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A comparative study of the influence of the content and source of β-glucan on the rheological, microstructural properties and stability of milk gel during acidification

Marek Aljewicz [a,*], Ana-Isabel Mulet-Cabero [b], Peter J. Wilde [b]

[a] Department of Dairy Science and Quality Management, Faculty of Food Science, University of Warmia and Mazury in Olsztyn, 10-719, Olsztyn, Poland
[b] Quadram Institute Bioscience, Norwich Research Park, Norwich, Norfolk NR4 7UQ, UK

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ABSTRACT

The aim of the study was a comprehensive, comparative study evaluating the impact of the addition level (0.125%, 0.25%, 0.5%, 1%) of highly purified β-glucans isolated from bacteria (curdlan), fungi (scleroglucan) and oats on the stability, rheological and microstructural properties of milk gel during acidification with glucono-δ-lactone. Viscosity and susceptibility to shear thinning were lowest in aqueous solutions containing oat β-glucan (OBG) and highest in solutions containing curdlan. Regardless of their addition level, curdlan and scleroglucan produced pseudoplastic fluid. Coagulation was most rapidly induced by scleroglucan, and it was most delayed under the influence of OBG. The susceptibility of acidified milk gel to phase separation was significantly influenced by the hydration properties and source of β-glucan. Acidification of gels with the lowest concentration (0.125%) of scleroglucan and OBG promoted aggregation of the caseins and then phase separation. This behaviour was not observed in gels containing linear β-glucan (curdlan). The images acquired under a confocal microscope revealed that the all analysed preparations significantly affected the formation of protein complexes whose size and shape were closely linked with the type of added β-glucan. The morphology of samples containing curdlan most closely resembled the structure of the control gel. The addition (up to 0.5%) of scleroglucan and OBG resulted in a gel with low stability. Separate protein structures and clusters of β-glucan were found. The addition of higher levels of β-glucan resulted in a more homogeneous microstructure of the product, which was similar to the control acidified gels.

1. Introduction

Yoghurt is the most popular fermented dairy product, and its consumption continues to increase around the world. Yoghurt's popularity can be attributed to the health-promoting properties of fermented milk and the applied additives (e.g., prebiotics). The acidity of yoghurt increases during storage, which intensifies fracture strain inside the protein network. This process is accompanied by syneresis, namely the release of serum from the gel matrix (Lucey, Teo, Munro, & Singh, 1998; Rohart, Michon, Confiac, & Bosc, 2016). Syneresis can be inhibited by adding milk-based preparations, changing the starter culture or acidification conditions, or by adding gelatin and/or polysaccharides such as xanthan gum, starch or pectin (Aichinger et al., 2003; Everett & McLeod, 2005). However, thickening agents (i.e. guar gum, carrageenan) are less accepted by consumers. Due to their proven health benefits by decreasing the risk of diet-dependent diseases such as hyperinsulinemia, hyperlipidemia, impaired immunity and osteoporosis (Aljewicz et al., 2018; Aljewicz, Tońska, Juśkiewicz, & Cichosz, 2018; Henrion, Francey, Lê, & Lamothe, 2019; Verma et al., 2020), β-glucans could offer an acceptable alternative to the polysaccharides commonly used in the dairy industry.

β-glucans are non-ionic polysaccharides that occur naturally in eukaryotes and prokaryotes. The source from which β-glucans are isolated determines their structure. β-glucan isolated from *Agrobacterium* spp. bacteria (curdlan) has a linear structure where the molecules of β-D-glucopyranoside units are linked by β-(1 → 3) bonds (Verma et al., 2020). In turn, β-glucans isolated from oats show a more complex structure, as they are linear homopolysaccharides composed of D-glucopyranosyl residues linked by a mixture of β-(1 → 3) and β-(1 → 4) bonds (Lazaridou, Biliaderis, & Izydorczyk, 2003). β-glucan

* Corresponding author.
E-mail address: marek.aljewicz@gmail.com (M. Aljewicz).

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isolated from *Sclerotium rolfsii* fungi (scleroglucan) is a polysaccharide composed of a linear chain of D-glucopyranoside units held together by β-(1 → 3) bonds with individual D-glucopyranoside groups linked by β-(1 → 6) bonds (Farina, Sineriz, Molina, & Perotti, 2001). The source of β-glucans also determines their molecular weight. Among the tested preparations, scleroglucan is characterised by the highest molecular weight (2–3 × 10^6 g/mol) (Survase, Saudagar, Bajaj, & Singhal, 2007; Verma et al., 2020), whereas the molecular weight of curdlan and oat β-glucan (OBG) is lower at 5.3 × 10^4 to 5.8 × 10^6 g/mol (Farina et al., 2001) and at 0.56 × 10^5 to 2.5 × 10^6 g/mol, respectively (Stone, 2009). Due to their high molecular weight, β-glucans are more effective thickening agents than other polysaccharides (i.e. inulin) (Everett & McLeod, 2005; Verma et al., 2020). β-glucans induce a greater increase in the viscosity of a solution when added at lower concentrations than other thickening agents (i.e locust bean gum or guar gum). The rheological properties of aqueous solutions or hydrogels containing various polysaccharides have been widely researched, whereas β-glucans have been less extensively studied. In aqueous solutions, β-glucans can form single or triple helices stabilised by hydrogen bonds, depending on the location of glycosidic bonds and the number of branches. This is an important consideration because the rheological properties of gels are influenced by thermal processing parameters and the structure of β-glucan molecules in a solution (Survase et al., 2007; Lazaridou & Biliaderis, 2004). Scleroglucan is readily soluble in water and forms stable pseudoplastic fluid. In comparison to scleroglucan, OBG is less soluble, because its solubility in foods depends on the source of the material, processing conditions and changes during food preparation, the time required to achieve full hydration (Wang & Ellis, 2014). Unlike OBG and scleroglucan, curdlan is not water-soluble, but it has excellent hydration properties (1 curdlan molecule can absorb up to 100 water molecules) and forms pseudoplastic fluid at temperatures higher than 80 °C (Verma et al., 2020). There is evidence to suggest (Verma et al., 2020; Sharafbafi, Tosh, Alexander, & Corredig, 2014; Corredig, Sharafbafi, & Kristo, 2011; Guggisberg, Cuthbert-Steven, Piccinali, Bütikofer, & Eberhard, 2009; Lazaridou & Biliaderis, 2007; De Bont, Van Kempen, & Vreeker, 2002; Böhm & Kulicke, 1999a, 1999b; Fig. 1) that solutions containing polysaccharides only will have different rheological properties than those containing polysaccharides as well as proteins. Polysaccharide molecules are larger and differently shaped than milk proteins, and they significantly affect the rheological properties of dispersions. In addition to hydration properties, the rheological properties of protein-polysaccharide dispersions are significantly influenced by polysaccharide charge. Anionic hydrocolloids such as xanthan gum, carboxymethyl cellulose, pectin and κ-carrageenan interact with casein micelles, protect the network against local fractures and inhibit syneresis (Corredig et al., 2011). In turn, neutral hydrocolloids (guar gum, locust bean gum, starch and β-glucans) stabilise the system and strengthen the protein network by increasing the viscosity of the continuous phase (Laneuville & Turgeon, 2014). The addition of neutral hydrocolloids to milk (in contrast to anionic hydrocolloids) has to be strictly monitored. When β-glucans preparations are added at low concentrations or if their molecular weight is low, the protective layer around casein micelles can be depleted, which increases attraction between micelles, stimulates protein aggregation and, consequently, causes damage to a product's structure (Corredig et al., 2011; Everett & McLeod, 2005; Syrbe, Bauer, & Klostermeyer, 1998).

Studies conducted to date have analysed the effect of OBG and barley β-glucan (Kontogiorgos, Ritzoulis, Biliaderis, & Kasapis, 2006; Kontogiorgos, Tosh, & Wood, 2009; Sharafbafi et al., 2014; Zhao et al., 2014) on the stability of whey protein or casein dispersions. The stability of dispersions containing scleroglucan and curdlan remains insufficiently investigated. The effect of β-glucan on milk acidification kinetics has been analysed by a limited number of studies (Lazaridou, Serafeimidou, Biliaderis, Moschakis, & Tzanetakis, 2014; Lazaridou, Vaikousi, & Biliaderis, 2008; Vasiljevic, Kealy, & Mishra, 2007).

When designing the study, it was assumed that the rheological properties of acidified and non-acidified milk gels containing β-glucans are influenced by the type of the applied polysaccharide and its content. Therefore, the aim of this study was to compare the effects of different types and concentrations of highly purified β-glucans isolated from different sources on the interactions between milk proteins and β-glucans during milk acidification in a simulated yogurt making process.

2. Materials and methods

2.1. Materials

The experimental milk gels were produced with the addition of (1 → 3) (1 → 4) β-glucan (purity: 75%; Mw = 0.97 × 10^6 g/mol) isolated from oats (*Avena sativa* L.) (Beta Bio Technology, Poland); (1 → 3) β-glucan (curdlan; purity: 90%; Mw = 0.91 × 10^6 g/mol) isolated from *Agrobacterium* spp. bacteria (Xi'an Lyphar Biotech Co., Ltd, China) and (1 → 3) (1 → 6) β-glucan (scleroglucan; purity: 90%; Mw = 2.04 × 10^6 g/mol) isolated from *Sclerotium rolfsii* (Cargill, Inc., Germany).

Sodium hydroxide, hydrochloric acid, sodium azide, lecithin, D-(+)-gluconic acid δ-lactone (GDL), silicon oil (PMX 200/20 cSt) as well as dyes fluorescent brightener 28 (Calcofluor-white) and fast green FCE were purchased from Sigma (Poole, UK). Instant skimmed milk powder (protein: 35; carbohydrate: 52 and fat 1% w/w) was purchased from Mlekowita (Gostynin, Poland).

2.2. Preparation of aqueous solutions of the β-glucan

The hydrogel solution was prepared by dissolving 0.125, 0.25, 0.5, 0.75 or 1% w/v of the β-glucan preparation in Milli-Q® water, and an adequate amount of 0.1 M NaOH or 0.1 M HCl was added to give a pH of the solution of pH 6.6 at 20 °C (the amount of 0.1 M NaOH or 0.1 M HCl was determined according to previous tests). The dispersion was mixed at 1000 rpm with a magnetic stirrer (C-MAG HS 7, IKA® Werke, Fisher Scientific Ltd.), heated at 60 °C for 40 min, homogenised (Ultra-Turrax T25, IKA® Werke, Fisher Scientific Ltd.) for 20 s at 8000 rpm, and heated to 80 °C. At this temperature, the sample was mixed at 1000 rpm, heated at 80 °C for 15 min, and left to cool at room temperature for 1 h. The obtained gel was only used to determine the viscosity flows of aqueous solutions of the β-glucan.

2.3. Preparation of non-acidified milk gel

Milli-Q® water, instant skimmed milk powder (12.48% w/w in the control milk gel; ~12% w/w in the experimental milk gel), lecithin (antifoaming agent) (0.5% w/w) and sodium azide (antibacterial agent) (0.02% w/w). The experimental milk gel also contained 0.125; 0.25; 0.5; 0.75 or 1% w/w of OBG or scleroglucan or curdlan. Milk gel was prepared in the Thermomix® TM5 multipurpose food processor (Wuppertal, Germany) in several stages. 1st) Preparation of the milk base. The ingredients for each type of product (control gel, experimental gel) were weighed, then the prepared products were mixed at 2000 rpm at 45 °C for 45 min. Milk was cooled to 4±1 °C in an ice-water bath and aged overnight at 4 °C in a refrigerator. 2nd) The following day, the β-glucan was added to the milk, the sample was mixed at three different speed and temperature settings (2500 rpm at 50 °C for 80 min; 2500 rpm at 65 °C for 15 min and 2000 rpm at 70 °C for 15 min). Then, to remove air bubbles, the hot sample was transferred to a beaker and vigorously stirred for 2 min using a magnetic stirrer (C-MAG HS 7, IKA®, UK). The sample was placed in an ice-water bath and quickly cooled to 44 °C.

2.4. Rheological measurements

The rheological measurements of the gel samples were carried out with a rheometer (Advanced Rheometer AR-G2, TA Instruments, Herts, UK) equipped with a Peltier temperature control unit. The

measurements were run in controlled strain mode. Rheological data were collected using Rheology Advantage Data Analysis software v. 5.8.2 (TA Instruments). The measurements were performed in at least three replicates.

Viscosity flows were measured on a hydrogel made in section 2.2 in a plate-plate geometry system (plate Ø 60 mm) with a 1 mm gap at 42 °C after 1 min of temperature equilibration, with a shear rate of 0.1–100 s^{-1}, and ten measurement points per decade. The linear viscoelastic region (LVR) was determined with a strain sweep of 0.01–100% at a fixed angular frequency of 1 Hz. Storage (G′) and loss (G″) moduli were measured at 42 °C by dynamic frequency sweep conducted over an angular frequency range of 0.1–10 Hz at a constant strain of 0.5%, with ten measurement points per decade. Data collection started after 1 min of temperature equilibration.

The gelation was measured with non-acidified milk (produced in section 2.3). Before measurements, the milk base was vigorously stirred on a magnetic stirring plate at 1500 rpm for 60 s then 1.55% w/w GDL (based on preliminary experiments) was added, then the sample was vigorously stirred on a magnetic stirring plate at 1500 rpm for 60 s again. The test was performed in a cone-plate geometry system (plate Ø 60 mm, 1°) with a 1 mm gap using time-sweep oscillatory measurements just after addition of GDL at a frequency of 1 Hz and 0.5% strain. Silicon oil was placed on top of the sample to prevent evaporation. The measurements were carried out at 42 °C after 1 min of temperature equilibration and after 2 min after adding the GDL; data were collected every 30 s, by which time the gels had reached pH ~4.6.

The addition of OBG was normalised to ensure that β-glucan content was identical to that of the experimental milk gel containing curdlan or scleroglucan. The dry matter content of milk gels (control, experimental) was normalised to 13%.

2.5. Milk gel stability analysis

The stability of milk gels during acidification were measured using a laser scanning instrument (Turbiscan Lab Expert analyser, Formulaction SA, Toulouse, France) to determine the extent of phase separation (sedimentation or flocculation). To the non-acidified milk gels 1.55% w/w of GDL was added (based on preliminary experiments) and vigorously stirred on a magnetic stirring plate at 1500 rpm for 60 s. Then 20 mL of the sample was placed in a flat-bottomed cylindrical glass cell (sample height = 60 mm; inside diameter = 13 mm) and scanned at 5 min intervals for 1 h 5 min to determine the light scattered from the gels as a function of sample height (40 μm intervals). Under the operating instructions, before the measurement, test samples were filled to the same height, and the absence of air bubbles was confirmed (the presence of bubbles was visually checked). The sample was incubated at 42 °C throughout the entire incubation period. The gels were compared using the Turbiscan Stability Index (TSI), which provides information on the general behaviour of milk gels. The TSI was calculated in Turbisoft 2.3.1.125 based on backscattering/transmission changes which are indicative of macromolecule (polysaccharide or protein) aggregation and migration (Eg.1).

$$TSI = \sqrt{\frac{\sum_{i=1}^{n}(x_i - x_{BS})^2}{n-1}} \quad \text{(Eg.1)}$$

where x_i is the average backscattering for each minute of measurement, x_{BS} is the average x_i, and n is the number of scans. High values of TSI are indicative of changes in gel structure, the system's instability and a high probability of phase separation (application, 2020).

The analysis was performed in at least two replicates.

2.6. Confocal laser scanning microscopy

2.6.1. Preparation of samples for staining

Ten mL of non-acidified milk gels (as described in section 2.3) was transferred to a beaker. Next, 50 μL of fast green FCE solution (1 mg/mL made with Milli-Q® water; for the protein stain) was added to the beaker. The mixture was vigorously mixed at 1000 rpm for 20 s using a magnetic stirrer (C-MAG HS 7, IKA®, UK). After 15 min of incubation in dark at room temperature, 300 μL fluorescent brightener 28 solution (1 mg/mL made with Milli-Q® water; for β-glucan stain) was added. The mixture was vigorously mixed at 1000 rpm for 20 s using a magnetic stirrer. After 15 min of incubation in dark at room temperature, 1.50% w/v of GDL was added to the beaker, and the sample was stirred. A small amount (~15 μL) of the sample was quickly transferred to a well (9 mm diameter; 0.12 mm deep) and placed on a glass plate. The excess gel was removed, and a coverslip was applied immediately without excessive pressure.

2.6.2. CLSM observations

The samples were viewed under a confocal scanning laser microscope (CLSM; Zeiss LSM 880, Carl Zeiss Ltd, Cambridge, UK). The thermostatic chamber of the microscope was set to 42 °C before the analysis. A 60 × oil-immersion objective was used for all images. The samples were excited using an Ar + laser 405 nm (Fluorescent brightener 28) and HeNe laser 633 nm (fast green FCE). The fluorescence emitted by the samples was detected at 406–460 nm (Fluorescent brightener 28) and 650–710 nm (Fast Green FCE). Greyscale images were captured at a resolution of 4096 × 4096 pixels, each covering an area of 191,48 × 191,48 μm.

2.7. Statistical analysis

The results were verified for normal distribution and homogeneity of variance. The significance of differences between means was analysed by Tukey's test, and the interactions between factors (addition level and structure of β-glucans, and the interactions between factors) were determined by two-way ANOVA. At this stage, data were presented as means ± standard deviation. All results were processed in Statistica 13.5 PL software (Statsoft 2017; Krakow, Poland) at a significance level of 0.05. The experiment performed in at least three replicates.

3. Results and discussion

3.1. The influence of β-glucans on aqueous dispersions viscosity

The relationship between the apparent viscosity and shear rate of aqueous dispersions with different content and structure of β-glucans is presented in Fig. 1.

The rheological properties of aqueous dispersions were significantly influenced by the type and content of β-glucans. Similar results were reported by Verma et al. (2020), Malaka, Ohashi, and Baco (2013), Kontogiorgos, et al., 2006 and Moresi, Presti, and Mancini (2001) who examined aqueous dispersions containing curdlan, scleroglucan and OBG. In aqueous dispersions with 1% of β-glucan, viscosity was highest (η = 105 Pa s) in products containing curdlan and lowest (η = 1.5 Pa s) in gels containing OBG (Fig. 1). The viscosity of the OBG gel was higher than that reported by Zhao et al. (2014) in aqueous dispersions containing the same amount of the polysaccharide. The above authors as well as Vaikousi, Biliaderis, and Izydorczyk (2004) observed that despite the same content (%) and similar molecular weight of the added β-glucan, the resulting aqueous dispersions can significantly differ in viscosity. These variations could be attributed to the use of the polysaccharide isolated from cereals grown in different locations, different methods of aqueous dispersions preparation and different storage times before measurement. According to Vaikousi et al. (2004), the polysaccharide was hydrated or fully dissolved, thus the structure was stabilized and viscosity increases when gels are left to mature for several hours before analysis. Higher viscosity of the received aqueous dispersions could also result from changes in polysaccharide structure after thermal processing (85 °C/15 min) (Verma et al., 2020; Sharafbafi et al., 2014; Zhao et al., 2014). In the present study, susceptibility to shear

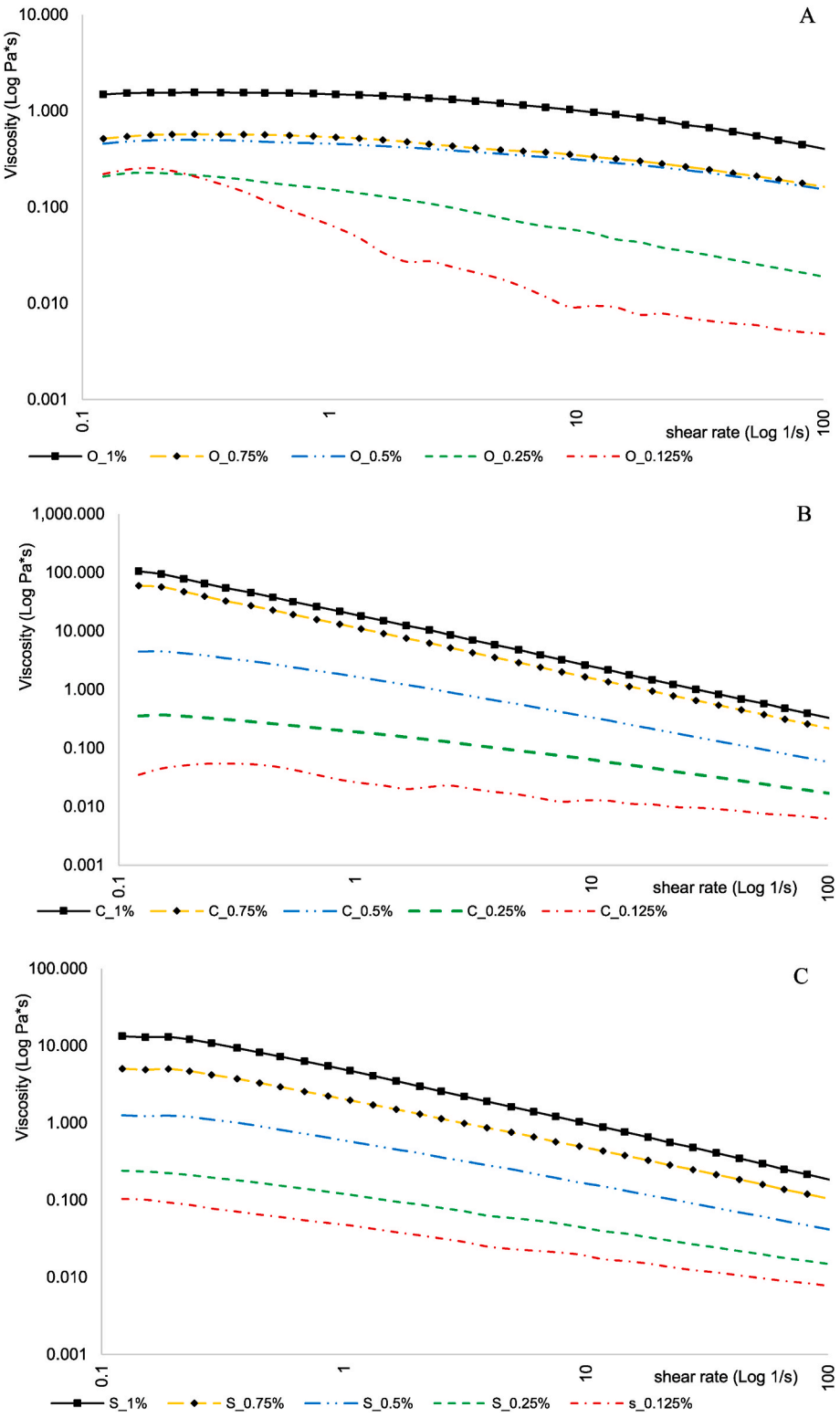

Fig. 1. Viscosity of aqueous solutions with different addition level (0.125%; 0.25%; 0.5%, 0.75% and 1%) of β-glucan preparation (A: oat β-glucan; B: curdlan; C: scleroglucan) determined at 42 °C and shear rates of 0.1–100 s^{-1}.

thinning was highest in aqueous dispersions containing curdlan and lowest in aqueous dispersions containing OBG (Fig. 1A–C). In products with low OBG concentrations (0.25%) and $\dot{\gamma}$ values of up to 0.3 s^{-1}, intermolecular interactions which are broken during applied shear can be rapidly reformed between polysaccharide chains due to low viscosity and high molecular mobility. In products with high polysaccharide content, interactions which are broken by the applied shear forces will only reform slowly due to high viscosity and low molecular mobility. As a result, the apparent viscosity decreases rapidly with a rise in $\dot{\gamma}$, and these gels behaved like non-Newtonian, pseudoplastic fluids, which is consistent with the findings of Skendi, Biliaderis, Lazaridou, and Izydorczyk (2003). However, unlike in other studies where 1% addition of

scleroglucan was used, the viscosity in this study was η = 105 Pa s ($\dot{\gamma}$ ~0.1 s-1) which was significantly lower than that (10,000 Pa s) reported by Moresi et al. (2001), but higher than that noted by Farina et al. (2001) and Mazzuca et al. (2017) (~1 Pa s and ~0.6 Pa s at $\dot{\gamma}$ ~0.1 s^{-1}, respectively). Similar observations were made by Moresi et al. (2001) who attributed their findings to differences in the size of scleroglucan molecules resulting from, for example, longer fungal culture times (Farina et al., 2001). To determine the effect of polysaccharides on the formation of milk protein gels, elastic modulus (G′) and viscous modulus (G″) values were compared at a frequency of 1 Hz. OBG dispersions and as well as aqueous dispersions containing 0.125% and 0.25% of curdlan and 0.125% of scleroglucan behaved like viscoelastic fluids (G ″> G′) with few or no entanglements between molecules. Similar results were reported in aqueous dispersions of oat (Lazaridou & Biliaderis, 2007; Skendi et al., 2003; Zhao et al., 2014) and barley β-glucan (Vaikousi et al., 2004) (Table 1). Viscosity decreased with a rise in elasticity (Fig. 1A–C; Table 1), which corroborates the findings of Survase et al. (2007) and Moresi et al. (2001).

3.2. The influence of β-glucans on the rheological properties of milk gels during acidification

The impact of β-glucan on the formation of milk protein gels was analysed over time, and the results are presented in Fig. 2A–C. Only G′ values were compared to increase the readability of the results.

In the control samples, protein coagulation started after 20 min of acidification when pH was below 5.3 and approximated the isoelectric point (pI) of β-lactoglobulin, and G′ increased by more than 1 Pa. The addition of β-glucans, regardless of their type, significantly ($p < 0.01$) accelerated the start of coagulation. The time interval to the beginning of the coagulation process was decreased with a rise in polysaccharide content. The addition of 0.125%, 0.25%, 0.5% and 1% of scleroglucan accelerated the beginning of coagulation by 5 min, 11 min, 15 min and 19 min, respectively (Fig. 2C). A similar relationship was noted in milk gels containing curdlan, where the application of 1% and 0.125% of curdlan shortened the time interval to the onset of coagulation by around 15.5 min and 7.5 min, respectively (Fig. 2B). Such a relationship was not observed in samples containing less than 0.5% of OBG (Fig. 2C). The above results indicate that the molecular weight, type, and consequently, the structure of β-glucan significantly affect the aggregation of milk proteins and gelation of dispersions. These findings corroborate the results reported by Pang, Deeth, and Bansal (2015). In contrast, Kontogiorgos et al. (2006) observed that barley β-glucan prolonged protein aggregation. Gelation time of cereal β-glucans (mostly from oat and barley) was dependent on the source (barley has higher gelling capacity than oat), molecular weight and concentration. With decreasing β-glucan concentration and increasing molecular weight the gelation time increase due to reduction of chain mobility (Lazaridou et al., 2003; Vaikousi et al., 2004). In addition, scleroglucan and OBG, curdlan is not water-soluble, and its effect on coagulation could probably be attributed to the high hydration properties of β-glucan molecules. As a result of water absorption by the β-glucan molecule, the amount of free water available to the protein in the system was reduced as the concentration of β-glucan in the solution increases. Compared to the water-soluble polysaccharide, the use of highly absorbent polysaccharides resulted in stronger and denser three-dimensional, water-holding networks. Thus the reduced availability of free water exerts a more substantial influence on the onset of coagulation of milk proteins than protein attraction resulting from changes in their zeta potential and increased in their hydrophobicity during acidification. However, these effects were less pronounced in products with low polysaccharide concentrations due to a greater distance between molecules and a lower rate of intermolecular interactions. Similar observations were made by Mleko, Li-Chan, and Pikus (1997) in gels containing κ-carrageenan.

In the beginning, in the experimental gels containing 1% of β-glucan, G′ values were significantly higher (~32 Pa for scleroglucan; ~10 Pa for OBG and curdlan) than in the control gel (G′ ~ 0.1 Pa) (Table 2).

The obtained results were consistent with those presented in the studies of Pang et al. (2015) and Kontogiorgos et al. (2006), which also showed a significant increase in the initial viscosity of gels containing various polysaccharides, including barley β-glucan. β-glucans decreased the free water content in the system, which increased bond resistance to shear stress, and the resulting dispersions display solid-phase behaviour (Everett & McLeod, 2005). Coagulation can be significantly impaired in products with a low content of β-glucans. The size of protein aggregates increased during acidification, but phase separation occurred when the polysaccharide-dependent energy barrier was crossed (Lazaridou et al., 2014, 2008, 2007, Kontogiorgos et al., 2009, 2006, Everett & McLeod, 2005). The above was observed in samples containing 0.125% of scleroglucan and OBG (Fig. 2A/C). Similar results were reported by Kontogiorgos et al. (2006) and Spagnuolo, Dalgleish, Goff, and Morris (2005), who observed that polysaccharide molecules were embedded into the protein network in early stages of coagulation. The study showed that fewer intermolecular bonds and lower elasticity characterised the resulting network, and it was rapidly degraded during measurements. The acidification promoted the phase separation as the casein will have greater attractive forces than the β-glucan, driving a phase separation and aggregation of the caseins. This could lead to syneresis of liquid from the casein gel, resulting in a depletion of the layer on the measuring geometry surfaces and caused sliding. These processes were typically observed in gels containing soluble (OBG and scleroglucan) β-glucans. The addition of insoluble curdlan (Fig. 2C), decreased the values of G′ in the initial stages of acidification. However, structural degradation was only temporary, and G′ began to increase after 60 min of acidification when pH approximated the pI of casein. Applied heat treatment (85 °C/15 min) led to the irreversible triple-helix conformation of polysaccharides and caused partial denaturation and whey protein aggregation (Malaka et al., 2013; Verma et al., 2020). It should be noted that in the first 30 min of acidification, a fragile network of coagulating milk proteins (mainly β-lactoglobulins) was formed. When pH reaches ~5.18, probably due to changes in electrostatic repulsion, rupture of hydrogen bonds, and macromolecules' incompatibility, phase separation occurs in products containing OBG and scleroglucan. In gels containing curdlan, the polysaccharide network was strong enough to stabilise the gel structure, which was why only a minor decrease in the value of G′ was noted. The system was stabilized, and the significance of the polysaccharide network increased with a rise in the concentration of polysaccharides due to a higher number of hydrogen bonds and shorter distances between molecules (Perrechil, Braga, & Cunha, 2009).

Table 1
Value of G′ and G″ for aqueous solutions with different addition level (0.125%; 0.25%; 0.5% and 1%) of different β-glucans preparation; oscillatory measurements at a frequency of 1 Hz (6.28 rad s^{-1}) and 0.5% strain.

		Sample		
Content	Moduli	Oat β-glucane	Curdlan	Scleroglucan
1.0%	G′	2.83 ± 0.07^{a}	8.20 ± 0.22^{a}	54.50 ± 0.64^{a}
	G″	5.92 ± 0.11^{a}	4.98 ± 0.22^{a}	14.29 ± 0.13^{a}
0.75%	G′	1.45 ± 0.04^{b}	4.57 ± 0.04^{b}	42.29 ± 0.435^{b}
	G″	2.54 ± 0.04^{b}	3.23 ± 0.16^{b}	11.07 ± 0.11^{b}
0.50%	G′	0.16 ± 0.04^{c}	1.26 ± 0.03^{c}	5.92 ± 0.02^{c}
	G″	0.52 ± 0.04^{c}	1.10 ± 0.01^{c}	3.17 ± 0.04^{c}
0.25%	G′	0.02 ± 0.01^{d}	0.22 ± 0.01^{d}	0.49 ± 0.01^{d}
	G″	0.05 ± 0.02^{d}	0.26 ± 0.01^{d}	0.46 ± 0.01^{d}
0.125%	G′	0.02 ± 0.00^{d}	0.07 ± 0.00^{e}	0.06 ± 0.00^{e}
	G″	0.02 ± 0.01^{e}	0.10 ± 0.02^{e}	0.09 ± 0.02^{e}

The values are represented as mean and standard deviation (n = 3).
[cba] Mean values in columns with different superscript letters are significantly different ($p \leq 0.05$) – experimental factor: content (each module is compared separately).

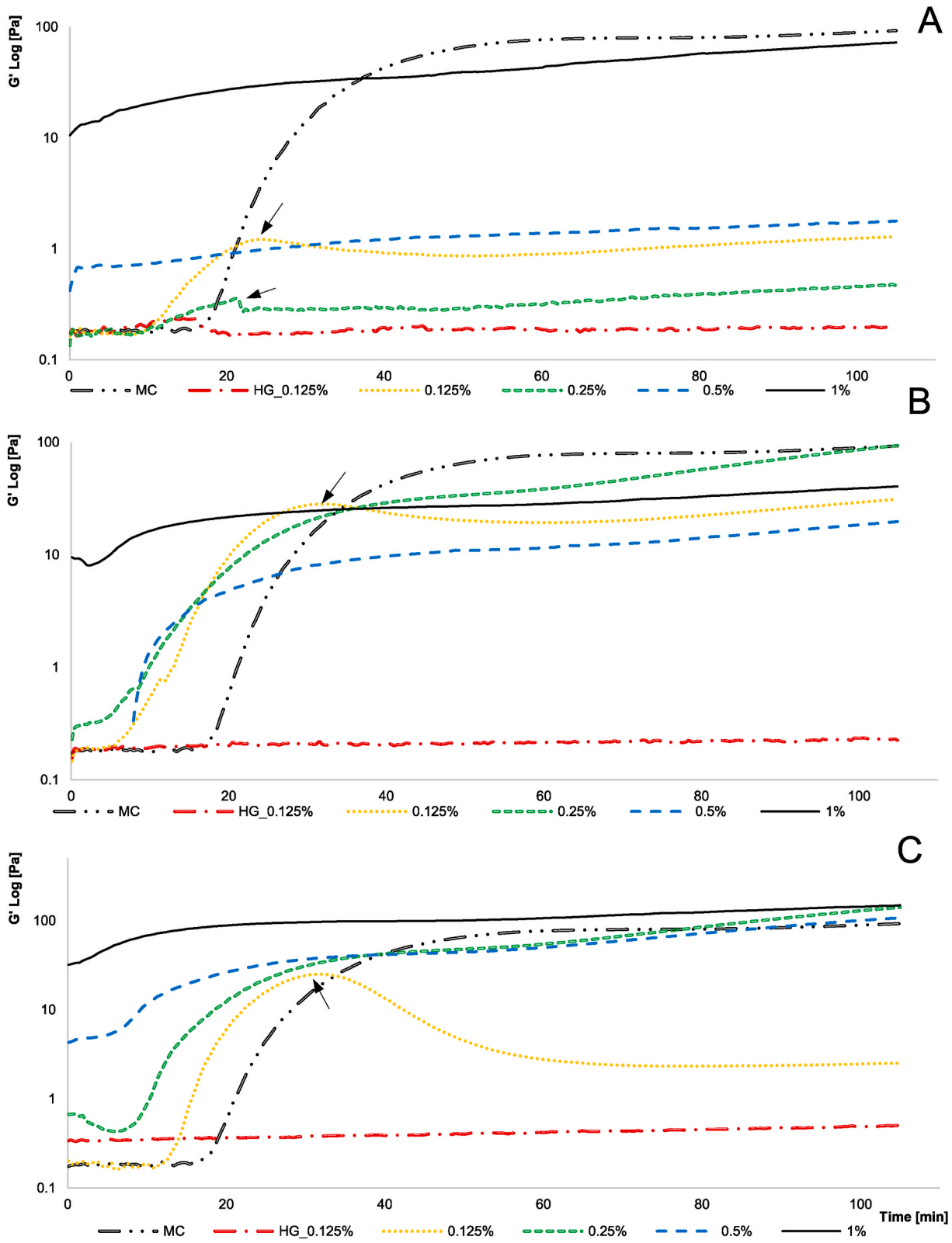

Fig. 2. Time dependence of G′ moduls for milk gels with different addition level (0.125%; 0.25%; 0.5% and 1%) of β-glucans preparation (A: oat β-glucan; B: curdlan; B: scleroglucan) during acidification. MC: control milk gels (without β-glucan); HG_0.125% - aqueous solutions produced only from water and 0.125% addition of β-glucan. The arrow in the graph indicates the breaking point of the structure.

Table 2
Value of G′ for milk gels with different addition level (0.125%; 0.25%; 0.5% and 1%) of different β-glucans preparation after 0.1 min and 105 min of acidification Control: milk gels (without β-glucan preparation); HG_0.125% - hydrogel produced only from water and 0.125% addition of β-glucan.

Sample	Oat β-glucan			Curdlan			Scleroglucan		
Moduli	G' [Pa]	G'' [Pa]	tanδ	G' [Pa]	G'' [Pa]	tanδ	G' [Pa]	G'' [Pa]	tanδ
Content	after 0.1 min								
HG_0.125%	0.194 ± 0.000^{aA}	0.079 ± 0.000^{Ab}	0.474 ± 0.000^{aB}	0.155 ± 0.000^{aA}	0.04 ± 0.000^{aA}	0.255 ± 0.000^{bA}	0.34 ± 0.000^{abC}	0.258 ± 0.000^{bcC}	0.76 ± 0.000^{bC}
Control	0.172 ± 0.011^{aA}	0.019 ± 0.002^{aA}	0.107 ± 0.001^{aA}	0.172 ± 0.011^{aA}	0.019 ± 0.002^{aA}	0.107 ± 0.001^{aA}	0.172 ± 0.011^{abA}	0.019 ± 0.002^{aA}	0.107 ± 0.001^{cA}
0.125%	0.164 ± 0.01^{aA}	0.042 ± 0.012^{aA}	0.25 ± 0.056^{aA}	0.148 ± 0.009^{aA}	0.055 ± 0.006^{aA}	0.375 ± 0.056^{dB}	0.202 ± 0.003^{aB}	0.1 ± 0.005^{abB}	0.495 ± 0.027^{aA}
0.25%	0.135 ± 0.005^{aB}	0.083 ± 0.003^{aA}	0.616 ± 0.001^{abA}	0.458 ± 0.000^{aA}	0.084 ± 0.000^{aA}	0.182 ± 0.000^{abB}	0.61 ± 0.118^{bA}	0.414 ± 0.056^{cB}	0.683 ± 0.041^{bA}
0.5%	0.422 ± 0.176^{aA}	0.818 ± 0.167^{aA}	2.032 ± 0.452^{bB}	1.511 ± 0.077^{bB}	0.761 ± 0.021^{aA}	0.504 ± 0.013^{cA}	4.306 ± 0.005^{cC}	1.943 ± 0.012^{dB}	0.452 ± 0.003^{aA}
1.0%	10.57 ± 0.722^{Ba}	13.355 ± 1.210^{bC}	1.263 ± 0.029^{cC}	9.497 ± 0.331^{cA}	4.764 ± 0.459^{bA}	0.502 ± 0.031^{cB}	31.945 ± 0.121^{dB}	9.122 ± 0.083^{eB}	0.286 ± 0.004^{dA}
	after 105 min								
HG_0.125%	0.194 ± 0.000^{aA}	0.077 ± 0.000^{aB}	0.394 ± 0.000^{abB}	0.231 ± 0.000^{dB}	0.05 ± 0.000^{dA}	0.215 ± 0.000^{bA}	0.503 ± 0.000^{aC}	0.335 ± 0.000^{bC}	0.667 ± 0.000^{dC}
Control	92.366 ± 5.566^{cA}	36.837 ± 2.244^{bA}	0.399 ± 0.001^{abA}	92.366 ± 5.566^{cA}	36.837 ± 2.244^{cA}	0.399 ± 0.001^{aA}	92.366 ± 5.566^{cA}	36.837 ± 2.244^{aA}	0.399 ± 0.001^{aA}
0.125%	1.283 ± 0.497^{aA}	0.352 ± 0.245^{aA}	0.257 ± 0.092^{bA}	30.905 ± 0.474^{abB}	12.294 ± 0.081^{abB}	0.398 ± 0.004^{aA}	2.511 ± 1.198^{aA}	1.051 ± 0.453^{bA}	0.424 ± 0.022^{aA}
0.25%	0.479 ± 0.017^{aA}	0.277 ± 0.01^{aA}	0.578 ± 0.001^{aB}	92.625 ± 4.674^{cB}	35.9 ± 2.009^{cB}	0.388 ± 0.003^{aA}	139.85 ± 2.051^{bC}	55.145 ± 1.153^{cC}	0.395 ± 0.003^{aA}
0.5%	1.775 ± 0.322^{aA}	1.678 ± 0.119^{aA}	0.955 ± 0.106^{cB}	21 ± 0.085^{abB}	9.697 ± 0.078^{aB}	0.462 ± 0.006^{cA}	106.85 ± 3.748^{dC}	37.705 ± 0.842^{aC}	0.353 ± 0.005^{cA}
1.0%	72.15 ± 1.472^{bB}	45.624 ± 7.911^{bB}	0.634 ± 0.123^{aB}	40.355 ± 3.713^{bA}	15.66 ± 0.764^{bA}	0.389 ± 0.017^{aAB}	147.62 ± 0.962^{bC}	33.58 ± 0.651^{aAB}	0.228 ± 0.003^{bA}

The values are represented as mean and standard deviation (n = 3);
^{cba} Mean values in columns with different superscript letters are significantly different ($p \leq 0.05$) – experimental factor: content;
^{CBA} Mean values in rows with different superscript letters are significantly different ($p \leq 0.05$) – experimental factor: type of β-glucan.

3.3. Evaluation of milk gel stability

During milk acidification, as mentioned earlier, a gradual aggregation of milk proteins occurs. The formation of a polysaccharide network or polysaccharide and protein networks was confirmed by a positive BS (backscattering) value in gels containing 1% of OBG, scleroglucan and curdlan, the average BS was determined at +7.14%, +12.83% and +9.96%, respectively (Fig. 3A–C). At low β-glucan concentrations, the viscosity was low enough to allow some flocculation/sedimentation events. As β-glucan content increases, this is prevented and an increase in backscatter suggests a greater level of aggregation, or a higher number of small aggregates, compared to a lower number of larger aggregates that would potentially decrease the BS value. These interactions lead to the formation of variously sized aggregates, which determined the extent and rate of flocculation. An increase in flocculation rates and more significant incompatibility between system components lead to a decrease in BS values.

Regardless of the source of the analysed polysaccharide, the addition of 0.125% of β-glucan induced the most rapid changes in milk gels. In the gel with 0.125% of OBG, TSI value increased significantly by around 485% (Fig. 4). The most intensive changes were noted between 60 and 90 min of acidification when pH was below the pI of whey proteins and approximated the casein pI (data not shown). In this sample, the highest TSI value (16) and a significant decrease (−40%) in BS value in the top part of the measurement system were noted after 105 min of acidification. Similar observations were made in milk gels containing scleroglucan, where a BS peak confirmed the sedimentation of small molecules in the bottom part (0–1 cm) of the measurement system (Fig. 3C). The addition of other polysaccharides, such guar gum, locust bean gum (Bourriot, Garnier, & Doublier, 1999a), carrageenan (Bourriot, Garnier, & Doublier, 1999b), and amylopectin (De Bont et al., 2002), including at concentrations lower than 0.125%, also increased the flocculation rate and led to the separation of biopolymers due to low compatibility. The presented findings and the results reported by Sharafbafi et al. (2014) and Kontogiorgos et al. (2009) indicate that phase separation could be slowed down or inhibited by modifying the proportions of proteins and polysaccharides. However, changes in the electric potential of biopolymers could also increase a system's instability (phase separation) (Aichinger et al., 2003). Unlike other polysaccharides, β-glucans are not polyelectrolytes (Farina et al., 2001); therefore, rapid phase separation during acidification most probably resulted from thermodynamic incompatibility (β-glucans forms an extended molecular network, whereas the milk proteins form particulate gels with a much denser structure) of biomolecules and, to a lesser degree, by depletion flocculation (Kontogiorgos et al., 2009).

The addition of 0.125% of curdlan decreased BS by around 10% (sample flocculation), but unlike in products containing other β-glucans, phase separation or wheying off were not observed in this sample (visual assessment at the end of the measurement). This result was attributed to the hydration properties of curdlan, which suggests that unlike other soluble β-glucans, even small amounts of curdlan can be effectively used to produce fermented milks. The BS lines in milk gels containing 0.125% of OBG and scleroglucan were analysed to evaluate the products' stability. The bulging shape of BS lines indicated flocculation changes, which was a local aggregation of caseins and uneven incorporation of whey proteins into the network. The obtained results are consistent with those presented in the studies of Lucey et al. (1998) and Rohard, Michon, Confiac, & Bosc, (2016) which also showed that the formed aggregates caused local strain and local cracks in the protein network, which led to local syneresis along the vat wall that was not visible in the top part of the sample.

3.4. The influence of β-glucans on the microstructure of acidified milk gels

The CLSM images present samples after 5 min (counting from the addition of GDL to milk) and 105 min (when the pH of the control sample reached 4.6) of acidification.

Confocal microscopy supported observations of the influence of

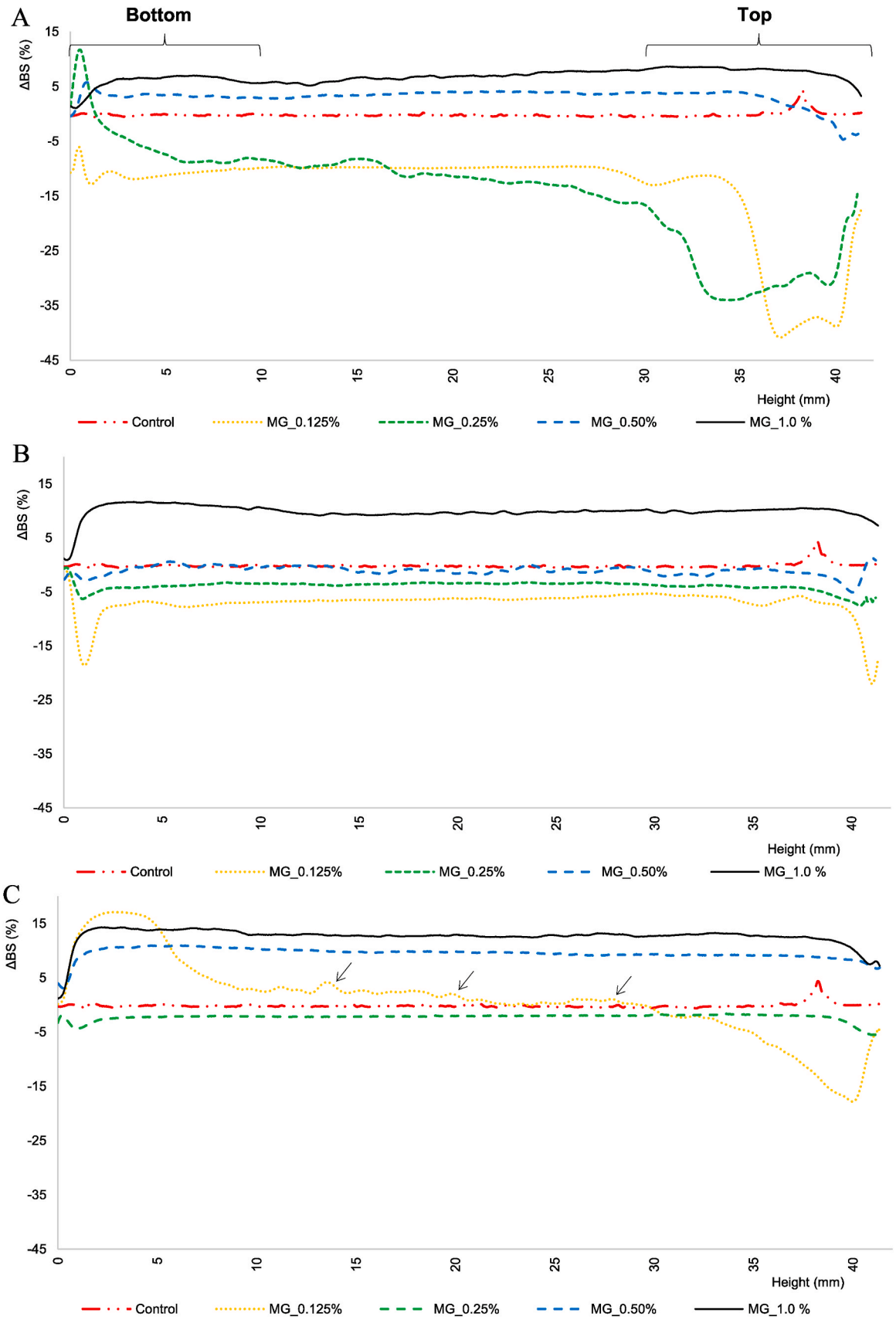

Fig. 3. The backscattered intensity (%) of milk gels with different addition level (0.125%; 0.25%; 0.5% and 1%) of β-glucans preparation (A: oat β-glucan; B: curdlan; C: scleroglucan) and after 105 min of acidification. Control milk gel (without β-glucan); The arrow in the graph indicates the breaking point of the structure. The horizontal axis is height along the tube.

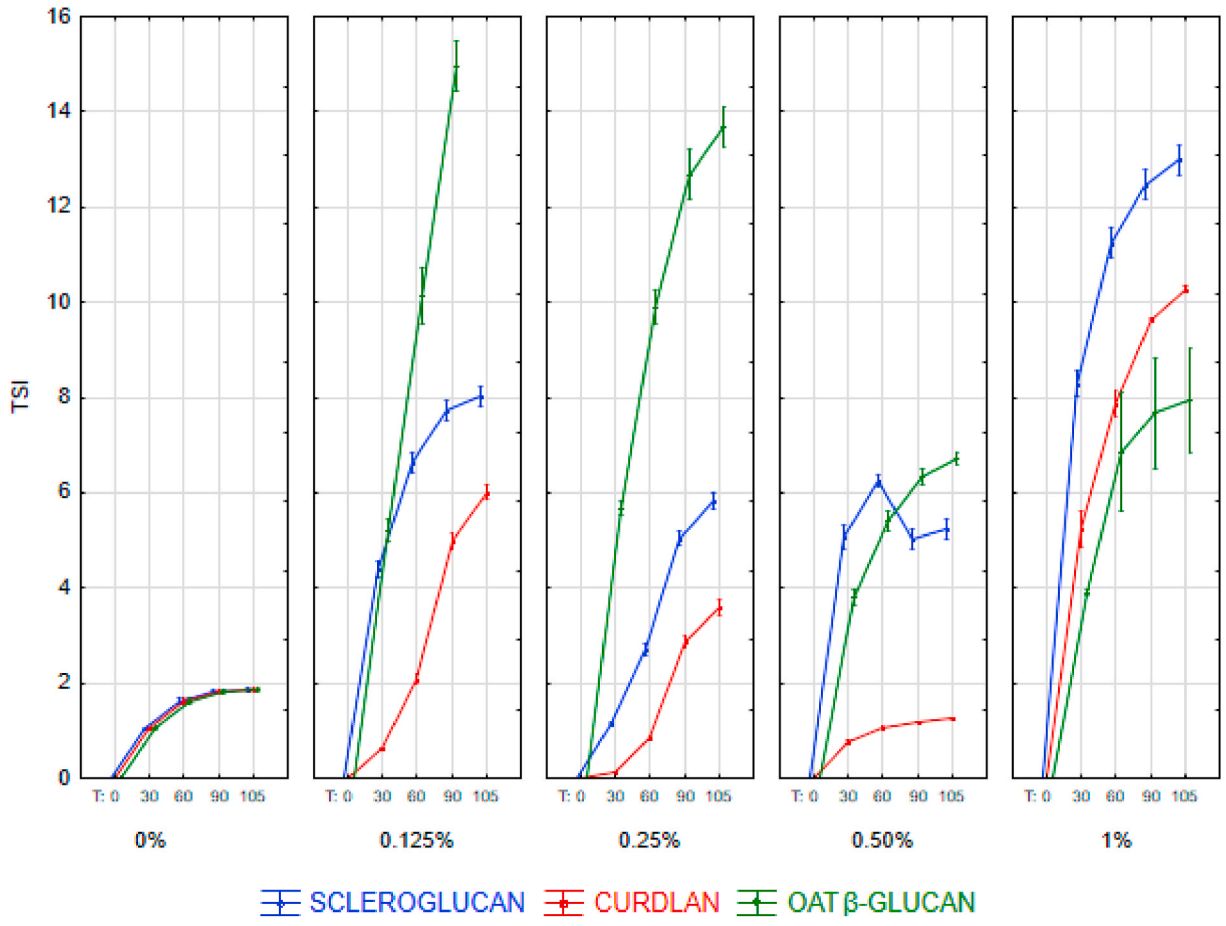

Fig. 4. Turbiscan Stability Index (TSI) of milk gels with different addition level (0.125%; 0.25%; 0.5% and 1%) of different β-glucans preparation (oat β-glucan; curdlan; scleroglucan) during acidification. 0% - control milk gel (without β-glucan preparation); Bars are represented standard deviation of the mean.

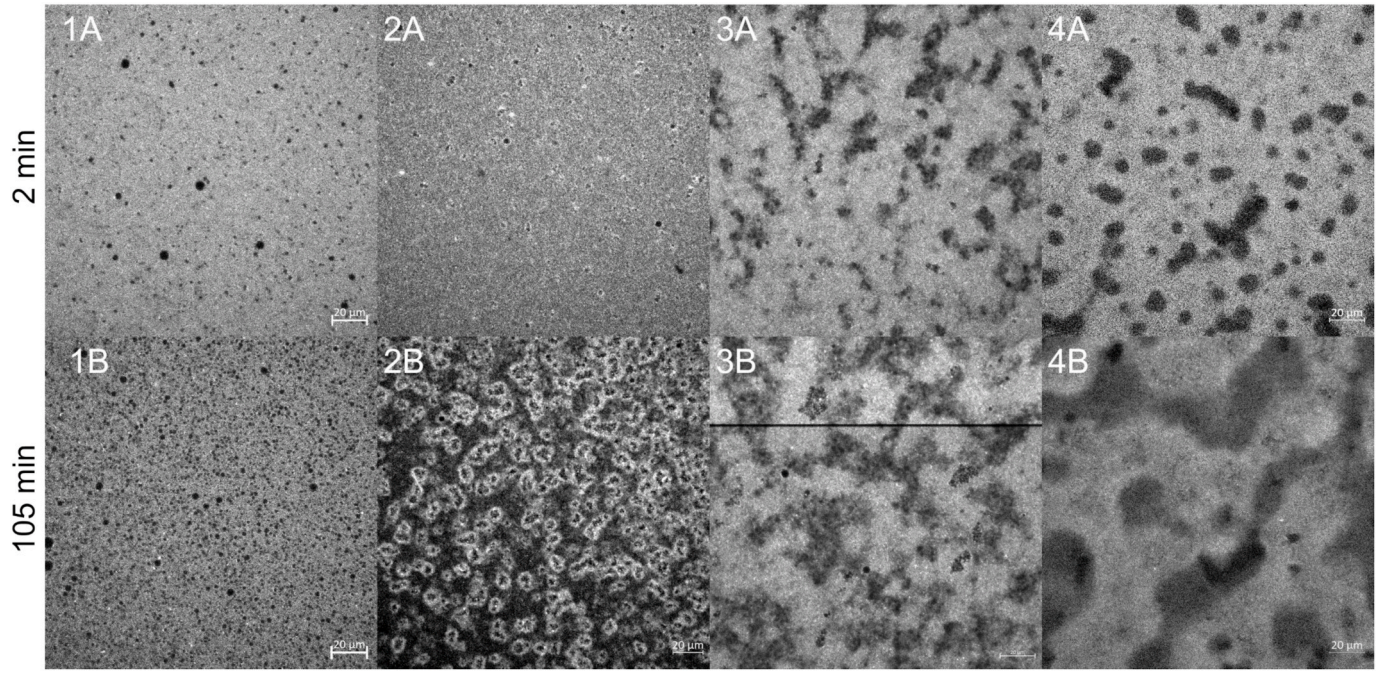

Fig. 5. Microstructure of acidified milk gels prepared only from milk (no 1) and with 0.125% addition of β-glucan (no 2: oat β-glucan; no 3: curdlan; no 4: scleroglucan) incubated at 42 °C. Confocal scanning laser micrographs were examined at the gelation time: 2 (A) and 105 min (B). Scale bar = 20 μm. Image size: 191.48 × 191.48 µm (4096 × 4096 pixels); The protein matrix is white, whereas mix of β-glucan and whey are dark.

various β-glucan preparations on the formation of protein-rich complexes (light areas) whose size and shape were highly correlated with the type of the applied β-glucan (black areas). The presence of characteristic networks which are characteristic of acidified milk gels, and similar observations were made by Guggisberg et al. (2009). The morphology of the experimental hydrogels was altered by acidification which decreased pH to 4.6–4.7. Curdlan contributed to the formation of a protein network which, despite higher porosity, was most similar to the structure of the control gel (Fig. 5 (3 A-B)). According to Wei et al. (2018), β-glucan can interact with proteins to prevent their uncontrolled aggregation and phase separation. Unlike curdlan, moderately soluble OBG induced the formation of small (10–20 μm), but numerous protein aggregates (Fig. 5 (2 B)). The important point here is that at this concentration, the OBG has dispersed the protein network into particulate structures, which can then sediment as shown by reduced BS at the top of the tube in Fig. 3A. In turn, the addition of readily soluble scleroglucan produced significantly larger (>20 μm) and irregularly shaped protein aggregates (Fig. 5 (4 B)). Similar results were also reported in studies examining guar gum (Tuinier, ten Grotenhuis, & De Kruif, 2000), xanthan (Hemar, Tamehana, Munro, & Singh, 2001), and xanthan and κ-carrageen (Li et al., 2019). Due to its neutral characteristics, OBG and, to a lesser extent, scleroglucan do not interact with milk proteins but can stimulate the agglomeration of casein micelles. The low compatibility of biopolymers increased the local concentration of β-glucan in each phase (black areas), depleted the protective layer surrounding casein micelles, and created an osmotic gradient that increased the attraction between micelles (Corredig et al., 2011; Galante, Boeris, Álvarez, & Risso, 2017). Unlike OBG and scleroglucan, inulin did not induce significant changes in yoghurt morphology in CLSM images (Guggisberg et al., 2009). These results indicate that polysaccharide structure, molecular weight, and the changes in polysaccharide molecules during hydration and thermal processing influence the morphology of gels during acidification.

4. Conclusions

In aqueous solutions, viscosity and susceptibility to shear thinning were lowest in solutions containing OBG and highest in hydrogels containing bacteria β-glucan (curdlan). At a shear rate of up to 0.3 s^{-1}, the hydrogel containing 0.125% of OBG displayed the behaviour of Newtonian fluids. The aqueous solutions behaved like pseudoplastic fluids at higher shear rates and higher β-glucan concentrations. Such a relationship was not observed in gels containing scleroglucan and curdlan. The study demonstrated that the type of β-glucan significantly affect the aggregation of milk proteins and the gelation of dispersions. Coagulation was most rapidly induced by scleroglucan, whereas the reverse was observed under the influence of OBG. The hydration properties of β-glucan exert a significant effect on the susceptibility of acidified milk gels to phase separation. The lowest concentration (0.125%) of scleroglucan and oats β-glucan led to significant negative changes in gel structure (increase in TSI values, formation of large and unstable biomolecule aggregates, and protein phase sedimentation) during coagulation. These changes proceeded most rapidly between 60 and 90 min of acidification when pH decreased below the pI of whey proteins and approximated the casein pI. No such adverse changes were observed in gels containing curdlan. The system's stability and the significance of the polysaccharide network increased only at higher polysaccharide concentrations when hydrogen bonds were formed.

The results of rheological, turbidimetric and microstructural analyses were highly consistent. The current findings indicate that changes in gel structure that are not visible to the naked eye can be detected by light scattering. These changes cannot be identified with standard rheological measurements, either. This study sheds new light on the factors and relationships influencing the rheological properties of acidified milk gels. The presented research has important practical implications, and the results can be used to develop production technology for a new dairy product.

The application of different types and concentrations of polysaccharides can enhance the functional properties and health benefits of fermented dairy products, thus increasing their consumer acceptance.

CRediT authorship contribution statement

Marek Aljewicz: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Visualization, Writing - original draft, Writing - review & editing, Funding acquisition. **Ana-Isabel Mulet-Cabero:** Resources, Methodology. **Peter J. Wilde:** Supervision, Writing - review & editing, Resources, Funding acquisition.

Declaration of competing interest

We declare that this work has not been published elsewhere and is not under consideration by another journal. The manuscript is approved by all authors to be submitted to Food Hydrocolloids. The authors have no conflicts of interest to declare.

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