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Physicochemical and antioxidant properties of a gastroprotective exopolysaccharide produced by *Streptococcus thermophilus* CRL1190

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ABSTRACT

The exopolysaccharide EPS1190 was isolated with high purity from skim milk fermented by the strain *Streptococcus thermophilus* CRL1190, the major EPS amount being recovered at stationary growth-phase (70.7 mg/L after 16 h). This polymer revealed moderate antioxidant activity *in vitro* and useful technological properties such as good emulsifying and flocculating capacity at low concentrations, high aqueous solubility (9.1 mg/mL), water and oil holding capacity (528.5% and 1266%, respectively) as well as thermal behavior. Thermogravimetric, Differential Thermal Analysis and Differential Scanning Calorimetry curves showed that the biopolymer was thermally stable up to 216 °C with a crystalline deformation in an endothermic process ($\triangle$H = 284.46 J) at a melting temperature of 74.08 °C. Furthermore, a degradation temperature (Td) of 295.4 °C was observed. These results postulate that EPS1190 could be used in the food industry due to its special microstructure (scanning electron microscopy of EPS1190 demonstrated a homogeneous matrix with micro-porous and rough structure), its good performance and functionality in both aqueous and oil systems, which would remain at high temperatures.

1. Introduction

Streptococcus (*S.*) *thermophilus* is one of the most valuable homofermentative lactic acid bacteria (LAB) which is widely used as starter culture for home-made and industrial fermented dairy products. *S. thermophilus*'s exopolysaccharides (EPS) received special attention over recent decades since they improve the texture of dairy products and confer beneficial health effects (Cui et al., 2016; Cui, Jiang, Hao, Qu, & Hu, 2017).

S. thermophilus CRL1190 is a probiotic LAB isolated from home-made fermented milks, that produces both a capsular polysaccharide (CPS) covalently bound to the cell surface and an exopolysaccharide secreted into the environment (EPS1190) which confers viscosity to fermented milks (Marcial et al., 2013; Mozzi et al., 2006; Rodriguez et al., 2008). In the last years, several studies were carried out to know the role of EPS1190 in gastritis, an inflammatory disorder of the lining of the stomach mainly due to an excess of acid secretion (Marcial, Rodríguez, Medici, & de Valdez, 2011). In this context, *S. thermophilus* CRL1190-fermented milk was able to prevent and treat chronic gastritis caused by non-steroidal anti-inflammatory/analgesic drugs in experimental animal models. The beneficial effect obtained was related to the EPS1190 since its administration induced mucus formation, an increase in regulatory cytokines (IL-10) and a decrease in pro-inflammatory cytokines (INF-γ and TNF-α) (Rodríguez, Medici, Mozzi, & de Valdez, 2010; Rodriguez, Medici, Rodriguez, Mozzi, & Font de Valdez, 2009). On the other hand, EPS1190 was able to block the adhesion receptors on human stomach AGS cell against *Helicobacter pylori* infection, a pathogenic bacterium that causes severe gastritis and even ulcer and gastric cancer. The EPS1190 also stimulates the regeneration of human epithelial cells of the stomach and even of the buccal cavity, being efficiently absorbed into the stomach cells by an endosomal mechanism of transport (Marcial et al., 2013).

Studies performed *in vitro*-gastric systems evinced a partial degradation of the EPS1190 when subjected to this harsh conditions (Mozzi, Gerbino, Font de Valdez, & Torino, 2009). From results of *in vivo* and *in vitro* studies, it is assumed that the biopolymer may still exert its beneficial properties in the stomach even partially degraded. Moreover, Pescuma et al. (2009) studied the effect of EPS1190 on whey

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peptides digestibility (β-Lactoglobulin hydrolyzed by *Lactobacillus acidophilus* CRL636) using an *in vitro* gastric-pancreatic system. When the mixture β-Lactoglobulin-EPS1190 was previously hydrolyzed by the *Lactobacillus* strain, the pepsin digestion was meaningfully higher than without EPS1190. As a consequence of this smaller size peptides were released, which are known to be less allergenic and immune-reactive.

The use of both the EPS1190 and the strain CRL1190 as starter culture may improve structural (natural bio-thickeners) and healthy properties of novel functional dairy products. In this study, we reported some physicochemical properties, i.e., aqueous and oil holding capacity, thermal stability, emulsifying and flocculating activities, as well as the antioxidant ability of the EPS produced by *S. thermophilus* CRL1190 in skim milk, which have not been yet reported.

2. Materials and methods

2.1. Milk fermentation

S. thermophilus CRL1190 (EPS and CPS producer strain) is hold at the culture collection of CERELA (Tucumán, Argentina). The strain was grown in LAPTg broth (Raibaud, Caulet, Galpin, & Mocquot, 1961) modified as follows: peptone, 15 g/L; tryptone, 10 g/L; yeast extract, 10 g/L; glucose, 1 g/L; lactose, 20 g/L; Tween80, 1 mL/L, and sub-cultured in reconstituted skim milk (RSM, 100 g/L). The fermented milk (FM) was prepared in sealed bottles containing 1 L sterile RSM (115 °C, 20 min, cooled down to 37 °C) inoculated with *S. thermophilus* CRL1190 (1.0%, v/v) and incubated at 37 °C. During fermentation, aliquots were withdrawn at 2, 4, 6, 12, and 16 h for compositional analysis. The FM aliquots were diluted and spread on the modified LAPTg supplemented with agar at 1.5% (w/v) for cell count. Titratable acidity (g lactic acid/kg FM) was determined by neutralization of 10 g cultures with 0.1 N NaOH (Dornic solution; Cicarelli Laboratories, Argentina) to pH 8.6 (digital pH-meter Sartorius model PT-10). The pH of FM was also determined. Sugars (lactose, glucose, and galactose) were quantified by HPLC with a Knauer Chromatograph (Knauer Wissenschaftliche Geräte GmbH, Berlin, Germany) equipped with a Rezex RPM-Monosaccharide Pb^{+2} (300 × 7.8 mm) column and refractive-index detector. The EPS production was determined by quantification of the isolated polymers as previously described (Mozzi et al., 2006). Briefly, samples of FM were deproteinized with trichloracetic acid (12%, w/v) incubated at 4 °C for 2 h and centrifuged (9500 × *g*, 12 min, 4 °C). The EPS1190 was precipitated from the clear supernatants by adding 3 vol of cold ethanol (96%) and incubating at −20 °C for 48 h. Precipitated EPS1190 obtained by centrifugation (20000 × *g*, 30 min, 4 °C) was dissolved in 5 mL deionized water and subjected to a second deproteinization and precipitation to eliminate remnant proteins. Finally, the polysaccharides were dissolved into deionized water, dialyzed (cellulose membrane, MWCO 10000, Thermo Scientific Inc., USA) at 4 °C for 48 h, lyophilized and weighted. The net EPS production was expressed as milligrams of polymer dry mass (PDM) per liter, subtracting the amount of unspecified material isolated from acidified non inoculated RSM treated in the same way (control). EPS1190 from 16 h cultures was stored at 4 °C for further analysis.

2.2. Purity analysis

EPS solutions (5 mg/mL) were prepared with deionized water, stored overnight at −20 °C, thawed quickly, and centrifuged (20000 × *g*, 20 min, 4 °C) to examine the precipitate formation. The residual protein content in the EPS sample was analyzed using a protein assay kit according to the instructions of the manufacturer (Bio-Rad Laboratories Inc., USA). In addition, ultraviolet (UV) absorption spectrum was recorded using a UV microplate reader (Synergy HT, Biotek, USA) between 200 and 550 nm to determine the presence of proteins and nucleic acids (Ye, Liu, Wang, Wang, & Zhang, 2012).

2.3. Solubility, water, and oil holding capacity

Solubility of EPS1190 in water was determined according to the procedure described by Wang, Cheung, Leung, and Wu (2010), with some modifications. A solution of EPS1190 was prepared by dissolving the polymer with deionized water (concentration of 50 mg/mL) under continuous agitation with a magnetic stir bar at room temperature (25 °C for 24 h). The solution obtained was placed into 1-mL microcentrifuge tube and centrifuged (20000 × *g*) at room temperature for 60 min to remove any insoluble particles. The undissolved residue was separated from the supernatant, freeze-dried, and its weight was subtracted from the original sample weight (50 mg).

Water holding capacity (WHC) of EPS1190 was determined by following the procedure of Ahmed, Wang, Anjum, Ahmad, and Khan (2013) with some modifications. EPS1190 (40 mg) was suspended in 2 mL deionized water with a vortex mixer. Dissolved material was centrifuged at 20000 × *g* for 45 min, and unbound water not held by the EPS material was discarded. All EPS material was dropped on filtering meshes for complete drainage of water and the weight of precipitated EPS was recorded. The percentage of WHC of EPS1190 was calculated through the following Eq. (1):

WHC (%) = [total sample weight after water absorption]/[total dry sample weight] x 100 (1)

The oil holding capacity (OHC) was calculated in a similar manner by adopting the method of Guil-Guerrero, Navarro-Juárez, López-Martınez, Campra-Madrid, and Rebolloso-Fuentes (2004). For the purpose, sunflower oil was used as dispersing media. The other steps were identical as for WHC.

2.4. Thermal analysis

The thermal analysis was carried out by TGA, DTA, and DSC (Thermogravimetric Analysis, Differential Thermal Analysis, and Differential Scanning Calorimetry) to know the behavior of the EPS and their structural modifications against temperature changes. TGA and DTA were recorded on Shimadzu TGA-DTA 50 equipment (Kyoto, Japan) from room temperature to 600 °C. The sample was located in a platinum crucible and it was heated at a rate of 10 °C/min under nitrogen atmosphere, using a constant flow of 20 mL/min. DSC was carried out on a PerkinElmer DSC 6 equipment under nitrogen gas purge (constant flow of 30 mL/min). The sample was heated in alumina crucible from 10 to 350 °C, at a rate of 10 °C/min.

2.5. Scanning electron microscopy (SEM)

The surface morphology of EPS1190 was determined by scanning electron microscopy (SEM, microscope Zeiss, model Supra 55vp, Germany) at an accelerating voltage of 10KV147. The samples were air-dried, sputter-coated with gold using a fine-coat ion sputter JFC-1100 (JEOL Ltd., Japan). SEM belongs to facilities of CISME (Centro de Investigaciones y Servicios de Microscopía Electrónica) –CCT CONICET Tucumán and UNT (Universidad Nacional de Tucumán).

2.6. Anti-oxidant capacity

2.6.1. Total antioxidant assay

Aliquots (0.1 mL) of EPS1190 solutions (0.5, 1, 2, 4, 6, and 8 mg/mL) was combined with 1 mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tube was capped and incubated in a boiling water bath at 95 °C for 90 min. After cooling to room temperature, the absorbance of the aqueous solution was measured at 695 nm against blank in a UV microplate reader (Synergy HT, Biotek, USA). Ascorbic acid (Vc) was used as a standard and the results were expressed as Vc equivalents.

2.6.2. Reducing power method

Aliquots of 0.250 mL of 0.2 M phosphate buffer (pH 6.6) and 0.250 mL of $K_3Fe(CN)_6$ (1%, w/v) were added to 0.1 mL of EPS1190 solutions (0.5, 1, 2, 4, 6, and 8 mg/mL). The resulting mixture was incubated at 50 °C for 20 min, followed by the addition of 0.250 mL of trichloroacetic acid (10%, w/v). The mixture was centrifuged at 3000 × *g* for 10 min to collect the upper layer of the solution (0.250 mL), which was mixed with distilled water (0.250 mL) and 0.050 mL of $FeCl_3$ (0.1%, w/v). The absorbance of the aqueous solution was measured at 700 nm against blank in a UV microplate reader (Synergy HT, Biotek, USA). Vc was used as a standard and the results were expressed as Vc equivalents. A higher absorbance indicates greater reducing power.

2.6.3. $ABTS^{\bullet+}$ radical scavenging activity

The $ABTS^{\bullet+}$ radical scavenging activities of EPS1190 were measured with the method reported by Liu et al. (2016) with some modifications. The stock solution of $ABTS^{\bullet+}$ was diluted to an absorbance of 0.99 at 734 nm and stocked before use. Aliquots of 0.010 mL of samples solutions (0.5, 1, 2, 4, 6, and 8 mg/mL) were mixed with 0.200 mL of $ABTS^{\bullet+}$ diluent. The reaction was kept at room temperature for 6 min and the absorbance was measured at 734 nm in a UV microplate reader (Synergy HT, Biotek, USA). Vc was used as a standard. $ABTS^{\bullet+}$ scavenging activity was calculated as a scavenging rate (%) using Eq. (2):

$$\text{Scavenging rate (\%)} = [1 - (A_1 - A_2)/A_0] \times 100 \quad (2)$$

Where A_0 was the absorbance of the control (without sample), A_1 was the absorbance in the presence of the sample and $ABTS^{\bullet+}$, and A_2 was the absorbance of sample blank (without $ABTS^{\bullet+}$).

2.7. Emulsifying activity and microscopic evaluation

The emulsifying activity of EPS1190 was assayed as described by (Bramhachari, Kishor, Ramadevi, Rao, and Dubey (2007) with some modifications. 1-ml of the EPS solutions (0.1; 0.5; 1.0, and 2.0 mg/mL prepared with phosphate-buffered saline) was vortexed for 1 min after the addition of 0.25 mL of hexadecane. The absorbance at 540 nm was read immediately before and after vortex agitation (A_0). The fall in absorbance was recorded after incubation at room temperature for 30 and 60 min (A_t). PBS was used as a negative control. The emulsification activity was expressed as the percentage retention of the emulsion during incubation for time, according to Eq. (3). Xanthan and guar gum were used for comparison. The morphology of the emulsions formed by those concentrations that obtained the best values of activity was examined through a 10× objective lens of light microscope Axiolab Carl Zeiss microscopy.

$$\text{Emulsifying activity (\%)} = (A_t/A_0) \times 100 \quad (3)$$

2.8. Flocculating activity

The flocculating activity was measured by using the method as described by Ahmed et al. (2013), with some modifications. Charcoal-activated carbon that was used as a testing material was suspended in deionized water and EPS solution (0.1, 0.5, 1, 2, 4, and 6 mg/mL) at a concentration of 5 g/L. In a test tube, 1 mL of a charcoal-activated carbon suspension was added and mixed with 0.1 mL of $CaCl_2$ solution (6.8 mM). These mixtures were vortexed for 30 s and allowed to stand for 10 min at room temperature. The turbidities of the upper 1 ml phase were measured at 550 nm. A control experiment without the EPS was also pursued in the same manner. The flocculating activity (%) was defined and calculated according to the following Eq. (4) Xanthan and guar gums were used for comparison.

$$\text{Flocculating activity (\%)} = [(A_c - A_s) / Ac] \text{ x } 100 \quad (4)$$

Where A_s = absorbance (A_{550}) of EPS1190, xanthan, and guar gums suspensions; and A_c = absorbance (A_{550}) of control.

2.9. Statistical analysis

All data were analyzed by one-way ANOVA test and significant differences were determined by Turkey-HSD at $p < 0.05$ (Di Rienzo et al., 2011). Assays and determinations were performed by two replicates (n = 2).

3. Results and discussion

3.1. Milk fermentation

S. thermophilus CRL1190 is a probiotic LAB that produces capsular- and exopolysaccharides in RSM (10%); the latter (EPS1190) composed of glucose and galactose is high molecular weight (HMW, 1782 kDa) and has proven gastroprotective activity (Mozzi et al., 2006; Rodríguez, Medici, Rodríguez, Mozzi, & de Valdez, 2009; Rodríguez et al., 2010). In industrial fermentation processes, the rate of milk acidification by *S. thermophilus* species is of major technological importance (Cui et al., 2017). The EPS production, growth, acidification and carbohydrate metabolism of the strain CRL1190 in RSM (10%) during fermentation are represented in Fig. 1. The strain displayed a high growth rate (μ_{max} = 0. 514 h^{-1}) linked to a rapid decrease in pH (Fig. 1A) after 4 h incubation (exponential phase), the viable cell count after 16 h was 9.36 log CFU/mL with pH values about 4.3. The EPS production was associated to the culture growth; it increased to 38.6 mg/L after 4 h (exponential growth phase) and reached 70.7 mg/L at the end of the period evaluated (16 h) corresponding to the stationary phase. *S. thermophilus* CRL1190, as many strains of this specie, prefers lactose as a major carbon and energy source. Fig. 1B shows the variation of milk sugars related to the titratable acidity (Fig. 1A) both occurring mostly

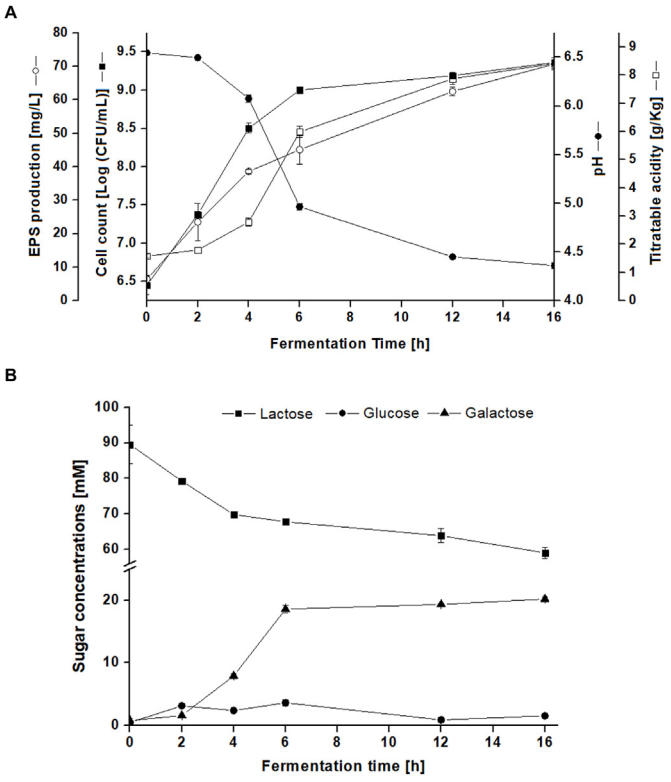

Fig. 1. Milk fermentation by *S. thermophilus* CRL1190 at 37 °C for 16 h
(A) EPS production, bacterial growth, pH and titratable acidity; **(B)** Variation of milk's sugars.

along with the exponential and late-exponential growth phases. As can be seen, the glucose moiety of lactose is fully consumed while galactose is accumulated in the growth medium.

3.2. Purity of EPS1190

When samples of the aqueous solution of EPS1190 at 5 mg/mL were kept in cold, no precipitate was observed after centrifugation and no protein was detected, the amount was lower than the detection limit of the method (< 10 μg). The UV spectra of purified EPS1190 isolated from 16-h fermented RSM showed only a single peak in the range of 190–210 nm, which is characteristic of carbohydrates (Yang et al., 2018). There was no absorption at 280 and 260 nm in the UV 235 spectrum indicating the absence of proteins and nucleic acids in the EPS sample (Ye et al., 2012).

3.3. Physical properties of EPS1190

The aqueous solution of EPS1190 after 24 h of vigorous mixing formed a uniform solution of moderate consistency with light white color similar to the milky dispersion observed for the EPS produced by the medicinal fungus *Cordycepssinensis* Cs-HK1 (Chen, Siu, Cheung, & Wu, 2014). EPS1190 has a good water solubility (9.65 mg/mL) and higher water and oil holding capacity (WHC 528.51% and OHC 1266.04%) compared to the biopolymer produced by *Lactobacillus* (*L.*) *kefiranofaciens* ZW3, which also contains glucose and galactose but in different monomers molar ratio (Ahmed et al., 2013). The solubility index corresponding to 9.6 mg/mL (19.3%) is in the order of those reported for the α-glucans produced by *L. plantarum* (21.6%) and *Leuconostoc lactis* (14.2%), but much lower when comparing to the α-glucan synthesized by *Enterococcus hirae* (46.5%); WHC being, respectively, 316.9, 117 and 202.04% for the aforementioned homopolysaccharides (Das & Goyal, 2014; Saravanan & Shetty, 2016; Jayamanohar et al., 2018). WHC is an efficient parameter commonly used to describe the physicochemical behavior of soluble and insoluble polysaccharides in aqueous solution, being the sum of bound water, hydrodynamic water and physically trapped water (Ghiribi et al., 2015; Ramasamy, Gruppen, & Kabel, 2015). The high WHC of EPS1190 could be linked to the molecular weight (1782 kDa) and porous microstructure of the polymer (Fig. 4) which could hold and entrap large amounts of water molecules (Das & Goyal, 2014; Mao, Tang, & Swanson, 2001). The OHC is another important industrial characteristic to be considered because the oil acts as a flavoring retainer agent and increases the mouthfeel of foods (Devi, Kavitake, & Shetty, 2016; Du et al., 2018; Feng et al., 2018). EPS1190 showed high OHC value probably due to the permeable biopolymer structure which might bind the hydrocarbon side chains of oil. The low intrinsic viscosity (ηH_2O) of EPS1190 (3.51 dL/mg) reported by Marcial et al. (2013) suggests it has a poor thickening capacity in comparison with gums commonly used as additives in the food industry such as guar gum (13.7 dL/mg) and xanthan gum (54.1 dL/mg). However, *S. thermophilus* CRL1190 has a great potential to be used in the dairy industry together with non-EPS producing strains because of its ropy phenotype and proven functional properties *in situ*, as well as the high WHC and OHC of its EPS1190. These attributes may improve certain characteristics of dairy products such as smoother consistency, lower susceptibility to syneresis or higher extensibility (Mozzi et al., 2006; Ruas-Madiedo & De Los Reyes-Gavilán, 2005).

3.4. Analysis of thermal properties

Thermostability of EPS is an essential characteristic for dairy applications as well as the behavior of its structure (Fig. 2).

TGA curve shows that EPS1190 is decomposed mainly in two stages: the first one has a mass loss of 13.47% (temperature range 30–110 °C) and is attributed to the elimination of polymer surface-bound water molecules. Water absorption by EPS1190 is due to the presence of polar

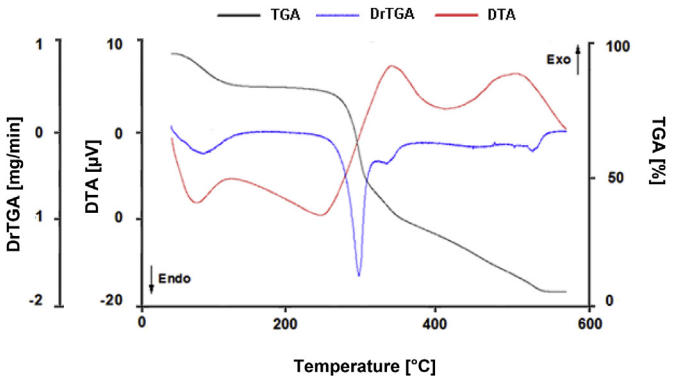

Fig. 2. Thermal analysis of EPS1190 with simultaneous TGA, DrTGA and DTA curves.

groups in its structure: OH capable to establishing hydrogen bonds with water molecules (Chowdhury, Basak, Sen, & Adhikari, 2011; Faria et al., 2011). The presence of only one peak at 67.4 °C in the first derivative (DrTGA) indicates that all the water is bonded in the same way as hydration water. In contrast, Gil et al. (2011) and Navarro, Jorge, Negri, Medina, and Gómez (2015) reported curves for water elimination showing two peaks which indicated that the sample had two kinds of water, coordinated water, and water linked by hydrogen bonds. In the DTA curve obtained for EPS 1190, this mass loss was associated with an endothermic peak located at 59.77 °C.

After dehydration, the polymer remained stable up to 215 °C suggesting that the macromolecular structure is conserved, and therefore its gastroprotective functionality. The second stage of weight loss involves two consecutive processes that occurred between 216 °C and 565 °C with a weight loss of 84.06%. The first process took place rapidly until 305 °C with a weight loss of 37.5%; the second one began at 305 °C and proceeded gradually at a slow rate. Initially, the breakdown of the C–O and C–C bonds may be the main cause of this loss; later, the slow decomposition may be attributed to the formation of thermally stable saccharides along the process (Chowdhury et al., 2011; Sajna et al., 2013).

Degradation temperature (T_d) found for EPS1190 was 295.4 °C; it is represented by the more intense peak in DrTGA curve indicating that this step is the fastest of the whole process. In addition, DTA curve (Fig. 2) revealed that this exothermic process is the one releasing most of the energy. These results agree with Ahmed et al. (2013), who reported an EPS of monomer composition similar to EPS 1190 produced by *L. kefiranofaciens* ZW3, which has a Td value of 299 °C and was thermally more stable than gums commonly used in the food industry (xanthan and locust gum). Finally, the decomposition process of the biopolymer EPS1190 ends at 565 °C through an endothermic process, the mass loss being of 45.8%. During this phase, a polynuclear aromatic and graphitic carbon structure could be formed as the temperature increases (Chowdhury et al., 2011) remaining only 3% of the solid residue until 600 °C.

As heat absorption and emission also affect thermal characteristics of exopolysaccharides linked to phase changes (i.e. melting) or deformation of the crystalline structure, subsequent analysis of melting point and energy changes of the EPS1190 structure was evaluated by DSC (Fig. 3) with heat flow from 10 to 350 °C.

The melting point was an endothermic peak started at about 74.08 °C, and the enthalpy change (ΔH) needed to melt 1 g of EPS1190 was 284.46 J. These results are different to those reported for EPSs produced by different LAB strains. Ahmed et al. (2013) observed that the melting point and enthalpy changes of EPS from *L. kefiranofaciens* ZW3 (also composed of glucose and galactose) were about 93.38 °C and 249.7 J, respectively. On the other hand, Wang et al. (2010) and Kanmani, Yuvaraj, Paari, Pattukumar, and Arul (2011) showed that the melting point and enthalpy changes of EPS isolated from *L. plantarum*

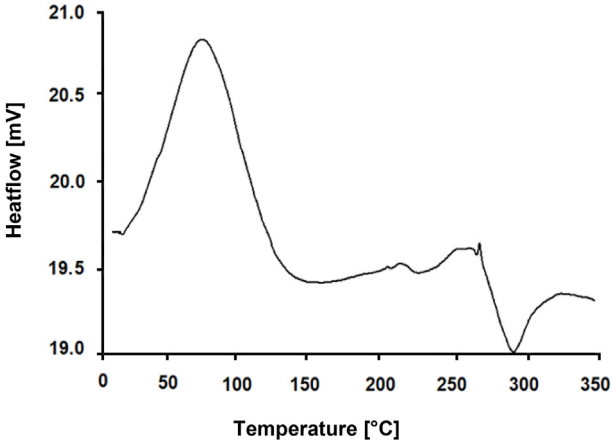

Fig. 3. DSC thermogram of EPS1190 from 10 to 350 °C.

KF5 and *S. phocae* PI80 were 88.35 °C/133.5 J and 120.09 °C/404.6 J, respectively.

Another prominent exothermic peak appeared from 215 °C (temperature of maximum stability exposed in TGA curve, Fig. 2) corresponding to thermal decomposition processes of the EPS1190. The first signal is at 232.07 °C associated to enthalpy change of −7,85 J, and a second one is between 290.8 °C with −50,81 J. These exothermic signals of thermal decomposition processes are in agree with DTA presented in Fig. 2.

3.5. SEM image

Scanning electron microscope (SEM) is a useful tool to study the surface morphology of polymer and helps to predict about its common physical properties (Ahmed et al., 2013; Chowdhury et al., 2011; Wang et al., 2010). The microstructure and surface morphology micrographs of EPS1190 are shown in Fig. 4.

From SEM scan it can be predicted that EPS1190 exhibits a homogeneous matrix with a micro-porous and rough structure. Owing to its small pore size distribution, the polymer can hold water and can be used as a texturing agent in the food industry as thickening, gelling, stabilizing, emulsifying and water-binding agents (Khan, Park, & Kwon, 2007). The hydroxyl groups present in the polymer increases its crystallinity. The small cubical pore structure may also be responsible for the compactness of the polymer, the stability of the gel structure when subjected to external forces, and the maintenance of the texture properties during storage (Purama, Goswami, Khan, & Goyal, 2009). Due to its high molecular weight (1782 kDa) and porosity, the EPS from *S. thermophilus* CRL1190 is a biopolymer of industrial importance.

3.6. Anti-oxidant capacity of EPS1190

3.6.1. Total antioxidant assay

Total antioxidant capacity is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acid pH (Alam, Bristi, & Rafiquzzaman, 2013). Pan and Mei (2010) reported that total antioxidant capacity reflects the capacity of a non-enzymatic, antioxidant defense system. Fig. 5A represents the total antioxidant capacity of EPS1190 at each concentration; expressed in terms of equivalents of Vc (a unit of ascorbic acid was 1 mg/mL). The antioxidant potential of EPS exhibited a dose-dependent activity within a concentration range of 0.5–8.0 mg/mL. However, the antioxidant capacity was lower than that of Vc, indicating that the EPS has a moderate antioxidant capacity.

3.6.2. Reducing power

Reductive ability is used to investigate the Fe^{3+} – Fe^{2+} transformation in the presence of EPS samples (Kanamarlapudi & Muddada, 2017). Aforementioned, Vc, a common antioxidant with great reducing power was used as the reference to examine the EPS1190 produced by the LAB strain. Fig. 5B represents the reducing power of the biopolymer at each concentration, expressed in terms of Vc equivalents (a unit of ascorbic acid was 1 mg/mL). Similar to the total antioxidant capacity, the reducing power of EPS1190 was lower than Vc and exhibited a dose-dependent response (range of 2.0–8.0 mg/mL). Concentrations of EPS lower than 2.0 mg/mL (0.5 and 1.0 mg/mL) showed no activity.

3.6.3. $ABTS^{\bullet+}$ radical scavenging ability

The $ABTS^{\bullet+}$ decoloration method is widely used to estimate the radical scavenging capacities of hydrophilic and lipophilic antioxidants (Sánchez-Moreno, 2002). The $ABTS^{\bullet+}$ radical scavenging abilities of EPS1190 compared to Vc are shown in Fig. 5C. EPS1190 showed positive responses at each concentration evaluated (0.5–8.0 mg/mL) reaching 50% activity at 4.0 mg/mL and 56.6% at 8.0 mg/mL. However, $ABTS^{\bullet+}$ radical scavenging ability of EPS1190 was lower than Vc at the concentration range evaluated. The drop in activity at concentrations greater than 4.0 mg/mL may be due to the restriction of mobility and accessibility to the radicals in solution by increasing the concentration of large and moderate viscosity of HMW-EPS1190 (Marcial et al., 2013; Mozzi et al., 2006). On the other hand, the EPS1190 samples are remarkably protein-free, thus $ABTS^{\bullet+}$ radicals scavenging responses are largely linked to the neutral structure of the biopolymer, mainly for its hydroxyl group OH (Leung, Zhao, Ho, & Wu, 2009). These results indicated that EPS1190 had a positive scavenging effect on the $ABTS^{\bullet+}$ radical due to their ability to convert reactive free radicals into a stable state and to complete the free radical chain reaction by donating electrons to radicals (Liu et al., 2016).

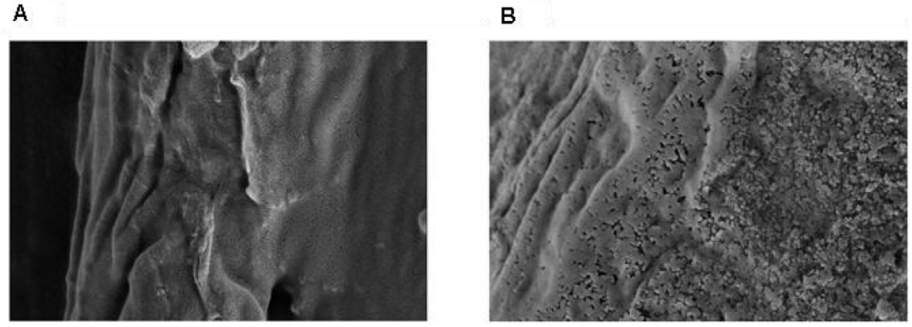

Fig. 4. Scanning Electron Microscopic images of EPS1190.
(A) 25000 X
(B) 40000 X.

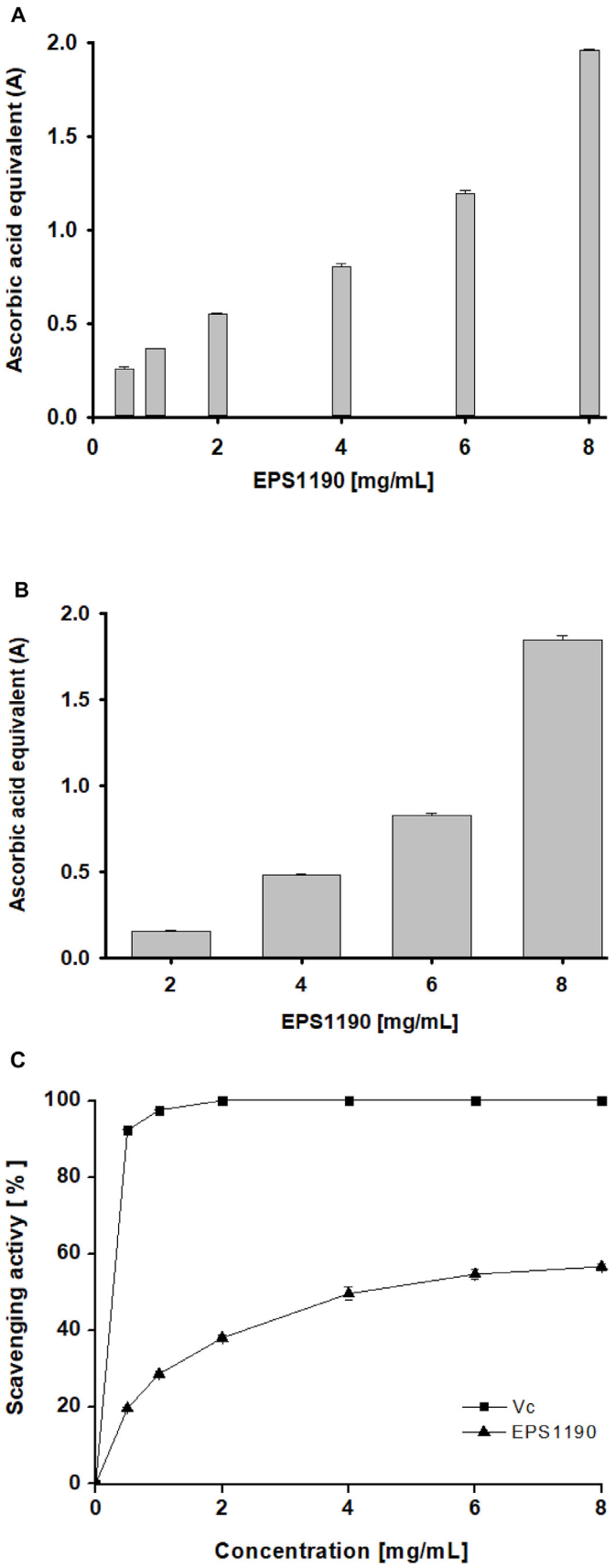

Fig. 5. Anti-oxidant capacity of EPS1190 evaluated *in vitro* by different methodologies.
(A) phosphomolybdenum; **(B)** reducing power; **(C)** $ABTS^{\bullet+}$ radical scavenging ability.

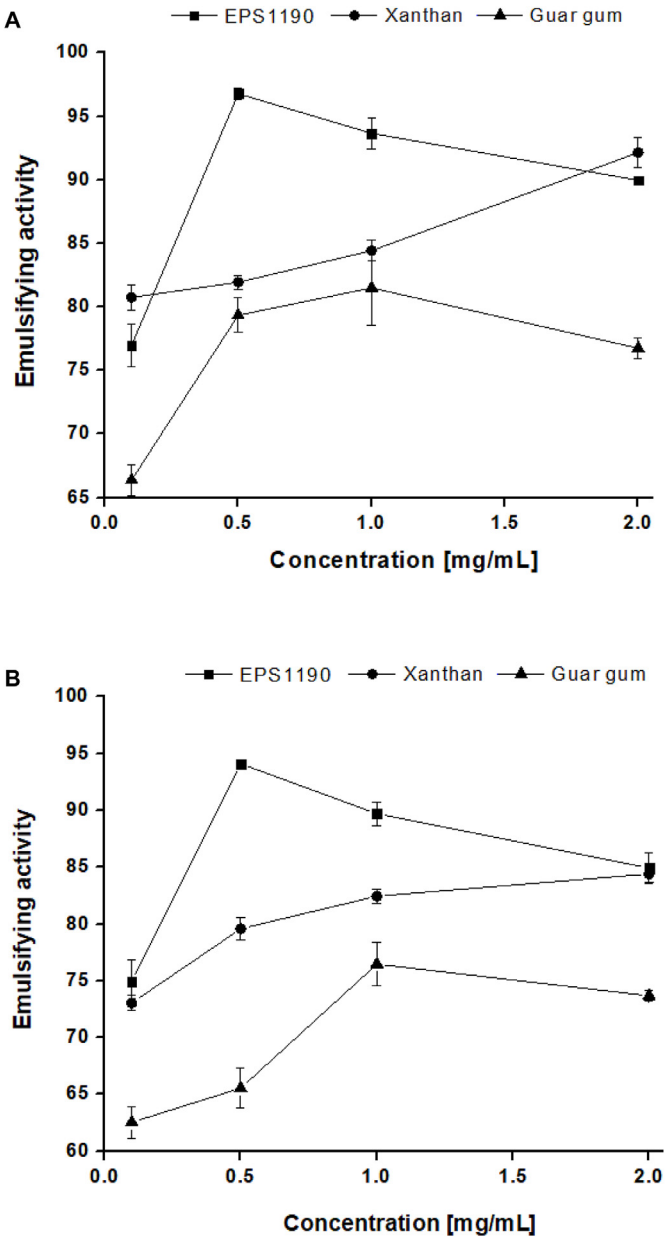

Fig. 6. Emulsifying activity of EPS1190, xanthan and guar gum.
Measurements of suspensions containing polymers' between 0.1 and 2.0 mg/mL, were performed after **(A)** 30 min and **(B)** 60 min.

3.7. Emulsifying activity and microscopic evaluation

Many microbial polymers may be useful for different industrial applications as bioemulsifiers due to their ability to stabilize emulsions between water and hydrophobic compounds. The use of bioemulsifiers is advantageous when compared to chemical counterparts since they are biodegradable, less toxic, and have activity under a wide variety of conditions (Freitas et al., 2009). These properties are important as oil acts as a flavoring retainer, increases the mouthfeel of foods and improve the palatability (Devi et al., 2016). The emulsifying activity of EPS was determined by holding up the emulsion of the hydrocarbon (hexadecane) in water. Generally, the emulsion breaks rapidly within an initial incubation of 30 min. The absorbance reading after 30 and 60 min gives a fairly good indication of the stability of the emulsion (Royan, Parulekar, & Mavinkurve, 1999). The emulsion stability of EPS1190 was compared with commercial polysaccharides such as xanthan gum and guar gum. Results are shown in Fig. 6. The EPS1190

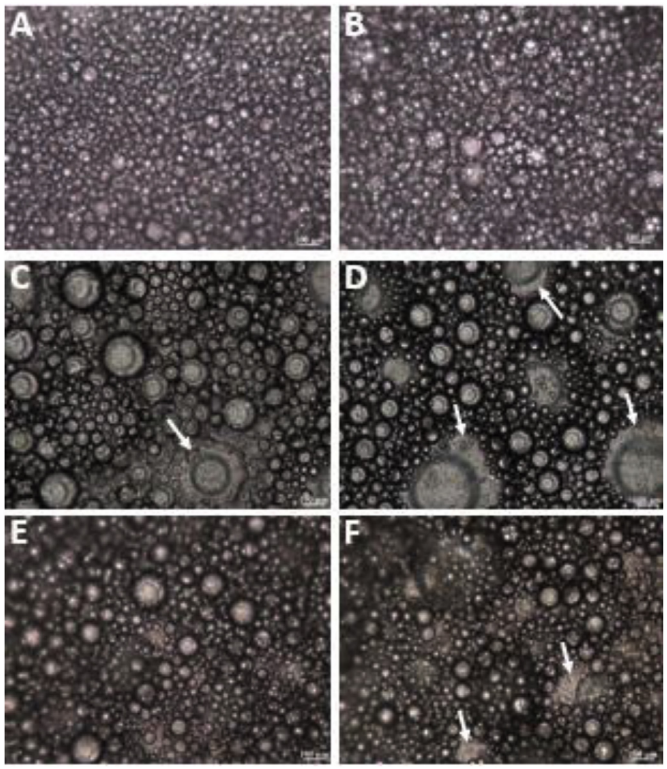

Fig. 7. Photomicrograph of polymers' emulsions formed with hexadecane at 30 and 60 min EPS1190 **(A and B)**, guar gum **(C and D)** and xanthan **(E and F)**. White arrows indicate disintegration areas of the emulsions.

retained 96.78% and 94.10% of the emulsification activity after 30 and 60 min at 0.5 mg/mL, respectively. The functional groups in the molecular chains of the polymer are considered as important determinants for emulsification activity (Bramhachari et al., 2007). The commercial polysaccharides showed relatively poor emulsifying activity as compared to CRL1190 biopolymer at each of the concentration evaluated. The optimal activity of guar gum (1 mg/mL) retained 81.50% and 76.47% whereas xanthan gum (2 mg/mL) presented 92.15% and 84.41% emulsion activity after 30 and 60 min, respectively. Moreover, Wang, Ahmed, Feng, Li, and Song (2008) reported an emulsifying activity (91 and 88% at 30 and 60 min) for the EPS (1 mg/mL) of *L. kefiranofaciens* ZW3 higher than xanthan and guar gum.

The droplet size of the emulsion is considered another critical parameter that determines the physical stability such as flocculation and creaming rate of different emulsions (Devi et al., 2016). Fig. 7 shows typical photomicrographs for the emulsions prepared with the EPS1190, xanthan and guar gum. The emulsion prepared with the CRL1190 biopolymer was significantly different from the other emulsions: the droplets remained small (between 20 and 50 μm) for the first 30 min, a small part of the population increased in size up to 80 μm after 60 min (Fig. 7A and B). In both cases, the emulsions formed remained densely packed as evenly distributed droplets. Presence of smaller droplets may result in more stable emulsions (Horozov, Binks, & Gottschalk-Gaudig, 2007). The emulsions prepared by commercial polysaccharides exhibited population drops of larger sizes. Guar gum formed two kinds of drops sets at 30 min, one group of 36–64 μm and another of 120–150 μm (Fig. 7C). At 60 min, emulsions droplets remained mostly among 45–75 μm and 97–140 μm (Fig. 7D). In addition, deformation areas of the emulsions were observed in the microphotographs at 30 and 60 min probably due to the disintegration aforementioned; there are low emulsifying activity and a remarkable decrease in stability for all the concentrations of guar gum tested with the time progresses. On the other hand, emulsions prepared with xanthan (Fig. 7 E and F) showed a more compact and less irregular structure than that observed for guar gum during the evaluation times. Although slight disintegration zones of the emulsions are observed, the sizes of the drops remained mostly among 35–55 μm and between 70 and 95 μm during 30 and 60 min, respectively. According to the above, xanthan would maintain a greater emulsifying capacity than guar gum at the concentrations and times evaluated in this study. Therefore, the ability of high stability micro-emulsion formation by EPS1190 indicates its potential application in the food industry as a bioemulsifier.

3.8. Flocculating activity

Flocculating agents are prevalent in various industrial processes such as wastewater treatment, drinking water purification and downstream in food and fermentation procedures (Sajayan, Kiran, Priyadharshini, Poulose, & Selvin, 2017). The flocculants are mainly of three types, e.g., inorganic flocculants (aluminum sulfate, poly-aluminum chloride, ferric chloride, and ferrous sulfate), organic flocculants (polyacrylamide derivatives and polyethyleneimine) and natural flocculants or biofloculants (gelatin, chitosan, sodium alginate, etc.) (Salehizadeh & Yan, 2014). The first two groups are most commonly used due to their good flocculating performance and low cost although they produced serious environmental and health problems (neurotoxic and carcinogenic monomers). For this reason, there is an increased interest in biofloculant (safe and biodegradable) to replace chemical flocculants (Devi et al., 2016; Salehizadeh & Yan, 2014).

Flocculation reactions were evaluated at different EPS1190 concentrations in the ranges of 0.50–1.00 mg/mL. Fig. 8 showed the results of the flocculating activity of EPS1190 compared with the commercial flocculants xanthan gum and guar gum, used as control. Flocculating capability increased with increasing the EPS1190 concentration up to 0.25 mg/mL (optimal concentration) with 82% activity; from this point on, a decreasing performance was observed. EPS1190 contains a large number of OH groups that act as electrostatic binding forming a complex with the sites for divalent cations (Ca^{2+}) which are easily adsorbed to suspended particles surface to form stable floccule (Devi et al., 2016). However, the incomplete adsorption due to an excess of flocculants destabilized the particles and only particles around it participated in the flocculating reaction. A high molecular weight bioflocculant is usually long enough and has a sufficient number of free functional groups that may act as bridges to bring many suspended biopolymers together and hence causes a larger floc size in the flocculation reaction (Lim, Kim, Kim, Yoo, & Kong, 2007). In the case of EPS1190, it showed a better flocculating activity at lower concentrations (0.25 mg/mL) than guar

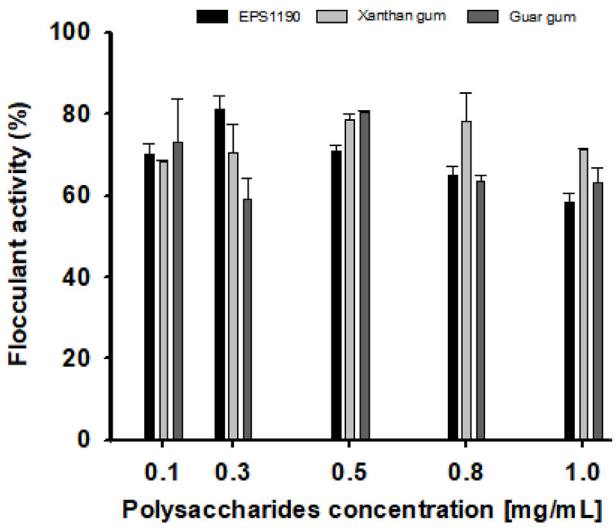

Fig. 8. Flocculating capacity of EPS1190, xanthan and guar gum.

gum and xanthan gum (optimal concentration of both gums at 0.5 mg/mL). A similar trend was observed by Wang et al. (2008) with ZW3 EPS (0.10–0.6 mg/mL) produced by *L. kefiranofaciens* ZW3 which decreased the flocculation activity from the optimal concentration (78% at 0.4 mg/mL).

4. Conclusion

S. thermophilus CRL1190 produces a heteropolysaccharide with proved gastroprotective activity. This strain exhibited an efficient behavior in skim milk displaying a fast lactose consumption, high viable cell numbers (9.65 log CFU/mL), decrease in pH (4.3) and high EPS production (70.7 mg) after 16 h of fermentation. EPS1190 isolated and used in this study has no appreciable impurities (proteins and nucleic acids). The biopolymer presented good performance in aqueous and oil matrices showing a higher water and oil holding capacity (WHC and OHC) than other EPS of the similar composition produced by LAB. The microporous structure of the EPS1190 could interact with water enhancing texturing properties in foods matrixes. Additionally, the polysaccharide remained thermally stable up to 215 °C (T_d = 295.4 °C) and the crystalline deformation of the polymer took place through an endothermic process (ΔH = 284.46 J) at melting temperature of 74.08 °C. The mentioned characteristic ensures the stability of its functional properties even if the process is carried out at high temperatures frequently used in the food industry. EPS1190 displayed moderate antioxidant properties *in vitro* assays mainly $ABTS^{\bullet+}$ radical scavenging ability and the formation of phosphate Mo (V) complex. On the other hand, EPS1190 was more effective as emulsion stabilizer than commercial polysaccharides such as xanthan and guar gum that are commonly used as emulsifiers in the food industry. A stable emulsion remained densely packed as uniformly distributed droplets at an optimum concentration of 0.5 mg/mL of EPS1190 after 60 min of testing. Besides, EPS1190 showed better flocculant activity than xanthan and guar gum at a lower concentration. From these results, good performance of EPS1190 in the dairy industry as a healthy food grade additive with potential antioxidant, emulsifying and flocculant activity is expected.

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