morphology of EPSs in aqueous solutions. In addition, similar to previous observation a high level of aggregation was observed for dextran S6 due to the potential large-sized molecules [39] and this property was suggested to be an advantage in terms of uniformity in size and distribution as well as unique dispersing ability [57]. Overall, AFM images together with SEM data revealed the promising properties of dextran S6 in the way of improvement of the technological properties of food products.

4. Conclusion

In this study, EPS producer sucuk LAB strains were isolated from traditional sucuk samples and all identified strains were belonged to *W. confusa* as potential starter cultures for meat industry. Among the identified strains, *W. confusa* S6 was shown to produce a dextran type homopolymeric glucan containing (1 → 2) and (1 → 3)-linked α-D-glucose chains as branching units. In terms of thermal features, dextran S6 demonstrated high thermal characteristics with a degradation temperature of 289.25 °C revealing its high level of stability required for distinct thermal applications of food industry. Dextran S6 exhibited amorphous nature which suggested its high level of solubility characteristics for different biological and technological applications. Importantly, microstructural and morphological properties of dextran S6 indicated its suitability to shape the required formations to obtain enhanced physicochemical features during production of different foods especially fermented ones. Studies are underway to test the role of dextran production by *W. confusa* S6 during sucuk production.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2024.130507.

CRediT authorship contribution statement

Fatma Beyza Özpınar: Methodology, Investigation, Formal analysis. **Hümeyra İspirli:** Methodology, Investigation, Formal analysis. **Selma Kayacan:** Validation, Methodology, Investigation, Formal analysis. **Kader Korkmaz:** Formal analysis, Investigation, Methodology. **Sevda Dere:** Investigation, Resources. **Osman Sagdic:** Writing – original draft, Investigation. **Zuhal Alkay:** Methodology, Investigation. **Yunus Emre Tunçil:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Mutamed Ayyash:** Writing – review & editing, Writing – original draft, Methodology. **Enes Dertli:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors of the manuscript entitled as 'Physicochemical and structural characterisation of a branched dextran type exopolysaccharide (EPS) from Weissella confusa S6 isolated from fermented sausage (Sucuk)' have no conflict of interests related to this manuscript.

Data availability

Data will be made available on request.

Acknowledgments

This work has been supported by Yildiz Technical University Scientific Research Projects Coordination Unit under project number TSA-2023-5314. We thank Dr. Murat Topuzoğulları for the critical help during the GPC analysis.

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