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Gellan gum: a new member in the dysphagia thickener family

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Abstract

In this study, gellan gum (GG) (0.075-0.3 wt%) is proposed as a new dysphagia thickener and compared against commercial starch-based thickeners (modified starch with or without gums, 5 wt%) and xanthan gum (XG, 0.5-1.0 wt%) using apparent viscosity, oral tribology using polydimethylsiloxane (PDMS) ball-on-disc set up and ζ-potential measurements. The measurements were conducted in presence of artificial saliva containing mucin with or without α-amylase at 37 °C. Viscosity results suggested that the commercial starch-based thickeners behaved like water in orally relevant shear, largely associated with the hydrolysis of modified starch by α-amylase, while, XG and GG showed no responsiveness to α-amylase. In the case of oral tribology, artificial saliva containing mucin adsorbed to the PDMS surfaces reducing friction as compared to water. The increase in boundary friction coefficients in commercial starch-based thickeners was likely associated with α-amylase-induced hydrolysis, increasing the PDMS-PDMS asperity contacts. Interestingly, the tribological behaviour of XG and GG was dictated mainly by viscous lubrication. However, in simulated oral conditions, the increase in friction coefficients in presence of XG and GG was influenced by depletion of artificial saliva from the PDMS surfaces due to electrostatic interaction between the gums and mucin. A combination of rheological and tribological techniques in orally relevant conditions appears as a reliable approach to understand the potential of GG (0.3 wt%) to act as a dysphagia thickener that offers similar mechanical properties as XG (1.0 wt%) at a lower concentration. Extensional viscosity measurement of GG is needed to understand its applications in dysphagia management.

Keywords: Rheology, Oral tribology, Dysphagia, Thickener, Xanthan gum, Gellan gum.

Introduction

Oral processing is a dynamic and multi-scale deformation process that involves breakdown of food into small particles, mixing with bio-lubricant saliva, resulting in formation of a food bolus followed by swallowing through the oesophagus into the stomach [1-4]. For healthy individuals, this food bolus is swallowed by propelling the food bolus to the back of the oral cavity into the pharynx via the combination of tongue movements as well as a series of muscle contractions. Abnormality in neuromuscular sequences or motor power caused by aging, stroke, Alzheimer’s disease, Parkinson disease or cancer can lead to swallowing disorders, such as dysphagia, which is shown to affect 8% of the world’s population [5-8]. Patients affected by dysphagia have difficulty controlling the flow of food bolus or liquid through the pharynx resulting in severe complications to those affected, such as malnutrition and increased risks of aspiration, choking and pneumonia. Aspiration is the major risk of dysphagia that occurs when food residues accidentally enters the respiratory pipe blocking the airways causing coughing and possible infections [5, 6, 9, 10].

Several considerations have been taken into account to overcome dysphagia that mainly include increasing the cohesiveness of the bolus during the flow and slowing down the speed of bolus flow and reducing the stickiness of the food bolus formed against the oral tissues. Altering the mechanical and textural properties of the food or liquid *via* the use of thickeners, such as modified starches and gums [11, 12] or introducing textural complexity [13, 14] have been shown to increase the oral residence time, slow down the swallowing process, providing sufficient time for the airways of dysphagia patients to close. Thickened food and liquids have also been shown to provide more cohesion to the food bolus reducing the amount of food bolus debris left in the pharynx after swallowing. The rheological characterization of these thickened foods and fluids has been studied extensively, via viscosity and viscoelasticity as a function of time and shear rate, to allow an understanding of the effect of viscosity on the swallowing process [10-12, 15].

Modified starch has been most commonly studied as dysphagia thickeners in literature. Over the last few decades, there has been a shift away from starch towards gum-based thickeners in dysphagia research. The key mechanism of fluid viscosity enhancement in starch is that the granules composed of amylose and amylopectin can swell in presence of water, resulting in an increase of viscosity. On the other hand, gums create an entangled network that can hold water molecules in their mesh. Among gums, xanthan gum has received a lot of attention in the recent years in dysphagia research domain due to its high viscosity and shear-thinning properties [16, 17]. Xanthan gum is produced industrially by fermentation using the gram-negative bacterium *Xanthomonas campestris*. The hetero-polysaccharide consists of repeating glucose units, alpha-D-mannose with an acetyl group, beta-D-glucuronic acid, and beta-D-mannose linked to pyruvate resulting in a high molecular weight macromolecule. The large number of side chains results in the stability of xanthan gum towards pH, ionic strength and temperature, allowing xanthan gum to be used as a commercial dysphagia thickener, either by itself or in combination with other ingredients [9, 12, 18-21].

Similarly, gellan gum is a bacterial exopolysaccharide, prepared commercially by aerobic submerged fermentation from *Sphingomonas elodea*. Gellan gum is a linear tetrasaccharide, composed of 4)-L-rhamnopyranosyl-(α-1 to 3)-D-glucopyranosyl-(β-1 to 4)-D-glucuronopyranosyl-(β-1 to 4)-D-glucopyranosyl-(β-1 to 4) containing acetyl substituents on the 3-linked glucose. Gelation occurs upon cooling of a heat-treated gellan gum solutions under quiescent conditions through the aggregation of its double helices. Gellan gum has demonstrated excellent stability over a range of pH and temperature, allowing it to be used in a wide range of food products [22, 23]. However, to our knowledge, there has been no study that has examined the potential of gellan gum to act as a dysphagia thickener under physiologically relevant conditions.

It is also worth noting that previous studies examining the role of starch and gums in dysphagia thickeners have investigated the mechanical properties of thickened fluids under ambient conditions, without taking into consideration the potential effect that saliva might have on the behaviour of these thickened liquids under flow conditions. Due to the complex composition of saliva (ions, mucin, enzyme) it is highly likely that some sensitive thickeners could alter their mechanical properties when in contact of saliva. Additionally, viscosity alone might not allow a thorough understanding of the mechanical properties of a food product undergoing oral processing [2, 24-28]. Therefore, studying the lubrication properties of these thickeners, *via* oral tribology measurements which is currently gaining momentum in oral processing research, is required to provide understanding on the interaction of these thickeners with oral surfaces and therefore, indirect insights on the *stickiness* or *slipperiness* of different thickeners.

Therefore, the aim of this study was to compare the viscosity and oral lubrication properties of gellan gum in comparison to two commercial starch-based dysphagia thickeners and xanthan gum. The study was carried out under different conditions (*i.e.*, in absence or presence of artificial saliva containing mucin without or with α-amylase) at 37 °C to investigate the alteration (if any) of mechanical properties under orally relevant conditions.

2. Materials and method

2.1 Materials

Xanthan gum (XG) derived from *Xanthomonas campestris* was purchased from Sigma-Aldrich, Dorset, UK and high acyl gellan gum (GG) (KELCOGEL® LT100) was purchased from CP Kelco UK Ltd, Surrey, UK. Two commercial starch-based dysphagia thickeners were used based on a previous study [10], Nutilis® (NT) (Ingredients: maltodextrin, modified starch from wazy maize starch, E1442: hydroxypropyl distarch phosphate, tara gum, xanthan gum, guar gum) was purchased from Nutricia Ltd, UK and Resource® ThickenUp™ (MS) (Ingredients: modified starch from waxy maize starch, E1442: hydroxypropyl distarch phosphate) was purchased from Nestlé, Ltd, UK. Mucin from porcine stomach Type II and α-amylase from porcine pancreas Type VI-B (13 units mg^{-1}) were purchased from Sigma-Aldrich, Dorset, UK. The use of pancreatic amylase was based on a previous study on oral processing and flavour release [29]. All other chemicals used were of analytical grade and were purchased from Fisher Scientific, Leicestershire, UK unless otherwise specified. All dispersion were prepared with Milli-Q water having a resistivity of 18.2 MΩ.cm at 25ºC (Milli-Q apparatus, Millipore, Bedford, MA, USA). Smooth polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning, USA) tribo-couples *i.e.* ball (∅ 47 mm) and disc (∅ 19 mm – 4 mm thickness) with surface roughness of 50 nm were purchased from PCS instruments, London, UK.

2.2 Preparation of the thickener samples

Xanthan gum (XG) (0.5, 1.0 wt%) was dispersed into purified water and stirred at room temperature by magnetic stirrer for at least 24 h allowing the system to swell. Gellan gum (GG) (0.075, 0.15, 0.30 wt%) was dispersed in purified water and stirred for 5 min at room temperature by magnetic stirrer. The solution was then heat treated at 85 ºC for 30 min in a water bath. Subsequently, the gellan gum solutions were cooled to room temperature in a water bath for 2 h. Nutilis® (NT) (5.0 wt%) and Resource® ThickenUp™ (MS) (5.0 wt%) were dispersed into purified water and stirred for 2 h at room temperature by magnetic stirrer.

2.3 Preparation of artificial saliva formulation

‘Artificial saliva buffer’ used in this study composed of 0.16 g/L NaCl, 0.33 g/L NH_4NO_3, 0.64 g/L K_2HPO_4, 0.2 g/L KCl, 0.31 g/L $K_3C_6H_5O_7.H_2O$, 0.02 g/L $C_5H_3N_4O_3Na$, 0.2 g/L H_2NCONH_2, 0.15 g/L $C_3H_5O_3Na$ and pH was maintained at pH 6.8. [30]. Mucin from porcine stomach was added at a concentration of 3 wt% to simulate the viscosity of human saliva in some of the experiments. To understand the effect of enzymatic degradation (if any), α-amylase was added to achieve 75 U/mL in the final thickener + buffer solution based on a previous study [31] and has been referred as ‘artificial saliva’. Artificial saliva in the absence or presence of α-amylase was added to the different thickener solutions at 1:1 w/w ratio at 37 ºC and mixed for 2 h based on standardised static *in vitro* digestion method [32]. Freshly prepared artificial saliva containing mucin with or without α-amylase was used for all measurements

2.4 Apparent viscosity

Rheological characterization was performed using a Kinexus ultra rheometer under steady shear (Malvern Instruments Ltd, Worcestershire, UK). A parallel plate geometry system (50 mm, model: PU50 SC0006 SS) with a gap of 1 mm was used for all measurements. About 2.0 mL of thickener solution was placed onto the sample plate and sealed with the temperature cover to maintain the sample at specified temperature and prevent evaporation. Flow curves were obtained in absence or presence of artificial saliva buffer without or with α-amylase as a function of shear rate ranging from 0.01 to 1000 s^{-1} at 37 ºC. Data from the flow curves were fitted to a power-law fit *i.e.* Ostwald de Waele fit $\eta_a(\dot{\gamma}) = K\dot{\gamma}^{n-1}$), where η_a is the apparent viscosity, $\dot{\gamma}$ is the shear rate, K is the consistency index and n is the flow behaviour index. Linear regression analysis was applied to the data for each sample in order to calculate flow behaviour index and the consistency coefficient.

2.5 Zeta-potential

The ζ-potential of the different thickeners under different conditions (i.e., alone, in presence of artificial saliva and in presence of artificial saliva + α-amylase) was determined using a microelectrophoresis instrument (Zetasizer, Nano ZS series, Malvern Instruments, Worcestershire, UK). The different systems were diluted to 0.05 wt% biopolymer concentration using the respective buffers. It was then added to a folded capillary cell (Model DTS 1070, Malvern Instruments Ltd., Worcestershire, UK). The ζ-potential of the samples was measured ten times for each diluted sample.

2.6 Tribology

The tribological properties of the different thickener samples were measured using a Mini Traction Machine 2 (MTM2, PCS instruments, London UK) following the method from [24]. The surface used was hydrophobic with a water contact angle of 108° [26]. A normal load of 2 N was used in all tests. The slide-to-roll ratio was set at 50%, and the sliding speeds were varied from 2000 to 1 mm s^{-1} in all tests. The coefficient of friction as a function of entrainment speed was measured for all samples. The entrainment speed was calculated using equation (1):

$$U = \frac{1}{2}(U_1 + U_2) \quad (1)$$

where, U is the entrainment speed and U_1 and U_2 are the velocities of the two contacting surfaces (*i.e.* ball and disc). All test were carried out at 37 ºC ±1 ºC to mimic oral conditions. It is noteworthy that using this PDMS tribological set-up with a load of 2 N, the maximum contact Hertizian pressure is ~ 200 kPa [28], which is about an order of magnitude higher than that measured in oral-palate contact of healthy adults (30-50 kPa) [33, 34].

2.7 Statistical analysis

Mean and standard deviation for each sample were calculated from at least triplicate measurements on three individual test samples ($n = 3 \times 3$). Significant differences between samples were determined by one way ANOVA and multiple comparison test with Tukey's adjustment was performed using SPSS software (IBM, SPSS statistics, version 24) and the confidence level was 95%.

3. Results and discussion

3.1 Apparent viscosity

Figure 1 illustrates the visual appearance of the different thickeners examined in this study. Although this is qualitative, visual perception of samples provides an important indirect indication of flow properties of the samples and is a method that has been used by the dysphagia community [35]. In the powdered form, the commercial thickeners (MS and NT) showed a grainier texture and whiter aspect as compared to both the GG and XG. Dispersion of the commercial thickeners in artificial saliva buffer occurred in less than two minutes, although a particulate structure could be observed in the MS sample. In comparison, lumps of non-dispersed XG can be observed after two minutes of dissolution whilst GG was easily dispersed (**Figure 1**). After 10 minutes of hydration, all samples were evenly dispersed and had a white opaque appearance. Different flow behaviour could be observed visually by tilting the test tubes containing the different samples. The GG, XG and NT did not flow on slight tilting suggesting a high viscosity on a qualitative basis and perhaps the formation of a weak gel-like matrix. In comparison, MS had somewhat liquid-like flow behaviour. Upon dilution with artificial saliva + α-amylase, the appearance of the starch-based samples showed considerable alterations. The MS became translucent and appeared to flow easily when tilted indicating some interactions with the artificial saliva containing α-amylase. Interestingly, the appearance of NT was barely affected *i.e.*, the system remained still opaque on dilution, however it appeared to flow rather easily. The appearance and observable flow of GG and XG appeared to remain unaffected by the dilution with artificial saliva containing α-amylase.

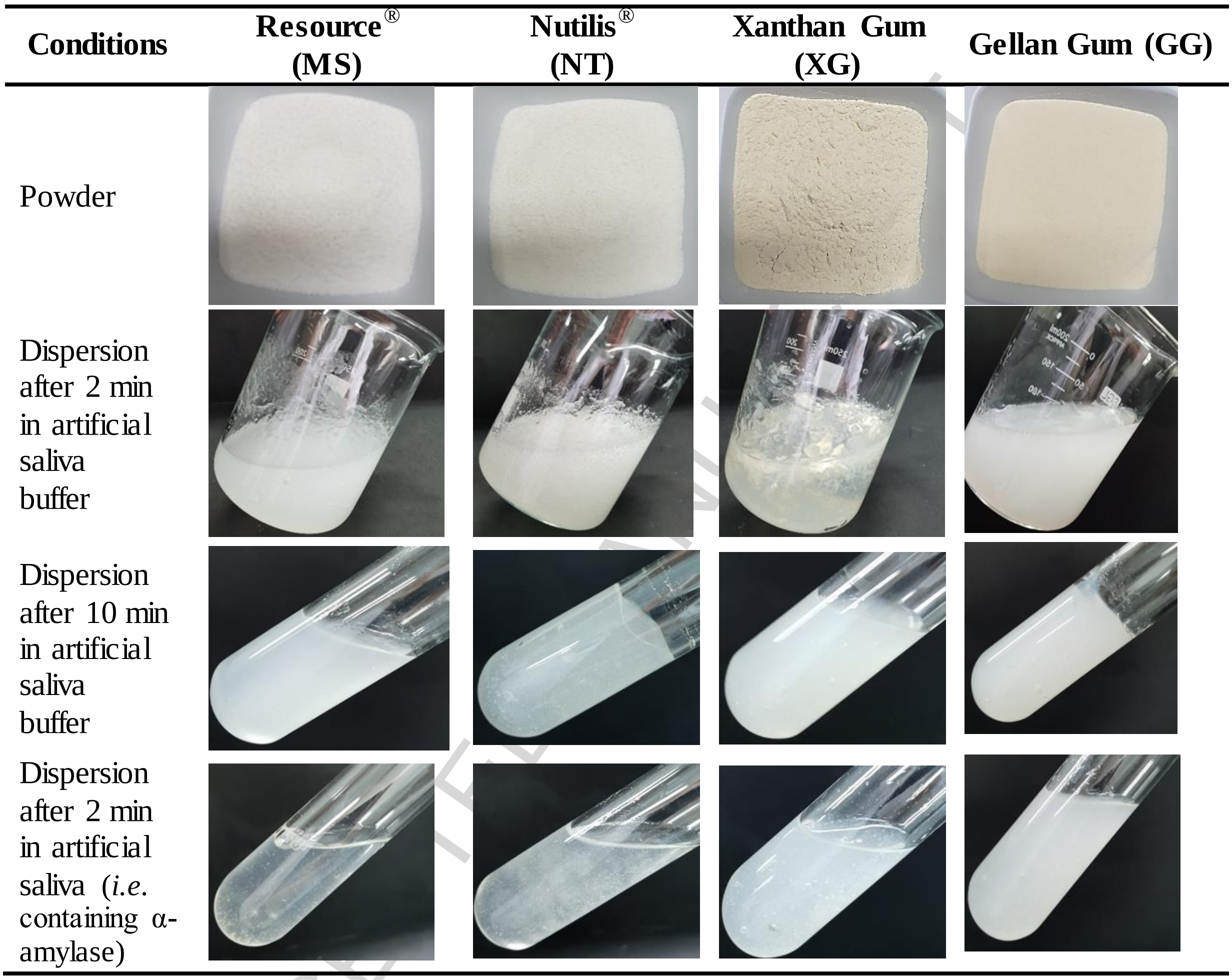

Figure 1. Visual images of the different thickener samples in powdered form and after dissolution in artificial saliva buffer or artificial saliva (*i.e.* containing α-amylase), respectively.

To assess the behaviour of the different samples under simulated oral processing conditions in absence or presence of α-amylase, their apparent viscosities were analysed. **Figure 2** shows the behaviour of the 5 wt% commercial dysphagia thickeners (MS and NT) and 0.5-1.0 wt% xanthan gum. In absence of artificial saliva or the buffer, both the starch-based commercial thickener samples demonstrated shear thinning behaviour (**Figure 2Ai**). Apparent viscosity was examined over a large shear rate range, although the discussion will be focusing on a range of shear rate from 1 to 100 s^{-1} corresponding to the orally relevant shear rate range established in recent studies [6, 36]. All the commercial starch-based thickeners showed typical pseudoplastic behaviour as their apparent viscosity decreased with an increase in shear rate (**Figure 2Ai**). The MS system had a lower viscosity as compared to NT. For instance, at 1 s^{-1} the viscosity NT (11.1 Pa s) was four-times higher than that of MS (2.6 Pa s) (see **Supplementary Table 1** for statistical analysis of apparent viscosities at shear rates of 1 and 100 s^{-1} shear rates for all samples). The flow curves of the starch-based thickeners is in accordance with previous studies [10, 12]. In fact neither of MS or NT showed a low shear rate Newtonian plateau, which has been also observed in literature [15].

From a compositional viewpoint, the key difference between the commercial thickeners (*i.e.*, NT and MS) is that MS is only composed of modified starch, whereas NT is composed of a mixture of the same modified starch and a range of polysaccharides, such as tara gum, xanthan gum and guar gum [10]. Even if the concentration of xanthan gum in NT is as low as 0.3%, it can lead to enhancement of mechanical properties of starch-gum system as compared to starch alone, when unperturbed or at extremely low shear rates [37]. In other words, in starch-gum systems, gums contributed to enhancement of viscosity as compared to those encountered in starch–water suspensions. It is noteworthy that gums in NT are thermodynamically incompatible with starch resulting in formation of a highly viscous phase-separated network [38] that disrupts as a function of shear. This explains the mechanism behind NT with higher shear-thinning properties as compared to starch network (MS), as observed in previous studies [10].

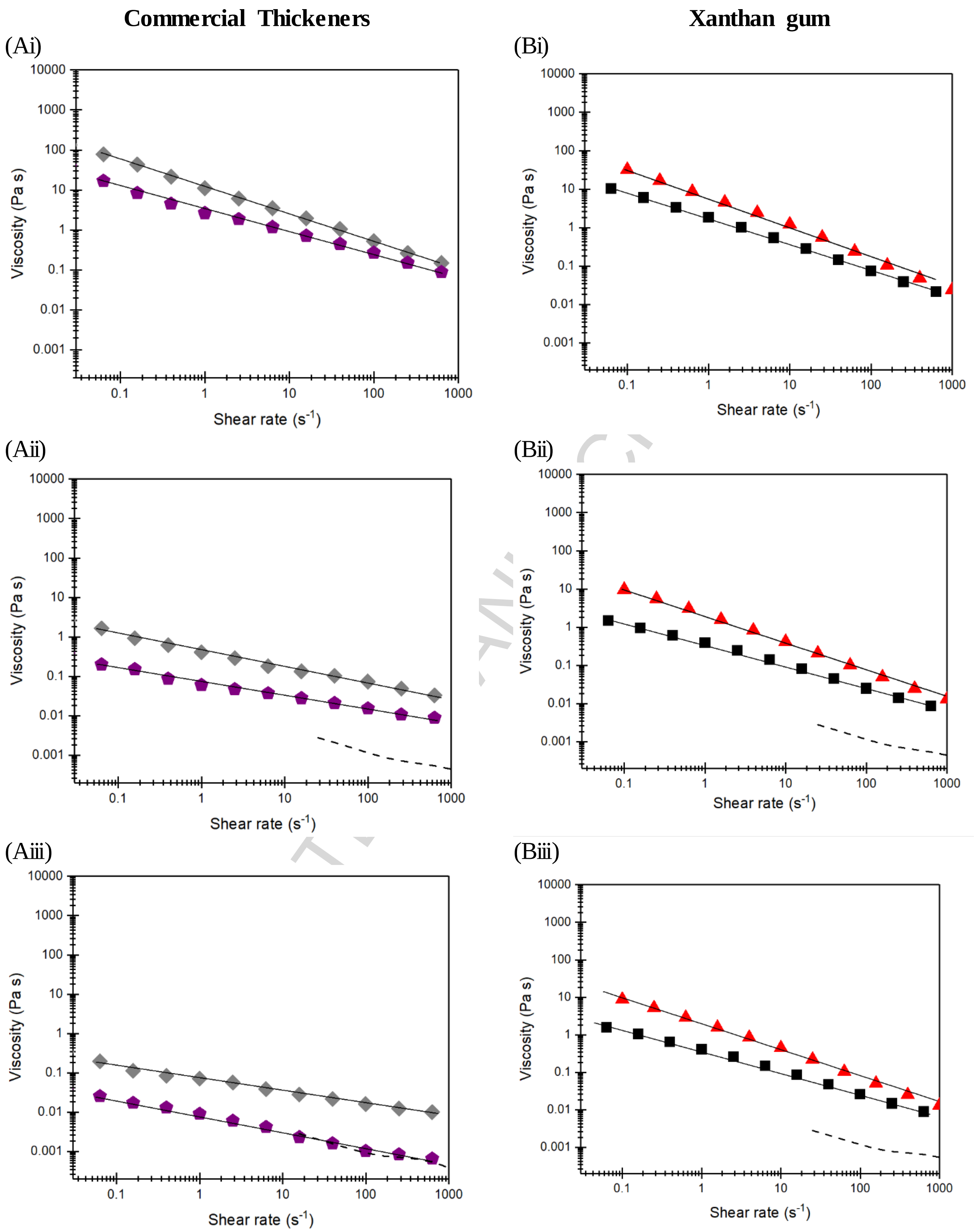

Figure 2. Flow curve of aqueous dispersions of different commercial thickeners *i.e.* Nutilis® (NT) ◆, Resource® (MS) ⬟ (A) and xanthan gum (XG) of different concentrations (0.5 wt% ■, 1.0 wt% ▲) (B) in absence of artificial saliva (i) at 37 °C, in presence of artificial saliva buffer (ii), in presence of artificial saliva (*i.e.* containing α-amylase) (iii), at 37 °C, respectively, dashed line are the control buffers *i.e.* artificial saliva buffer in (ii) and artificial

saliva containing α-amylase in (iii). Black solid lines are the best fits to the experimental data predicted using the Ostwald de Waele model.

Interestingly, mixing these commercial thickener dispersions with artificial saliva buffer (1:1 w/w ratio) resulted in an order of magnitude decrease in viscosity in both the commercial samples (**Figure 2Aii**). For instance at 100 s^{-1}, the viscosity of NT decreased by 85% (from 0.5 to 0.07 Pa s, $p < 0.05$), whilst the viscosity of MS decreased by 95% (from 0.3 to 0.02 Pa s, $p < 0.05$) in presence of artificial saliva buffer. The lesser changes in the viscous properties of NT might have resulted from the ability of the gums to interact to some extent with the artificial saliva buffer (*e.g.* mucins, ions). Noteworthy that both the samples had apparent viscosity about one-order of magnitude higher than the artificial saliva buffer (**Figure 2Aii**) at orally relevant shear rates (50-100 s^{-1}).

The addition of artificial saliva containing α-amylase to the commercial thickeners (1:1 w/w ratio) led to further liquefaction of the systems (**Figure 2Aiii**), which is congruent with the visual observation (**Figure 1**). The striking feature was that at shear rate above 50 s^{-1}, the viscosity of MS overlapped with the curve of artificial saliva ($p < 0.05$). In comparison, the viscosity of NT was still at least an order of magnitude higher than that of artificial saliva containing α-amylase ($p < 0.05$). Indeed, this same modified starch (*i.e.*, E1442: hydroxypropyl distarch phosphate) has been used in the formulation of both NT and MS. It is also worth noting that substitution of starch with hydroxypropyl phosphate has been demonstrated to delay enzymatic hydrolysis of starch by approximately 40% [39, 40]. However, such chemical substitution did not prevent the liquefaction of MS dispersion in presence of artificial saliva containing α-amylase under orally relevant shear rates in this study (**Figure 2Aiii**). Therefore, the main rheological differences between NT and MS might be attributed to the presence of gums in NT, preventing significant digestion of the starch by α-amylase. Such behaviour has been demonstrated previously where addition of guar gum to both normal and waxy starches led to a delayed hydrolysis by α-amylase [41].

Shifting our focus to the gums tested in the current study, XG (0.5 and 1.0 wt%) demonstrated a shear thinning behaviour (**Figure 2Bi**). Increasing the concentration of XG by twofold led to a 64% increase in viscosity at 1 s^{-1}, in accordance with previous studies [10, 25, 42]. The apparent viscosities of XG (0.5 and 1.0 wt%) were comparable to that of the commercial thickeners (NT and MS) (5 wt%) (**Figure 2Ai**) as corresponding shear rates ($p < 0.05$). Mixing of XG with artificial saliva buffer resulted in a 60% decrease in viscosity suggesting the formation of a more liquid system (**Figure 2Bii**). As expected, upon a 50% dilution with artificial saliva buffer, the flow curve of 1.0 wt% XG became similar to that of 0.5 wt% XG before dilution ($p > 0.05$). For instance, the apparent viscosities of 1.0 wt% XG + artificial saliva buffer and 0.5 wt% XG at 100 s^{-1} were 0.07 Pa s suggesting that the addition of salts, from artificial saliva buffer or mucin did not affect the bulk interactions between XG molecules significantly or lead to further gelation [19, 43]. The incorporation of artificial saliva containing α-amylase did not affect the apparent viscosity of XG, as expected from the visual observations (**Figures 1, 2Biii**) and the viscosity values were one-order of magnitude higher than artificial saliva at 50 s^{-1} shear rate in contrast to the behaviour of MS (**Figures 1, 2Aiii**). The viscosity of both the XG dispersions at 0.5 and 1.0 wt% concentrations with artificial saliva containing α-amylase were similar ($p > 0.05$) to that of XG gum + artificial saliva buffer, confirming no hydrolysis occurred in presence of artificial saliva as α-amylase can only hydrolyse α-(1→4) glycosidic bonds.

Similar to XG, GG (0.075, 0.15, 0.3 wt%) also demonstrated expected shear thinning behaviour irrespective of the conditions (**Figure 3**).

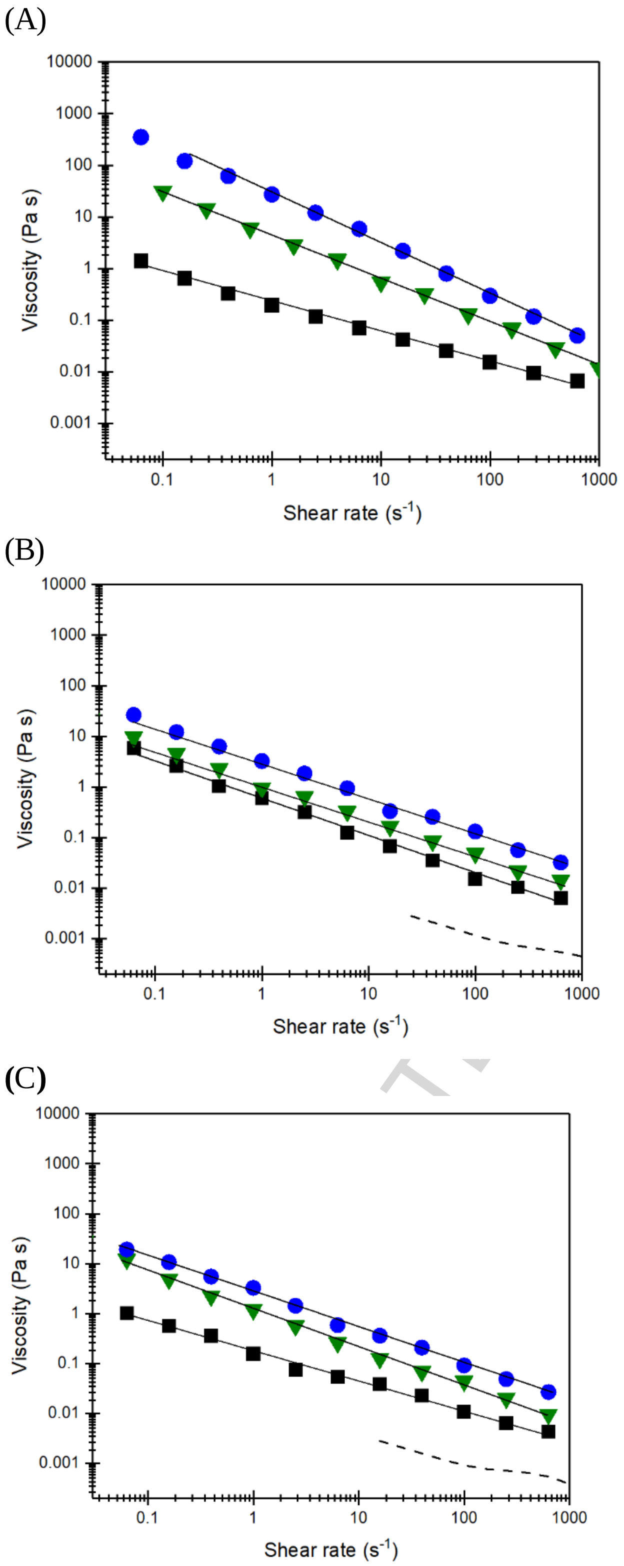

Figure 3. Flow curves of aqueous dispersions of gellan gum (GG) of different concentrations (0.075 wt% ■, 0.15 wt% ▼, 0.3 wt% ●) in absence of artificial saliva (A) at 37 °C in presence of artificial saliva buffer (B), in presence of artificial saliva (*i.e.* containing α-amylase) (C), at 37 °C, respectively, dashed line are the control buffers *i.e.* artificial saliva buffer in (B) and artificial saliva (*i.e.* containing α-amylase) in (C). Black solid lines are the best fits to the experimental data predicted using the Ostwald de Waele model.

The viscosity increased with an increasing concentration of GG (**Figure 3A**). This can be attributed to the increased the amount of double helical junction zones formed in presence of increased concentration of GG, resulting in the formation of a weak gel-like network [22]. At higher shear rates (> 100 s^{-1}), the rate of forced molecular disentanglements in GG might have been higher than the rate at which new entanglements of polymer chains were formed [44]. The disentangled polymer chains aligned in the direction of flow, thereby reducing the apparent viscosity. At low concentrations (0.075 and 0.15 wt%), the apparent viscosity of GG at 1 s^{-1} was similar ($p > 0.05$) to those of MS (5 wt%). However, at high concentration (0.3 wt%), GG had a significantly larger viscosity (η = 27.2 Pa s at 1 s^{-1}) as compared to both the commercial thickeners (5 wt%) (**Figure 3A**). The long gellan gum molecules led to the formation of a more shear-thinning system as compared to the commercial thickeners [23].

Interestingly, the apparent viscosities of 0.075 wt% GG at shear rates ranging from 1 to 100 s^{-1} was not affected by dilution with artificial saliva buffer ($p > 0.05$), this suggests that GG might have interacted with the mucins and/ions in the buffer and overshadowed the dilution effect (**Figure 3B**). However, at higher concentrations (0.15 and 0.3 wt%), the viscosity of GG significantly decreased. All the GG samples had one-to-two orders of magnitude higher viscosities than that of artificial saliva buffer at orally relevant shear rates, as also observed in XG (**Figure 2Bii**). Considering that α-amylase only hydrolyses α-(1→4) glycosidic bonds [45, 46] and not β-(1→4) glycosidic bonds that are present in GG, the curves of GG in presence of artificial saliva containing α-amylase should not vary as compared to those in presence of the artificial saliva buffer. Such similarity in presence (**Figure 3C**) and absence (**Figure 3B**) of α-amylase was indeed noticed in 0.3 wt% GG ($p >$ 0.05), the behaviour was also similar to that observed in XG (**Figure 2Biii**). This is an important finding that GG maintained a relatively high viscosity even in presence of saliva containing ions, mucin and α-amylase at orally relevant shear rates, which has previously never been reported in literature.

All the data obtained using shear-sweep measurement for the commercial starch-based thickeners, XG and GG (**Figures 2 and 3**) were satisfactorily fitted to the Ostwald de Waele model (R^2 ranging from 0.984-0.998) **(Table 1**).

Table 1. Consistency index (*K*) and flow behaviour index (*n*) of commercial thickeners (NT and MS), gellan gum (GG) and xanthan gum (XG) in absence of artificial saliva, in presence of artificial saliva buffer or in artificial saliva containing α-amylase, at 37 °C, respectively.

Thickeners	**wt%**	**Conditions**	**Ostwald de Waele fit** $\eta_a(\dot{\gamma}) = K\dot{\gamma}^{n-1}$	
			***K* (Pa s^n)**	***n***
Resource® (MS)	5.0	Alone	3.41	0.44
		+ artificial saliva buffer	0.06	0.59
		+ artificial saliva (α-amylase)	0.006	0.63
Nutilis® (NT)		Alone	11.74	0.32
		+ artificial saliva buffer	0.50	0.57
		+ artificial saliva (α-amylase)	0.05	0.73
Xanthan Gum (XG)	0.5	Alone	1.44	0.31
		+ artificial saliva buffer	0.33	0.39
		+ α-amylase	0.34	0.41
	1.0	Alone	7.55	0.17
		+ artificial saliva buffer	2.15	0.26
		+ artificial saliva (α-amylase)	2.14	0.26
Gellan Gum (GG)	0.075	Alone	0.15	0.54
		+ artificial saliva buffer	0.53	0.31
		+ artificial saliva (α-amylase)	0.19	0.44
	0.15	Alone	8.25	0.17
		+ artificial saliva buffer	1.86	0.22
		+ artificial saliva (α-amylase)	1.48	0.24
	0.30	Alone	16.59	0.14
		+ artificial saliva buffer	2.69	0.29
		+ artificial saliva (α-amylase)	2.86	0.28

From **Table 1**, it can be observed that all the commercial thickeners and gum dispersions possessed shear thinning behaviour as $n < 1$. Commercial dysphagia thickeners formed a network-like architecture mainly due to swollen and fragmented starch granules (MS) or a phase separated network with gums (NT) [10] with high *K* values (**Table 1**). Under oral processing conditions, hydrolysis by α-amylase resulted in one-order of magnitude decrease in *K* and a subsequent increase in the *n* (>0.6). These values of *K* and *n* are comparable to those observed in a previous study done with starches digested by pancreatic amylases [41].

The value of *n* represents the degree of shear thinning flow behaviour of gum dispersion as it deviates from 1. It is an indication of an increase in gum dispersion's pseudoplastic behaviour at higher concentrations. The values of *n* for GG at 0.15 wt% and 0.3 wt% were comparable to that of XG at 0.5 wt% and 1.0 wt% respectively in absence or presence of artificial saliva buffer or artificial saliva containing α-amylase (**Table 1**). The *K* is a measure of the dispersion viscosity taking into account its deviation from Newtonian flow behaviour. Even upon dilution with artificial saliva buffer, values of *K* remained were higher than 1.5 for XG (1.0 wt%) and GG (0.15, 0.3 wt%) due to biopolymer entanglement and water binding capacity, unlike the behaviour of the commercial starch-based thickeners (MS and NT). Interestingly, at low GG concentration (0.075 wt%), the increase in the *K* value and corresponding decrease in *n* upon dilution with artificial saliva buffer suggest the occurrence of further entanglement in GG polymer chains. The presence of salts in artificial saliva buffer might have increased the association between the double helices of the GG, where calcium bridges can form with glucuronic acid residues, increasing the number of polymerization points [47, 48], thus overshadowing the dilution effect caused by the buffer.

Both the gums, GG and XG maintained a very high *K* and low *n* values under oral processing conditions that did not change in presence of α-amylase. The absence of liquefaction, a constant shear thinning behaviour and a controlled consistency under simulated oral processing conditions in presence of artificial saliva (ions, mucins and α-amylase) shows potential for GG to be considered as a candidate thickener for impaired swallowing management as compared to that of commercial starch-based thickeners. Furthermore, GG shows similar flow behaviour to XG at a significantly lower concentration under simulated oral conditions.

3.2 ζ-potential

To understand possible electrostatic interaction (if any) of the thickeners with artificial saliva from a charge perspective, ζ-potential measurements were carried out in the samples in absence or presence of artificial saliva buffer and artificial saliva containing α-amylase (**Figure 4**). The controls *i.e.* artificial saliva with and without the presence of α-amylase showed a ζ-potential value of -10 mV, which can be attributed to the negatively charged sialic acid and sulphated residues in the mucin in neutral pH [30]. Interestingly, MS also showed a negative charge owing to the presence of hydroxypropyl groups adding hydrogen bonds to the amylopectin molecules of the waxy maize starch [49]. The MS showed a decline in its net negative charge in presence of artificial saliva buffer, which might be attributed to the ion-induced charge screening effects [30, 50, 51]. Therefore, the decrease in the ζ-potential of MS corroborates with the fact that upon addition of artificial saliva, a less cohesive system might be obtained resulting in a decrease of viscosity [20].

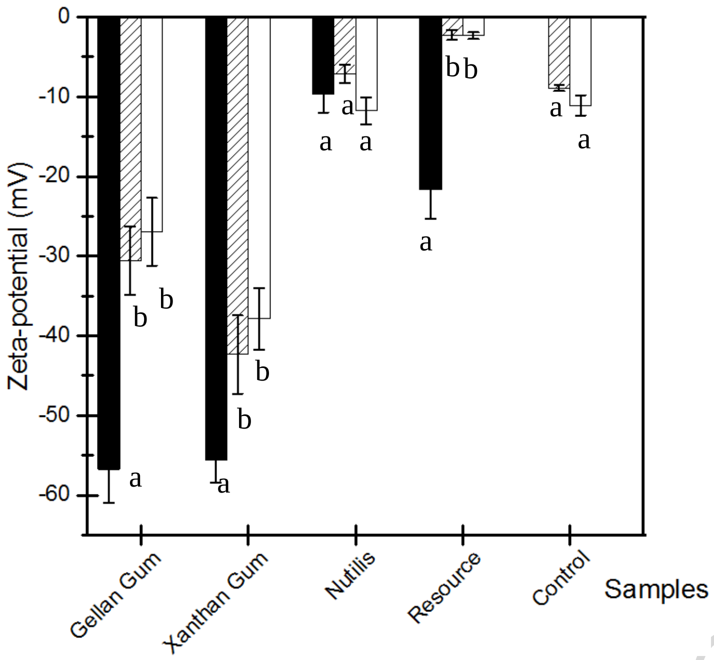

Figure 4. Mean ζ-potential of the different thickener samples in absence of artificial saliva (black), in presence of artificial saliva buffer (dashed) and in presence of artificial saliva (*i.e.* containing α-amylase) (white), respectively. Error bars represent standard deviation. Means at each conditions for a given thickener not sharing the same letter are significantly different ($p < 0.05$) according to Tukey test.

The NT was not affected by the addition of salts as it is composed of a mixture of starch and gums. Under ambient condition, GG and XG had a high negative surface charge at the shear plane (**Figure 4**), in accordance with previous studies [18, 52]. The addition of salts (*i.e.,* calcium ions) from the artificial saliva (pH 7) decreased the net negative ζ-potential of XG and GG, possibly due to ion-binding or electrostatic charge screening effects as well as possible interactions with mucin, which is discussed later. There was no change in the ζ-potential of XG and GG in presence of α-amylase as compared to the corresponding systems in the buffer ($p > 0.05$). Nevertheless, XG and GG still had considerably higher surface charge as compared to MS under the same conditions ($p < 0.05$), highlighting better dispersibility in the presence of artificial saliva as compared to the starches.

3.2 Oral tribology

Oral processing is a highly dynamic process, where in the early stages the bulk properties, such as apparent viscosity of the food product may dominate [27]. Whilst in the later stages, surface properties, such as thin film lubrication properties of soft tissues (*i.e.*, tongue and palate or oesophagus lining) by the broken food products and food-saliva mixture may become dominant affecting mouthfeel perception [1, 2, 27, 28, 53-55]. To date, most literature in the dysphagia research domain have focused on rheology [56]. However, understanding the surface interactions of thickeners with oral tissues and therefore, the friction performance of the food product under oral processing has attracted relatively little research attention [57-59]. Hence, the study of the lubrication properties of these different systems (commercial thickeners, XG and GG) *via* oral tribology measurements, under different conditions was of great interest to further understand and compare their lubrication properties. The tribological properties of the different systems were investigated at $1 < \overline{U} <$ 2000 mm s^{-1}, with a clear emphasis on the boundary and mixed regimes, which are more relevant to oral processing conditions, considering that the average speed of a human tongue is ~ 20 mm s^{-1} [60].

Figure 5 shows the friction coefficients of the commercial thickeners and XG with or without the addition of artificial saliva. In absence of salivary buffer (**Figure 5Ai**), the friction coefficients of the commercial thickeners followed the same behaviour ($p > 0.05$, see **Supplementary Table 2** for statistical analysis of coefficients of friction at entrainment speeds of at 3 and 50 mm/s shear rates for all samples). The contacts exhibited the characteristics of working in two lubrication regimes (*i.e.*, mixed and hydrodynamic).

Commercial Thickeners **Xanthan gum**

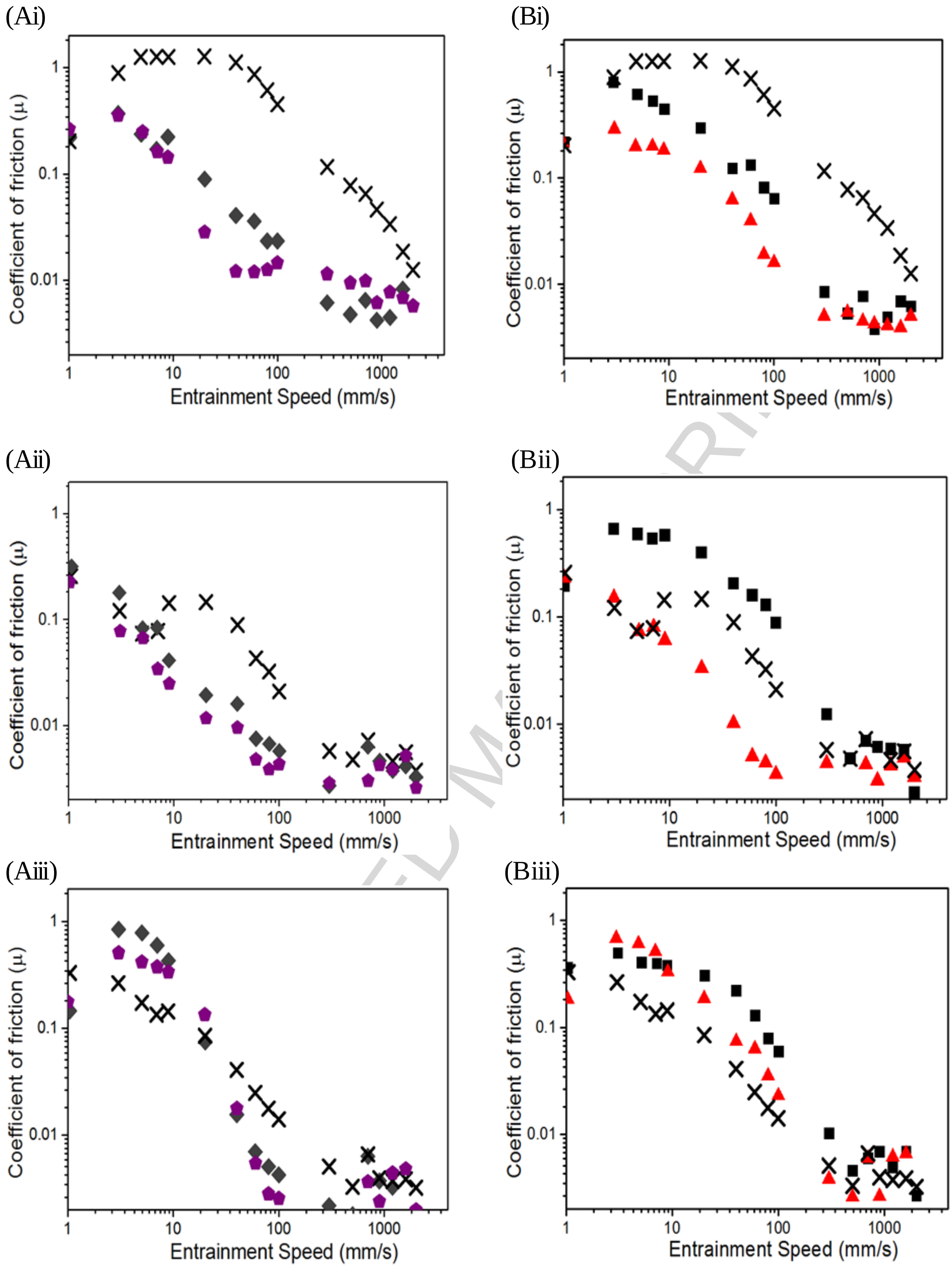

Figure 5. Coefficients of friction (µ) of aqueous dispersions of different commercial thickeners Nutilis® (NT) ◆, Resource® (MS) ⬟ (A) and xanthan gum (XG) (0.5 wt%:■, 1.0 wt%: ▲) (B) as a function of entrainment speed, in absence of artificial saliva (i) at 37 °C, in presence of artificial saliva buffer (ii), in presence of artificial saliva (*i.e.* containing α-amylase) (iii), at 37 °C, respectively, **X** are the control buffers *i.e.* water in (A), artificial saliva buffer in (B) and artificial saliva (*i.e.* containing α-amylase) in (C).

In comparison to water ($\mu = 1.0$), the commercial thickener samples ($\mu < 0.04$) had a significantly lower friction coefficient, at 50 mm s^{-1}, suggesting that as the entrainment speed increased the commercial thickeners entered into the contact area reducing the friction force. This confirms that water showed practically no lubrication performance at low entrainment speed ($\overline{U} < 100$ mm s^{-1}), as compared to that of commercial thickeners [26].

Mixing of the thickeners with artificial saliva buffer did not alter the lubrication trend of the NT or MS and no significant differences were observed with or without artificial saliva buffer (**Figure 5Aii**, $p > 0.05$). It is worth noting that artificial saliva buffer had an order of magnitude lower friction coefficient (at 50 mm s^{-1}, $\mu = 0.076$) as compared to water, which is in agreement with previous studies [24, 27]. This is largely associated with the lubricating properties of negatively charged mucin molecules present in the artificial saliva buffer (**Figure 4**) that adsorbed to the hydrophobic PDMS surfaces and allowed the formation of a hydrated layer. The macromolecule-mediated hydrated layer would have limited the PDMS-PDMS asperity contact under the low loads used in this study, consequently lowering the friction coefficients [27, 61]. Hence, it was expected that the addition of artificial saliva buffer to the thickeners would have contributed to increase their lubrication potential, however, this was not the case. Instead, we hypothesize that mucin or ions in the artificial saliva buffer might have interacted with the thickeners, as suggested by the reduction in ζ-potential (**Figure 4**) and thus were not available to adsorb efficiently to the PDMS surfaces. For instance, before mixing NT or MS to artificial saliva buffer, these had ζ-potential values of −9.7, −21.7 and −8.8 mV, respectively. And after the addition of artificial saliva buffer, the ζ-potential values of NT and MS altered to −7.2 and −2.3 mV, respectively. The near neutral ζ-potential values of MS in presence of artificial saliva buffer suggested that mucin molecules might not have sufficient net charge and limited the formation of a hydrated layer between PDMS surfaces. Hence, the reduction of the friction coefficient of NT or MS with added artificial saliva buffer was not observed. However, it should be noted that the mucin used in this study was from porcine gastric mucosa, which is different from the salivary mucins due to their biochemical nature. This biochemical difference might impact the interactions between mucin-surfaces and mucin-sample under real oral conditions [62].

The addition of artificial saliva containing α-amylase to MS and NT resulted in a significant increase in their friction coefficient at low entrainment speed ($\overline{U}$ < 10 mm s^{-1}), as compared to the systems with artificial saliva alone (**Figure 5Aiii**) ($p < 0.05$). The increase in μ values of the commercial thickeners might be due to the α-amylase-induced hydrolysis of the MT and NS. For instance, at 3 mm s^{-1}, NT + artificial saliva buffer had μ value of 0.18, whilst with artificial saliva containing α-amylase, μ increased to 0.84, which is similar to that of water. These results corroborates with the flow behaviour results (**Figure 2Aiii**), indicating that the modified starches used in both commercial thickeners (MS and NT) were hydrolysed to a certain extent reducing the viscosity as well as potentially squeezing the hydrolysed starches out of contact and consequently increasing the μ values (**Figure 5Aiii)**. The other possible mechanisms behind such increase in friction in MS and NT is potential *stickiness* of the disaccharides generated during this limited amylolytic process as reported by a previous study [63], however this needs further investigation in future.

The XG at low concentration (0.5 wt%) did not lubricate the contact area in the boundary regime (*i.e.*, the friction coefficient of 0.5 wt% XG was similar to water (**Figure 5Bi**). This suggest that XG was not entrained in the contact area at low speed, probably due to the low viscosity of the system as well as obvious lack of surface interactions with the hydrophobic PDMS tribo-pairs, resulting in xanthan gum potentially “slipping-off” the tribo-pairs. Increasing the concentration of XG to 1.0 wt% significantly reduced the friction coefficient below the values of water in the boundary region, which has been also reported previously [64]. In other words, by increasing the concentration and thus the viscosity of XG

(**Figure 2Bi**), μ of PDMS contacts can be decreased, thereby suggesting that the dominant mechanism to be most likely viscous lubrication. Noteworthy, the dilution of XG with artificial saliva buffer or artificial saliva containing α-amylase had no significant effect on the lubrication properties of XG (**Figures 5Bii and iii**).

Interestingly, for GG, a clear dependence of μ on polymer concentration of GG was observed in the boundary region (**Figure 6A**). For instance, at 3 mm s^{-1}, 0.15 wt% GG had an order of magnitude higher friction coefficient (μ = 0.29) as compared to that of 0.3 wt% (μ = 0.03). This is congruent with the 85% increase in the viscosity when increasing the concentration of GG from 0.15 wt% to 0.3 wt% (**Figure 3A**). However, at low concentration (0.075 wt%), μ was not significantly different in presence of GG as compared to that of water at low entrainment speeds (μ = 0.731). These results suggests that the lubrication mechanism provided by GG is of viscous origin similar to that observed in case of XG (**Figure 5Bi**), allowing the system to support the load *via* an increase in fluid pressure with increased viscosity [65], instead of any surface interactions between GG and the PDMS contacts. Hence, in terms of the lubrication performance, GG has comparable benefits in terms of increasing the viscosity at a much lower concentration (0.3 wt%) and thus reducing friction as compared to commercial starches (5 wt%) under oral processing conditions.

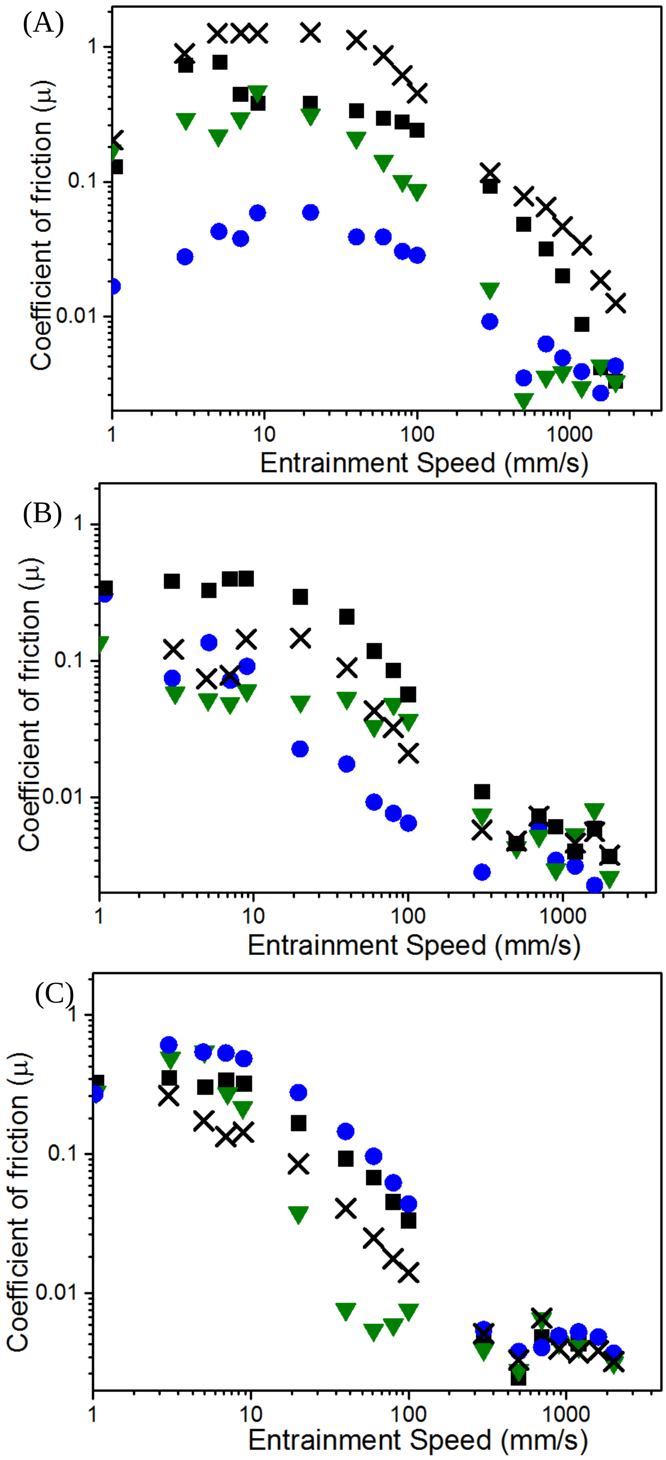

Figure 6. Coefficient of friction of aqueous dispersions of gellan gum (GG) (0.075 wt%:■, 0.15 wt%: ▼, 0.30 wt%: ●) as a function of entrainment speed, in absence of artificial saliva (A) at 37 °C, in presence of artificial saliva buffer (B), in presence of artificial saliva (*i.e.* containing α-amylase) (C), at 37 °C, respectively, **X** are the control buffers *i.e.* water in (A), artificial saliva buffer in (B) and artificial saliva (*i.e.* containing α-amylase) in (C).

Interestingly, the 1:1 w/w dilution of GG with artificial saliva buffer did significantly affect the lubrication behaviour of GG at higher concentration (0.3 wt%) ($p < 0.05$) whereas at other concentrations, the difference was rather subtle (**Figure 6B**). The viscosity of 0.3 wt% GG is decreased upon dilution with artificial saliva buffer (**Figure 3C**), which might play a key role in increasing the µ values. Also, in presence artificial saliva buffer containing mucin, mucin might have interacted with GG depleting the lubricious mucins from adsorbing to the hydrophobic PDMS contacts, which is congruent with reduction in net charge of GG in presence of artificial saliva buffer (**Figure 4**). Although the net surface charge of mucin in the pH tested in this study is negative (pH 7), it is worth noting that the neutral or the charged amino acids along the backbone of mucins are not uniformly distributed [66]. Thus, the interaction between GG and mucin at pH 7.0 may be predominantly electrostatic, involving patches of positive charge of arginine, histidine and lysine residues in the peptide backbone of mucin with negatively charged GG. Such electrostatic interactions of mucin with negatively charged polysaccharide is not specific to GG, but has been also reported in alginate [67, 68] or dextran sulphate [69].

One should note that besides interactions with mucin, In comparison to the commercial thickeners, the incorporation of α-amylase did not significantly affect the µ values of GG (**Figure 6C**) ($p > 0.05$) as compared to that in presence of artificial saliva buffer (**Figure 6B**). This is expected due to lack of responsiveness of GG to α-amylase and thereby not affecting the viscosity (**Figure 3C**) as discussed previously. Therefore, under different conditions, the lubrication properties of GG remained fairly constant except at 0.3 wt% GG.

It is evident from **Figures 5 and 6** that viscous lubrication played a dominant role in reducing the µ values of PDMS surfaces in most of the conditions. Hence, in order to understand the lubrication mechanisms of the different thickeners, µ values were plotted against the reduced viscosity, $U\eta_{eff}$, where η_{eff} is the effective viscosity of the different systems measured at 1000 s^{-1} (**Figure 7**) [70]. Interestingly the commercial thickeners (MS and NT) do not show a good collapse and this can be attributed to the estimation of viscosity. **Figure 2Ai** shows considerable shear thinning of both the curves (MS and NT) that continues at the highest shear rates explored. However, it is apparent that the high shear rate viscosities of these materials were very similar. Looking at **Figure 5Ai**, where friction is represented as function of entrainment speed, both MS and NT show very similar performance with lower μ values when compared to that of water. A faint decline at high speeds in the curve of NT is evidence of the hydrodynamic lubrication regime. The same dip can be observed in **Figure 7A**, suggesting that the viscosities used in the definition of the reduced viscosity are rather over estimations. In contrast to the behaviour of the thickeners, both the gums (XG and GG) show a good collapse, showing that the estimation of high shear rate viscosity plateau is reasonable and viscosity dominated the lubrication at least in the mixed lubrication regime [64] (**Figures 5Bi** and **6B**). Therefore, due to the large magnitude of apparent viscosity of the gums as compared to water (*i.e.*, approximately one order of magnitude at higher shear rates), these biopolymers were capable of supporting the load under entrainment speed reducing the μ values lower than that of water.

Under oral processing conditions (*i.e.*, in the presence of artificial saliva containing α-amylase), the lubrication behaviour of the saliva can be attributed to the mucin-adsorbed PDMS surfaces reducing asperity contacts. Interestingly, no collapse of the curves can be observed (**Figure 7B**). The trend of XG and GG were similar, suggesting that the lubrication of the gums under oral processing condition was influenced by the reduction of their viscosities, however, this was not the only effect. Interestingly, the lubrication behaviour of the gums were significantly different from the artificial saliva, preventing the surface-dominant lubrication mechanism of mucins to dominate in presence of the gums, which might be attributed to the electrostatic interactions between the gums and mucin (**Figure 4**).

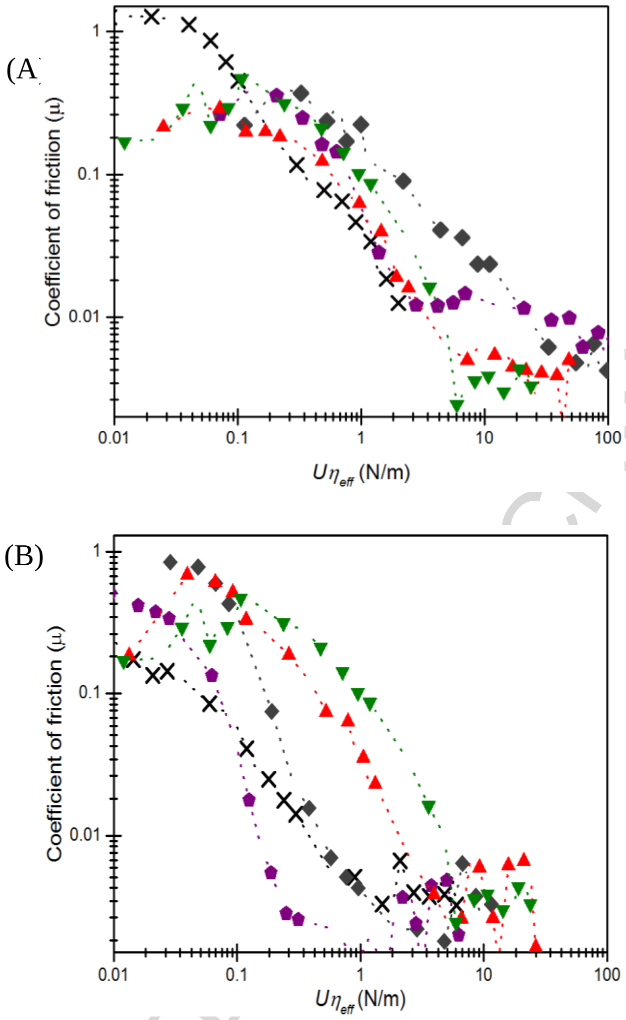

Figure 7. Coefficients of friction (µ) as a function of $U\eta_{eff}$ in absence of artificial saliva (A) and in presence of artificial saliva (*i.e.* containing α-mylase) (B) of commercial thickener Nutilis® (NT) ◆, Resource® (MS) ⬟, 1.0 wt% xanthan gum (XG) ▲, and 0.15 wt% gellan gum (GG) ▼. Water or artificial saliva (*i.e.* containing α-amylase) are represented by × in (A) and (B), respectively. The effective viscosity (η_{eff}) of each thickener was chosen at 1000 s^{-1}.

In the mixed lubrication regime, the commercial thickeners did not collapse with the gums but have very similar trends as that of artificial saliva containing α-amylase, resulting in a lower friction coefficient **(Figure 7B)**. This is particularly noticeable with MS (composed solely of modified starch), which has the lowest friction coefficient under the mixed lubrication, as compared to NT (mixture of gums and modified starch), which had values in between the ones of XG and MS. As mentioned previously, mucin might not be interacting with commercial modified starches (MS) possibly due to the hydrolysis of the latter (**Figure 4**). Therefore, the negatively charged mucin molecules might still be still available for adsorbing to the PDMS surfaces and thus, in case of thickeners, the artificial saliva appears to be mostly responsible for the lubrication properties of these thickeners.

4. Conclusions

This study examined apparent viscosity, ζ-potential and oral tribology measurements of commercial dysphagia thickeners, xanthan gum and gellan gum under oral processing conditions. Under simulated oral processing conditions, apparent viscosities of the commercial starch-based thickeners significantly reduced due to the hydrolysis by α-amylase. In comparison, the apparent viscosities of xanthan and gellan gums were unaffected by α-amylase. From a tribological perspective, the lubrication profile of all thickeners was found to be controlled mainly by their viscous component. Hence, samples with high viscosities (> 0.1 Pa s at 100 s^{-1} shear rates, *i.e.* one to two orders of magnitude higher than water) showed promising lubrication performances (*i.e.* $\mu \leq 0.07$ in the mixed regimes) as compared to water ($\mu \sim 1.0$ in the mixed regimes) due to their larger drag force to allow entrainment as compared to water. Under oral processing conditions, the hydrolysis of the commercial thickeners resulted in increasing the boundary friction, which might be attributed to the starch hydrolysis reducing the viscosity. Interestingly, in case of the xanthan and gellan gums, the increase in friction coefficients under simulated oral conditions was partly dictated by reduction in viscosity of due to dilution and partly by depletion of mucins from the PDMS surfaces by electrostatic interaction, thereby increasing μ to ≥ 0.6 in the boundary regimes. It is noteworthy that similar material properties of gellan gum (0.15-0.3 wt%) can be achieved at a significantly lower concentration as that of xanthan gum (0.5-1.0 wt%) highlighting the promise of gellan gum as a potential dysphagia thickener. Noteworthy that extensional viscosity analysis of gellan gum in comparison to starch-based dysphagia thickeners and xanthan gum was not conducted in this study. Hence, further studies on extensional viscosity measurement of gellan gum in simulated oral processing conditions and relationship of instrumental data with sensory measurements (*e.g.* thickness, cohesiveness, stickiness) are needed to warrant its applications in dysphagia management.

5. Acknowledgement

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Conflict of Interests

'Declarations of interest: none

6. References

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Highlights

- Commercial starch-based thickeners showed liquefaction in presence of α-amylase
- Viscosities of gellan gum (GG) and xanthan gum (XG) were unaffected by α-amylase
- No polymers adsorbed onto the hydrophobic polydimethylsiloxane (PDMS) surfaces
- Lubrication was controlled by the viscosity parameter and complexation with mucin
- Lower concentration of GG could achieve similar material properties as XG