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Published in:
Journal of Texture Studies

DOI:
[10.1111/jtxs.12264](https://doi.org/10.1111/jtxs.12264)

2017

Document Version:
Peer reviewed version (aka post-print)

[Link to publication](#)

Citation for published version (APA):
Waqas, M. Q., Wiklund, J., Altskär, A., Ekberg, O., & Stading, M. (2017). Shear and extensional rheology of commercial thickeners used for dysphagia management. *Journal of Texture Studies*, 48(6), 507-517.
<https://doi.org/10.1111/jtxs.12264>

Total number of authors:
5

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1 Shear and extensional rheology of commercial thickeners used for dysphagia management

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8

9 Keywords:

10 Dysphagia, thickeners, extensional viscosity, fluid elasticity, microstructure, velocity profile

11 Abstract

12

13 People who suffer from swallowing disorders, commonly referred to as dysphagia, are often
14 restricted to a texture-modified diet. In such a diet, the texture of the fluid is modified mainly
15 by the addition of gum or starch-based thickeners. For optimal modification of the texture,
16 tunable rheological parameters are shear viscosity, yield stress, and elasticity. In this work,
17 the flow properties of commercial thickeners obtained from major commercial suppliers were
18 measured both in shear and extensional flow using a laboratory viscometer and a newly
19 developed tube viscometry technique, termed Pulsed Ultrasound Velocimetry plus Pressure
20 Drop (PUV+PD). The two methods gave similar results, demonstrating that the PUV+PD
21 technique can be applied to study flow during the swallowing process in geometry similar to
22 that of the swallowing tract. The thickeners were characterized in relation to extensional
23 viscosity using the Hyperbolic Contraction Flow (HCF) method, with microscopy used as a
24 complementary method for visualization of the fluid structure. The gum-based thickeners had
25 significantly higher extensional viscosities than the starch-based thickeners. The rheological
26 behavior was manifested in the microstructure as a hydrocolloid network with dimensions in
27 the nanometer range for the gum-based thickeners. The starch-based thickeners displayed a
28 granular structure in the micrometer range. In addition, the commercial thickeners were
29 compared to model fluids (Boger, Newtonian and Shear-thinning) set to equal shear viscosity
30 at 50s^{-1} and it was demonstrated that their rheological behavior could be tuned between highly
31 elastic, extension-thickening to Newtonian.

32 Practical applications

33 Thickeners available for dysphagia management were characterized for extensional viscosity
34 to improve the understanding of these thickeners in large scale deformation. Extensional
35 deformation behavior was further explained by using microscopy as corresponding technique
36 for better understanding of structure/rheology relationship. Moreover the major challenge in
37 capturing human swallowing process is the short transit times of the bolus flow (<1 second).
38 Therefore the ultrasound based rheometry method; PUV-PD which measures the real-time
39 flow curve in $\sim 50\text{ms}$ was used in addition to classical shear rheometry. The two methods
40 complimented each other indicating that the PUV-PD method can be applied to study the
41 transient swallowing process which is part of our future research, where we are studying the
42 flow properties of fluids in an *in-vitro* swallowing tract.

1. Introduction

44 Dysphagia, which refers to swallowing disorders in general, has various causes, such as brain
45 damage, post-stroke complications, Parkinson's disease, and trauma (Clavé *et al.*, 2006).
46 Approximately 8% of the global population suffers from dysphagia (Steele, 2015). Dysphagia
47 is a growing concern in the developed world due to the aging population. Currently in Europe,
48 about 17% of the population is ≥ 65 years of age. This segment of society has increased by
49 28% during the past 10 years, as compared to the remainder of the population, which has
50 increased only 0.8%. It is estimated that in general, 30% of individuals who are ≥ 65 years of
51 age and 40% of persons who are registered at care facilities suffer from dysphagia (Ekberg *et*
52 *al.* (2002). Thus, there is an urgent need for research into new therapies for swallowing
53 disorders.

54

55 During normal swallowing, rapid transfer of food or drink from the mouth to the stomach
56 takes place without misdirection into the airways (Bülow, 2003). Dysphagics are challenged
57 by the fast and turbulent flow of liquids through their oropharynx (Cichero, 2013), and they
58 have been reported to aspirate when the velocity of water in the pharynx increases to 0.5 m/s
59 (Tashiro *et al.*, 2010). This makes texture modification an important consideration for
60 reducing the fast flow of fluid through the pharynx. A thickened diet is a common food-based
61 strategy to manage dysphagia. The underlying idea is that a viscous food bolus travels with
62 lower velocity, thereby providing more time for the oropharyngeal apparatus to close (Moret-
63 Tatay *et al.*, 2015; Steele, 2015; Tashiro *et al.*, 2010). However, highly viscous liquids
64 require the exertion of more force by the tongue to push the bolus through which may
65 increase the chances of post-swallow residues (Clavé *et al.*, 2006; Steele, 2015). A recent
66 White Paper published by the European Society for Swallowing Disorders suggests that more
67 rheological parameters should be investigated as part of the bolus modification strategy, such
68 as extensional viscosity and yield stress (Newman *et al.*, 2016). Thickener-based powders,
69 which can be either gum or starch-based, are available in the market (Mackley *et al.*, 2013).
70 These thickeners are added to liquids to thicken the texture and promote ease of swallowing
71 (Tatay *et al.*, 2015). Gum-based and starch-based thickeners differ in the way that they absorb
72 water. Starch-based thickener swells, while a gum-based thickener creates a network that
73 entraps water upon hydration. Gum-based thickeners are less susceptible to viscosity
74 modification during oral processing, whereas starch-based thickeners are modified by the
75 amylase enzyme in the saliva resulting in reduced viscosity (Leonard *et al.*, 2014).

76

77 Thickeners need to be classified to provide guidelines to healthcare professionals for the
78 preparation of foodstuffs with different consistencies. Different countries have developed
79 their scales for the consistency ranges of thickened products for treating dysphagia. The
80 protocol is set in the US by the National Dysphagia Diet (NDD), in Australia by the Dietitians
81 and Speech Pathology Associations, and in the UK by the British Dietetic Association (Popa
82 Nita *et al.*, 2013). The NDD guidelines, which are the most widely used, consider the shear
83 rate of 50 s^{-1} (as being relevant for swallowing) and a temperature of 25°C as reference.
84 Moreover, the NDD guidelines categorize the food products on the basis of apparent shear
85 viscosity at 50 s^{-1} on the range from as thin as water (1-50 cP) to the consistency of pudding

86 (>1750 cP). Alternative scales rely on subjective terms, such as nectar-like and pudding-like
87 and are not so popular (Quinchia *et al.*, 2011). The NDD scale has been criticized for not
88 considering the shear rate-dependence and the extensional properties of the food (Zargaraan *et*
89 *al.*, 2013), the latter referring to the ability of a material to resist extensional flow. Studies
90 have shown that a food bolus is subject to both shear and extensional flow during swallowing,
91 and also when the bolus is compressed between the tongue and the soft palate (Chen *et al.*,
92 2011; Hasegawa *et al.*, 2005; Salinas-Vázquez *et al.*, 2014). The swallowing literature refer to
93 this as cohesiveness of the bolus, and there is some confusion whether the mechanism is fluid
94 elasticity as expressed by the extensional viscosity or if there could also be an effect of the
95 yield stress. A cohesive food fluid has been concluded to resist disintegration during
96 swallowing reflux, thereby reducing the risk of post-swallowing residues (Chen *et al.*, 2011;
97 Ishihara *et al.*, 2011). However, unlike the shear response, the extensional rheology of the
98 food has been largely neglected (Chen, 2009). The main reason being the lack of appropriate
99 experimental techniques (Chen, 2009). A general challenge in extensional flow is to achieve a
100 steady state (Petrie, 2006), and the measured extensional viscosity is in reality always
101 transient. A measurement system such as the Hyperbolic Contraction Flow (HCF) technique,
102 has been developed which is suitable for medium-viscosity fluids (Stading *et al.*, 2001;
103 Wikström *et al.*, 1999a). This technique has been applied to various food systems such as
104 dough/dairy products (Andersson *et al.*, 2011), bread (Oom *et al.*, 2008) and ketchup (Berta *et*
105 *al.*, 2016) as well as to polymer melts (Köpplmayr *et al.*, 2016).

106
107 The technique utilizes a flow through a hyperbolic nozzle designed to have constant extension
108 rate. The calculation of the extensional viscosity from the measured force on the nozzle and
109 the given extension rate assumes a Power-law fluid (Debbaut & Crochet, 1988). The
110 contribution of shear is small for a shear-thinning fluid and is subtracted from the total
111 measured stress (Wikström *et al.*, 1999a). The HCF method gives the transient extensional
112 viscosity for a given extension rate at fixed Hencky strain. A precise determination of the
113 extensional flow in the hyperbolic nozzle requires comparative simulations, but the simplified
114 determination using the assumption of a Power-law fluid has proven to be good (Nyström *et*
115 *al.*, 2012). The method has also recently been validated against other methods for polymer
116 melt samples (Köpplmayr *et al.*, 2016).

117 The human pharynx has a complex geometry that ranges in shape from tubular to elliptical,
118 with dimensions 5–6 cm and 2.8–3.0 cm (Walsh *et al.*, 2008). Since swallowing is a dynamic
119 process, it is imperative to study it in real-time and in the context of a relevant geometry. In-
120 line Pulsed Ultrasound Velocimetry (PUV) combined with Pressure Drop (PD) is an advanced
121 version of tube viscometry. Originally developed to study human blood flow, this technique
122 has subsequently been applied successfully in many food applications (Reinhardt Kotzé *et al.*,
123 2013; Wiklund *et al.*, 2008). Examples of applications include: chocolate tempering (Dufour
124 *et al.*, 2007), dairy products/xanthan gum (Wiklund *et al.*, 2008); and improving the flow of
125 tomato ketchup (Dogan, 2002). An advantage as compared to rotational viscometers is the
126 real time determination of the flow curve which is measured approximately every 50 ms. In
127 the present study, we apply the technique for the first time towards characterizing the
128 thickeners used in dysphagia management, as well as the model fluids.

129 The main aim of this study was to characterize commercial thickeners for dysphagia
130 management for clinically relevant rheological parameters, such as shear viscosity,
131 extensional viscosity and yield stress. We further compared existing laboratory rheometry to
132 advanced tube PUV+PD viscometry, which mimics the nearly tubular geometry present in the
133 swallowing tract. The thickeners were also characterized for microstructural properties. The
134 results from the rheological analyses of the commercial thickeners were compared with model
135 fluids serving as a reference in the current study since they were standardized for elasticity
136 and viscosity. The present study is the first in a series aimed at the construction of a
137 laboratory-based human swallowing tract that could be used to study the flow properties of
138 model fluids, dysphagia products, and general foodstuffs in a pharyngeal swallowing
139 geometry using the PUV+PD method.

140 **2. Materials and Methods**

141 **2.1 Materials**

142 Five commercially available thickener products designed for patients with dysphagia were
143 kindly provided by the suppliers: Nutilis powder from Nutricia Nordic AB, Stockholm,
144 Sweden (denoted herein as Nutilis); Fresubin[®] Clear thickener from Fresenius Kabi GmbH,
145 Bad Homburg, Germany (Fresubin Clear); Findus thickener from Findus Sweden AB
146 (Findus); Nestlé Resource[®] Thicken-up[™] (Nestlé Thicken-up); and Nestlé Resource[®]
147 Thicken-up[™] Clear (Nestlé Clear) from Nestlé Health Science Center, Stockholm, Sweden.
148 The syrup used in the study was Lys Syrup (84% sugar) from Dan Sukker, Malmö, Sweden,
149 the xanthan gum (Grinsted Xanthan CLEAR 80) was supplied by Danisco France SAS (Melle,
150 France), and the poly(acrylamide) (PAA) was supplied by ACROS Organics.

151

152 **2.2 Sample preparation**

153

154 A large volume of the thickener solution (~2 liter) was prepared by mixing the sample with
155 tap water following the manufacturer's guidelines to achieve a honey-like consistency
156 (according to the NDD scale) using a magnetic stirrer until complete homogenization was
157 achieved (about 1 hour). The thickeners are used in elderly care centers to thicken fluid food
158 and therefore are expected to be highly soluble. Shear viscosity was adjusted within the honey
159 consistency range (0.35-1.75 Pa.s) specifically to 0.55 ± 0.03 Pa.s (Table 1) at a shear rate of
160 50 s^{-1} . The amounts of powder that were used to achieve the desired shear viscosities (Pa.s)
161 are listed in Table 1. The model Newtonian, Boger, and shear-thinning fluids were made by
162 diluting the syrup with water to achieve the targeted viscosity of 0.55 Pa.s at a shear rate of 50
163 s^{-1} , with subsequent mixing (in the case of the Boger and shear-thinning fluid) with a
164 concentrated solution of either the xanthan gum or PAA polymer (Table 1). Finally all the
165 samples mixed were degassed before mixing using a vacuum pump, D-82178 from ASF-
166 THOMAS: Munich, Germany.

167

168 [Table 1 here]

169

170 Separate sample preparation was performed for microscopy. The given thickeners were
171 dispersed in deionized water on % w/w basis in different concentrations depending upon the
172 thickener structure visualization (table 1) and stirred constantly for 4 to 8 hours until they
173 were dissolved completely. All the samples were mixed at room temperature ($\sim 25^{\circ}\text{C}$) except
174 for Findus where higher temperature ($\sim 65^{\circ}\text{C}$) was used since it was not easily soluble at room
175 temperature.

177 **3. Rheological measurements**

179 3.1 *Shear rheology measurement (Flow curves and yield stress)*

181 The shear rheology of the samples was measured for shear rates ranging between 1 to 1000s^{-1}
182 to cover the entire shear rate range mentioned in the literature, using an ARES-G2 (TA
183 Instruments, New Castle, DE, USA) equipped with a cone and plate geometry with a diameter
184 of 40 mm and cone angle of 0.04 rad. The yield stress was measured using the Reologica
185 Stresstech HR (Reologica AB, Lund, Sweden) stress-controlled rheometer. Flow
186 measurements were performed by the continuous increase of the shear stress. The stress value
187 at which the two tangents crossed was considered as the yield stress value (Moller *et al.*,
188 2006). The yield stress was also determined from the flow curve by curve fitting of the
189 Hershel-Bulkley model

$$191 \quad \tau = \tau_0 + K(\dot{\gamma})^n \quad (1)$$

193 where τ denotes the shear stress, τ_0 the yield stress, $\dot{\gamma}$ the shear stress while ‘K’ and ‘n’ are
194 constants. Stresstech instrument was equipped with concentric cylinders (bob and cup
195 geometry) with the cup radius, $R_c=13.5$ mm and bob radius, $R_b=12.5$ mm. All the rheological
196 measurements were performed at 25°C as recommended by the NDD standards.

198 3.2 *Advanced tube viscometry, PUV+PD (Flow curves and yield stress)*

200 The real-time velocity profile was monitored with advanced tube viscometry using PUV+PD
201 measurement to acquire the flow curve (Flow-Viz, Gothenburg, Sweden). The method and the
202 system are described in detail elsewhere (R Kotzé *et al.*, 2015; Wiklund *et al.*, 2007; Wiklund
203 *et al.*, 2008). In the present study, the sample was mixed in the product tank with an agitator
204 (Fig. 1).

206 [figure 1 here]

207 Flowing of the sample was initiated by the positive displacement pump through a stainless
208 steel pipe. The shear stress (τ) at the wall and the radial shear distribution inside the tube are
209 determined from the pressure drop (Δp) across a fixed length ($l=0.6\text{m}$) and radius ($r=0.011$
210 m) of the tube (Fig. 1) using the relationship:

211

212
$$\tau = \frac{r\Delta p}{2l} \quad (2)$$

213 Two ultrasound sensors were used to capture the velocity profile. The shear rate was
214 calculated from the gradient of the measured velocity profile. The PUV+PD software
215 measures the complete inline viscosity profile. Moreover, the software can post-process the
216 data and measure the yield stress from the pressure drop and plug radius data as

217

218
$$R = 2l\tau_0/\Delta P \quad (3)$$

219 where R is the radius of the plug, and l is length of the pipe in which the fluid is flowing.
220 Thus, real time estimation of the yield stress can be performed with PUV+PD.

221 3.3 *Extensional rheology measurement using Hyperbolic Contraction Flow*

222

223 The transient extensional viscosity of the thickened solutions was determined by the HCF
224 method using an Instron 5542 (Instron Corp., Canton, USA). The method used is thoroughly
225 described elsewhere (Nyström *et al.*, 2012; Stading *et al.*, 2001; Wikström *et al.*, 1999a).
226 The hyperbolic nozzle used for the measurement had an inlet radius of 10 mm and outlet
227 radius of 0.78 mm. The maximum Hencky strain was in the range of 3.6–8.7 depending on the
228 fluid tested. The power law parameters (K and n) from equation 4

229

230
$$\sigma = K\dot{\gamma}^n \quad (4)$$

231 Required to subtract the contribution of shear stress σ to the total measured stress were
232 determined from the flow curves measured in the ARES-G2 by fitting the data to the Power
233 law model. Each measurement was performed in triplicate and the relative standard deviation
234 was <3.7% between measurements for all samples tested.

235

236 3.4 *Microstructural characterization*

237

238 3.4.1 *Mica sandwich technique and transmission electron microscopy (TEM)*

239

240 The microstructure of a diluted thickener (Table 1) was determined using the Mica Sandwich
241 Technique described in detail by Barreto *et al.* 2013 (Barreto *et al.*, 2013). TEM was used to
242 analyze the replicas under the LEO 706E microscope (LEO Electron Microscopy Ltd.,
243 Cambridge, England).

244 3.4.2 *Light microscopy (LM)*

245 The starch-based thickeners, Nestlé Thicken-up and Findus, were analyzed using light
246 microscopy, revealing that they had structure at micro-meter scale compared to nano-meter
247 scale for the xanthan-based thickeners. Two staining methods were used: Lugol's iodine
248 solution for starch; and a mixture of Lugol's iodine and Light Green solution (1:1) for both
249 starch and protein. Lugol's iodine stains amylopectin a pink-to-brownish color and amylose

250 purple, whereas Light Green stains proteins green. The light microscope used was a Nikon
251 Microphot-FXA (Nikon, Tokyo, Japan), together with an Olympus Altra 20 color camera
252 connected to a computer and operated using the Olympus cellSens Dimension software
253 (Olympus Soft Imaging Solutions GmbH, Münster, Germany).

254

255 **4. Results and Discussion**

256 4.1 Microstructure

257 TEM and LM were used to visualize the fine structures of the xanthan gum and starch in the
258 commercial thickeners (Fig. 2). The xanthan gum-based thickeners, Fresubin (Fig. 2A and D),
259 Nestlé Clear (Fig. 2B and E) and Nutilis (Fig. 2C and F), formed transparent solutions which
260 meant that no microstructure could be observed by LM due to the limiting resolution of about
261 1 μm . With TEM at high magnification, a main mesh-like network structure was observed
262 (Fig. 2A, B and C). At even higher magnification thin filaments were observed as shown in
263 the micrographs (Fig. 2D, E and F). The main component of the gum based thickeners is
264 xanthan gum, while the manufacturers do not specify other biopolymers present. The thin
265 filaments correspond well with the structure of xanthan helices previously visualized with the
266 same microscopy technique (Lundin *et al.*, 1995). The starch-based thickeners were
267 analyzed at a lower magnification using LM to accommodate the starch-based microstructure.
268 They were not visualized by TEM because the microstructure is too heterogeneous. In Nestlé
269 Thicken-Up (Fig. 2G), only slightly swollen starch granules were noticed. Most of the
270 granules stained light-brown, indicating that they contain amylopectin, and a few starch
271 granules stained purple, which indicates that amylose had leached out. The granule structure
272 was at largely retained indicating a low degree of gelatinization. The Findus sample showed
273 some starch granules that stained purple and a protein network that stained green (Fig. 2H).
274 Moreover, unstained fat droplets were observed in the sample. The Findus thickener is not a
275 single thickener-based product, in addition to the starch, protein and fats contribute to the
276 microstructure and fluid consistency.

277 [Figure 2 here]

278 4.2 Shear rheology by viscometry and PUV+PD

279 The thickeners and the model fluids were characterized using laboratory-based viscometry as
280 well as advanced tube viscometry (PUV+PD) with tube dimensions that resemble those of the
281 pharynx. The latter was mainly included in the study to demonstrate to the clinical dysphagia
282 community that a flow in a tube, such as the pharynx can be evaluated with both methods, as
283 well as a basis for our future studies of flow in the pharynx using the ultrasonic techniques
284 where we want to utilize the real time ability to determine flow curves in transient flows.

285 4.2.1 Flow curves in shear rheology (Lab-based and PUV+PD)

286 Thickeners used for dysphagia management and model fluids were characterized using
287 laboratory-based viscometry as well as advanced tube viscometry (PUV+PD) with tube
288 dimensions that resemble those of the pharynx. Figure 3(A–C) and Table 2 show that the flow

289 curves derived from the two methods overlapped well with similar power-law K and n values
290 for all the thickener-based and model fluids. The gum-based thickeners were the most shear-
291 thinning (Fig. 3A), with the lowest shear thinning indices noted for Nestlé Clear ($n=0.19$) and
292 Fresubin Clear ($n=0.19$), followed by Nutilis ($n=0.33$). The starch-based thickeners (Fig. 3C),
293 Nestlé Thicken-up and Findus, were the least shear-thinning, showing higher n values of 0.39
294 and 0.61, respectively. The shear thinning index for the Newtonian and Boger fluids was as
295 expected 1 for both the methods. The model fluids (shear thinning with either PAA or xanthan
296 gum) were not measured using the PUV+PD method due to the limited capacity of the pump
297 used in the current study to propel such highly viscoelastic fluids.

298 [Figure 3 here]

299 [Table 2 here]

300 The results show that the PUV+PD method in a clinically relevant geometry gives the same
301 results as classical viscometry. Furthermore, the PUV+PD method gives a complete flow
302 curve in 0.19-1.35 ms and can thus be used in fast transient flows such as for a bolus passing
303 the pharynx in about a second. The main limitation of the method is that air may be
304 introduced during pumping thus transforming a surface active fluid to foam.

305 The flow behavior index for the model shear-thinning xanthan gum polymer was $n=0.22$ and
306 for the shear-thinning PAA was $n=0.79$, as assessed by the laboratory viscometry. The model
307 fluids used in the study serve as a reference, since they are based on a single elastic polymer
308 (PAA or xanthan gum) and a Newtonian fluid (either syrup or water) system, thereby
309 eliminating the interference effects of other polymers used in the commercial powders. In
310 addition to the food-grade elastic polymer xanthan gum, PAA was also used in the model
311 fluids (PAA). The PAA is not a food grade polymer but is much more elastic than xanthan
312 gum (Jones *et al.*, 1989). The use of PAA allows the study of high-level elastic effects. These
313 model fluids are also planned to be used in the future to study the influence of high
314 extensional viscosity in relation to swallowing.

315 All the fluids used in the present study were thickened to syrup consistency (range, 0.35–1.75
316 Pa.s), as recommended by the NDD, and more precisely to a viscosity of 0.55 ± 0.03 Pa.s at a
317 shear rate of 50 s^{-1} for the reason mentioned earlier. Gum-based xanthan solutions are strongly
318 shear-thinning owing to their rigid-rod polymer conformation in solution. This means that a
319 xanthan-based thickener is perceived as being less thick as the shear rate increases during oral
320 processing. It should also be noted that starch-thickened foods are susceptible to reductions in
321 thickness during oral processing through the action of the amylase in saliva, thus reducing the
322 effective viscosity during swallowing. Moreover, less xanthan gum than starch was needed to
323 acquire the set viscosity of 0.55 Pa.s in the current study.

324 It should be noted that shear-thinning is less pronounced in PAA-based model fluids. This is
325 because PAA is a highly compact molecule with very strong intramolecular bonding, and the
326 shear flow, which is considered to be a “weak flow”, is not sufficient to stretch the polymer.
327 Hence large extensional deformation, as applied in the present study, is needed to study the
328 flow properties of PAA in detail.

329 4.2.2 Yield stress in shear rheology (Viscometry and PUV+PD)

330 Yield stress for the thickeners and model fluids was considered during measurements since
331 previous publications (Marcotte, 2001; Steele, 2014) have proposed yield stress as a
332 contributing factor to bolus cohesiveness and therefore yield stress has to be considered in
333 addition to other parameters to alleviate aspiration. The yield stress values were determined
334 by viscometry and the PUV+PD method and are presented in Table 2. Figure 4 presents an
335 example of determination of yield stress with laboratory rheometer for the sample having the
336 highest yield stress value; Fresubin Clear. The value is taken at the stress value when two
337 tangent lines intersect each other at the point of sudden drop in shear viscosity and the
338 consequential increase in shear rate. The two methods gave similar values and the small
339 differences were not statistically significant ($P>0.05$).

340 [Figure 4 here]

341 Generally the samples composed of xanthan gum had higher values for the yield stresses in
342 the order Fresubin Clear>Nestlé Clear>Nutilis and no or negligible yield stress was detected
343 in the starch-based thickeners (Nestlé Thicken-up and Findus). The yield stress depends on
344 the structure of the thickener fluid and the exact composition of the commercial fluids is not
345 known. However, from the microscopy images in Fig. 2 it is clear that the gum-based
346 thickeners have a well-developed network structure at rest.

347 The model xanthan gum fluid had a yield stress of 13 Pa which is similar to the reported value
348 of 10 Pa by Marcotte and coworkers (Marcotte, 2001). Furthermore the yield stress noticed in
349 model xanthan-gum system confirms observed yield stresses noticed in the gum-based
350 thickeners. The yield stresses of the PAA based model fluids (Boger and shear thinning) were
351 negligible and similar results were reported by Yang (Yang, 2001). Yield stress has been
352 proposed to be responsible for the “binding properties” of xanthan gum in dysphagia
353 management (Marcotte, 2001). In the swallowing context, this binding property is expected to
354 promote a cohesive bolus structure, thereby reducing the risk of premature disintegration of
355 the bolus during swallowing. The pressure gradient created as the bolus is squeezed between
356 the tongue and palate is essential for causing the bolus to flow across the oropharynx (Steele,
357 2014). Therefore, the higher the yield stress, the greater the force needed to initiate the flow.
358 This means individuals with weak swallowing reflex may suffer from post-swallow residues
359 in case they swallow food of very high yield stress or they have reduced capacity to generate
360 appropriate tongue pressure as mentioned by the group of Becker (Becker *et al.*, 2015).
361 However, during oral processing and swallowing, the bolus is never static and therefore the
362 overall stress required exceeds the yield stress at the levels measured by Steele and coworkers
363 (Steele, 2014). While it has been shown by Alsanei and Chen (Alsanei *et al.*, 2014) that the
364 average maximum tongue pressure generation capacity decreases with growing age, the study
365 conducted by Steele (Steele, 2014) noted that the senior citizens (aged 70 years) still can
366 generate enough tongue pressure to handle a honey-thick consistency bolus at the shear rate of
367 50 s^{-1} studied herein. Therefore we believe the yield stresses noticed in the present work is
368 less likely to influence the swallowing process overall. While the yield stresses measured with
369 two different methods gives relatively identical values, the PUV-PD method has the

370 advantage of being independent of any possible wall slip, since the yield stress is determined
371 from the radius of the plug not in direct contact with the wall. Moreover PUV-PD mimics the
372 flow geometry of the pharynx.

373 Yield stress is an important characteristic in many food systems such as in ketchup and
374 mayonnaise, (Berta *et al.*, 2016) however the measurement of yield stress is not straight
375 forward. Many difficulties such as wall slippage arise during measurement and a detailed
376 discussion on these difficulties has been discussed elsewhere. (Barnes, 1995; Walls *et al.*,
377 2003). In the current work, bob and cup geometry was used since the results matches better
378 with the ones from PUV-PD method and we believe the PUV-PD method addresses the wall
379 slip condition in a better way.

380 4.3 Extensional flow

381 The HCF method was applied to measure the extensional viscosity of the given products.
382 Extension rates were varied from 1–100 s⁻¹ (Fig. 5) for all the fluids. The extensional
383 viscosity of the thickeners (Fig. 5A) was measurable even at an extension rate <10 s⁻¹, which
384 was not the case for the model fluids. The thickener-based and model fluids behaved
385 differently in extensional flow. The xanthan-based thickeners were more elastic than the
386 starch-based thickeners, while the model fluids (Fig. 5 b) made with PAA (Boger and shear-
387 thinning) showed extension-thickening, whereas 2% xanthan gum exhibited extension-
388 thinning behavior. The extensional viscosity corresponds well with the presence of the
389 xanthan dominated network structure shown in Figure 2A-F.

390
391 [Figure 5 here]

392
393 We have previously shown that the extensional viscosity of xanthan gum fluids promotes safe
394 swallowing, which means that extensional viscosity is an important parameter to consider
395 while designing fluid foods for persons with dysphagia (Nyström *et al.*, 2015). In the labeling
396 information for the thickeners, the precise amount of xanthan gum is not given, although it is
397 reasonable to assume that with a higher level of xanthan gum, greater elasticity is achieved, as
398 previously observed (Choi *et al.*, 2014). The extension-thinning behavior noticed for the
399 xanthan-based model fluids is consistent with a xanthan gum based commercial fluid. This is
400 likely due to the semi-rigid rod-like conformation of the xanthan gum. The extension-
401 thickening behavior of PAA is due to its coiled structure and the polymer uncoils and aligns
402 in the stretching direction (Ferguson *et al.*, 1990).

403
404 The fact that xanthan gum solutions both in the commercial thickeners and in water behaves
405 extension-thinning at higher extension rates possibly suggests that they are perceived less
406 slimy in the context of swallowing than PAA. While assigning a fixed shear rate during
407 swallowing of 50 s⁻¹ is an over-simplification, extension rates during swallowing have not
408 been described to date in the literature to the best of our knowledge. This makes predictions
409 about extensional viscosity with respect to swallowing even more complicated, and therefore
410 prompts further research. As noticed in TEM, the structure of network is more pronounced in
411 Fresubin and Nutilis than in Nestlé clear. It is however not possible to relate the nature of the
412 network to the individual components since the exact thickener composition is not known.

413 In the current study, we have characterized commercial thickeners and model fluids to
414 understand flow properties. However, further studies are required to determine the appropriate
415 level of elasticity and type of polymer to promote safe and easy swallowing, as well as to
416 define the most dominant shear and extension rates. Trouton ratio $Tr = \frac{\eta_{\dot{\epsilon}}}{\eta_{\dot{\gamma}}}$ estimates the
417 departure of ratio of extensional to shear viscosity from its Newtonian counterpart, which is
418 estimated around 3 (Sochi, 2010) for the Newtonian equivalent. Trouton ratios for the gum-
419 based thickeners were: Fresubin =~40, Nestlé Clear=~45 and Nutilis =~68 and for starch-
420 based thickeners: Nestle thicken-up=41.9 and Findus=~152. The ratio is higher than three for
421 all the fluids which confirms the elastic nature of the samples.

422

423 **5. Conclusions**

424

425 This study shows that the xanthan-based commercial thickeners used for dysphagia
426 management are slightly more shear-thinning and have considerably higher extensional
427 viscosities than starch-based thickeners. Moreover, with microstructural characterization
428 using light and electron microscopy, we further elucidated how the network structure of
429 xanthan gum influences the rheology in a different way than starch does. Model fluids can be
430 designed to mimic commercial thickeners as well as to set the upper limit for maximum
431 elasticity that will be tested in clinical studies in the future. The shear viscosity measured
432 using laboratory viscometry and the newly developed PUV+PD method gave similar results,
433 which means by using the PUV+PD method the flow curve and yield stress can be acquired in
434 less than 1.4 ms which is important for the short time scales involved in human swallowing.
435 Only low yield stresses were detected, considerably lower than expected to occur during
436 swallowing.

437

438 **Acknowledgments**

439 The Swedish Research Council Formas is gratefully acknowledged for financing this study.
440 We are also thankful to Marco Berta for help with the experimental rheology.

441 **Ethical statements**

442 The author declares no conflict of interest for this study, while this study does not involve any
443 animal or human testing.

444 **References**

- 445 Alsanei, W. A., & Chen, J. (2014). Studies of the Oral Capabilities in Relation to Bolus Manipulations
446 and the Ease of Initiating Bolus Flow. *Journal of Texture Studies*, 45(1), 1-12. doi:
447 10.1111/jtxs.12041
- 448 Andersson, H., Öhgren, C., Johansson, D., Kniola, M., & Stading, M. (2011). Extensional flow,
449 viscoelasticity and baking performance of gluten-free zein-starch doughs supplemented with
450 hydrocolloids. *Food Hydrocolloids*, 25(6), 1587-1595. doi:
451 <http://dx.doi.org/10.1016/j.foodhyd.2010.11.028>
- 452 Barnes, H. A. (1995). A review of the slip (wall depletion) of polymer solutions, emulsions and particle
453 suspensions in viscometers: its cause, character, and cure. *Journal of Non-Newtonian Fluid
454 Mechanics*, 56(3), 221-251.

455 Barreto, C., Altskär, A., Fredriksen, S., Hansen, E., & Rychwalski, R. W. (2013). Multiwall carbon
456 nanotube/PPC composites: Preparation, structural analysis and thermal stability. *European*
457 *Polymer Journal*, 49(8), 2149-2161.

458 Becker, B. J., & Connor, N. P. (2015). Effects of aging on evoked retrusive tongue actions. *Archives of*
459 *Oral Biology*, 60(6), 966-971.

460 Berta, M., Wiklund, J., Kotzé, R., & Stading, M. (2016). Correlation between in-line measurements of
461 tomato ketchup shear viscosity and extensional viscosity. *Journal of Food Engineering*, 173,
462 8-14. doi: <http://dx.doi.org/10.1016/j.jfoodeng.2015.10.028>

463 Bülow, M. (2003). Therapeutic aspects of oral and pharyngeal swallowing dysfunction (Phd Thesis).

464 Chen, J. (2009). Food oral processing—A review. *Food Hydrocolloids*, 23(1), 1-25. doi:
465 <http://dx.doi.org/10.1016/j.foodhyd.2007.11.013>

466 Chen, J., & Lolivret, L. (2011). The determining role of bolus rheology in triggering a swallowing. *Food*
467 *Hydrocolloids*, 25(3), 325-332. doi: <http://dx.doi.org/10.1016/j.foodhyd.2010.06.010>

468 Choi, H., Mitchell, J. R., Gaddipati, S. R., Hill, S. E., & Wolf, B. (2014). Shear rheology and filament
469 stretching behaviour of xanthan gum and carboxymethyl cellulose solution in presence of
470 saliva. *Food Hydrocolloids*, 40, 71-75. doi: <http://dx.doi.org/10.1016/j.foodhyd.2014.01.029>

471 Cichero, J. A. Y. (2013). Thickening agents used for dysphagia management: effect on bioavailability
472 of water, medication and feelings of satiety. *Nutrition Journal*, 12, 54-54. doi: 10.1186/1475-
473 2891-12-54

474 Clavé, P., De Kraa, M., Arreola, V., Girvent, M., Farre, R., Palomera, E., & SERRA-PRAT, M. (2006). The
475 effect of bolus viscosity on swallowing function in neurogenic dysphagia. *Alimentary*
476 *pharmacology & therapeutics*, 24(9), 1385-1394.

477 Dogan, N. (2002). In-Line Measurement of Rheological Parameters and Modeling of Apparent Wall
478 Slip in Diced Tomato Suspensions Using Ultrasonics. *Journal of Food Science*, 67(6), 2235-
479 2240. doi: 10.1111/j.1365-2621.2002.tb09533.x

480 Dufour, D., Windhab, E. J., Takeda, Y., & Jeelani, S. A. (2007). In-line monitoring of chocolate
481 crystallization by UVP-PD technique.

482 Ekberg, O., Hamdy, S., Woisard, V., Wuttge-Hannig, A., & Ortega, P. (2002). Social and psychological
483 burden of dysphagia: its impact on diagnosis and treatment. *Dysphagia*, 17(2), 139-146.

484 Ferguson, J., Walters, K., & Wolff, C. (1990). Shear and extensional flow of polyacrylamide solutions.
485 *Rheologica Acta*, 29(6), 571-579.

486 Hasegawa, A., Otoguro, A., Kumagai, H., & Nakazawa, F. (2005). Velocity of Swallowed Gel Food in
487 the Pharynx by Ultrasonic Method. *Journal of The Japanese Society for Food Science and*
488 *Technology-nippon Shokuhin Kagaku Kogaku Kaishi*, 52(10), 441-447. doi:
489 10.3136/nskkk.52.441

490 Ishihara, S., Nakauma, M., Funami, T., Odake, S., & Nishinari, K. (2011). Viscoelastic and
491 fragmentation characters of model bolus from polysaccharide gels after instrumental
492 mastication. *Food Hydrocolloids*, 25(5), 1210-1218. doi:
493 <http://dx.doi.org/10.1016/j.foodhyd.2010.11.008>

494 Jones, D. M., & Walters, K. (1989). The behaviour of polymer solutions in extension-dominated flows,
495 with applications to Enhanced Oil Recovery. *Rheologica Acta*, 28(6), 482-498. doi:
496 10.1007/BF01332919

497 Köpplmayr, T., Luger, H.-J., Burzic, I., Battisti, M. G., Perko, L., Friesenbichler, W., & Miethlinger, J.
498 (2016). A novel online rheometer for elongational viscosity measurement of polymer melts.
499 *Polymer Testing*, 50, 208-215. doi: <http://dx.doi.org/10.1016/j.polymertesting.2016.01.012>

500 Kotzé, R., Haldenwang, R., Fester, V., & Rössle, W. (2015). In-line rheological characterisation of
501 wastewater sludges using non-invasive ultrasound sensor technology. *Water SA*, 41, 683-690.

502 Kotzé, R., Wiklund, J., & Haldenwang, R. (2013). Optimisation of Pulsed Ultrasonic Velocimetry
503 system and transducer technology for industrial applications. *Ultrasonics*, 53(2), 459-469.
504 doi: <http://dx.doi.org/10.1016/j.ultras.2012.08.014>

505 Leonard, R. J., White, C., McKenzie, S., & Belafsky, P. C. (2014). Effects of Bolus Rheology on
506 Aspiration in Patients with Dysphagia. *Journal of the Academy of Nutrition and Dietetics*,
507 114(4), 590-594. doi: <http://dx.doi.org/10.1016/j.jand.2013.07.037>
508 Lundin, L., & Hermansson, A.-M. (1995). Supermolecular aspects of xanthan-locust bean gum gels
509 based on rheology and electron microscopy. *Carbohydrate Polymers*, 26(2), 129-140. doi:
510 [http://dx.doi.org/10.1016/0144-8617\(94\)00070-A](http://dx.doi.org/10.1016/0144-8617(94)00070-A)
511 Mackley, M. R., Tock, C., Anthony, R., Butler, S. A., Chapman, G., & Vadillo, D. C. (2013). The rheology
512 and processing behavior of starch and gum-based dysphagia thickeners. *Journal of Rheology*
513 (1978-present), 57(6), 1533-1553. doi: <http://dx.doi.org/10.1122/1.4820494>
514 Marcotte, M. (2001). Rheological properties of selected hydrocolloids as a function of concentration
515 and temperature. *Food Research International*, 34(8), 695-703. doi:
516 [http://dx.doi.org/10.1016/S0963-9969\(01\)00091-6](http://dx.doi.org/10.1016/S0963-9969(01)00091-6)
517 Moller, P. C. F., Mewis, J., & Bonn, D. (2006). Yield stress and thixotropy: on the difficulty of
518 measuring yield stresses in practice. *Soft Matter*, 2(4), 274-283. doi: 10.1039/B517840A
519 Moret-Tatay, A., Rodríguez-García, J., Martí-Bonmatí, E., Hernando, I., & Hernández, M. J. (2015).
520 Commercial thickeners used by patients with dysphagia: Rheological and structural
521 behaviour in different food matrices. *Food Hydrocolloids*, 51, 318-326. doi:
522 <http://dx.doi.org/10.1016/j.foodhyd.2015.05.019>
523 Newman, R., Vilardell, N., Clavé, P., & Speyer, R. (2016). Effect of Bolus Viscosity on the Safety and
524 Efficacy of Swallowing and the Kinematics of the Swallow Response in Patients with
525 Oropharyngeal Dysphagia: White Paper by the European Society for Swallowing Disorders
526 (ESSD). *Dysphagia*, 31(2), 232-249. doi: 10.1007/s00455-016-9696-8
527 Nyström, M., Jahromi, H. T., Stading, M., & Webster, M. (2012). Numerical simulations of Boger fluids
528 through different contraction configurations for the development of a measuring system for
529 extensional viscosity. *Rheologica Acta*, 51(8), 713-727.
530 Nystrom, M., W. M. Qazi, M. Bulow, O. Ekberg and M. Stading (2015). "EFFECTS OF RHEOLOGICAL
531 FACTORS ON PERCEIVED EASE OF SWALLOWING." *APPLIED RHEOLOGY* 25(6): 40-48.
532 Oom, A., Pettersson, A., Taylor, J. R., & Stading, M. (2008). Rheological properties of kafirin and zein
533 prolamins. *Journal of Cereal Science*, 47(1), 109-116.
534 Petrie, C. J. S. (2006). Extensional viscosity: A critical discussion. *Journal of Non-Newtonian Fluid*
535 *Mechanics*, 137(1-3), 15-23. doi: <http://dx.doi.org/10.1016/j.jnnfm.2006.01.011>
536 Popa Nita, S., Murith, M., Chisholm, H., & Engmann, J. (2013). Matching the Rheological Properties of
537 Videofluoroscopic Contrast Agents and Thickened Liquid Prescriptions. *Dysphagia*, 28(2),
538 245-252. doi: 10.1007/s00455-012-9441-x
539 Quinchia, L. A., Valencia, C., Partal, P., Franco, J. M., Brito-de la Fuente, E., & Gallegos, C. (2011).
540 Linear and non-linear viscoelasticity of puddings for nutritional management of dysphagia.
541 *Food Hydrocolloids*, 25(4), 586-593. doi: <http://dx.doi.org/10.1016/j.foodhyd.2010.07.006>
542 Salinas-Vázquez, M., Vicente, W., Brito-de la Fuente, E., Gallegos, C., Márquez, J., & Ascanio, G.
543 (2014). Early Numerical Studies on the Peristaltic Flow through the Pharynx. *Journal of*
544 *Texture Studies*, 45(2), 155-163. doi: 10.1111/jtxs.12060
545 Sochi, T. (2010). Non-Newtonian flow in porous media. *Polymer*, 51(22), 5007-5023. doi:
546 <http://dx.doi.org/10.1016/j.polymer.2010.07.047>
547 Stading, M., & Bohlin, L. (2001). Contraction flow measurements of extensional properties. *ANNUAL*
548 *TRANSACTIONS-NORDIC RHEOLOGY SOCIETY*, 8, 181-186.
549 Steele. (2014). Variations in Tongue-Palate Swallowing Pressures When Swallowing Xanthan Gum-
550 Thickened Liquids. *Dysphagia*, 29(6), 678-684. doi: 10.1007/s00455-014-9561-6
551 Steele. (2015). The Blind Scientists and the Elephant of Swallowing: A Review of Instrumental
552 Perspectives on Swallowing Physiology. *Journal of Texture Studies*, 46(3), 122-137. doi:
553 10.1111/jtxs.12101
554 Tashiro, A., Hasegawa, A., Kohyama, K., Kumagai, H., & Kumagai, H. (2010). Relationship between the
555 rheological properties of thickener solutions and their velocity through the pharynx as

556 measured by the ultrasonic pulse Doppler method. *Bioscience, Biotechnology, and*
557 *Biochemistry*, 74(8), 1598-1605.

558 Walls, H., Caines, S. B., Sanchez, A. M., & Khan, S. A. (2003). Yield stress and wall slip phenomena in
559 colloidal silica gels. *Journal of Rheology (1978-present)*, 47(4), 847-868.

560 Walsh, J. H., Leigh, M. S., Paduch, A., Maddison, K. J., Philippe, D. L., Armstrong, J. J., Sampson, D. D.,
561 Hillman, D. R., & Eastwood, P. R. (2008). Evaluation of pharyngeal shape and size using
562 anatomical optical coherence tomography in individuals with and without obstructive sleep
563 apnoea. *Journal of Sleep Research*, 17(2), 230-238. doi: 10.1111/j.1365-2869.2008.00647.x

564 Wiklund, J., Shahram, I., & Stading, M. (2007). Methodology for in-line rheology by ultrasound
565 Doppler velocity profiling and pressure difference techniques. *Chemical Engineering Science*,
566 62(16), 4277-4293.

567 Wiklund, J., & Stading, M. (2008). Application of in-line ultrasound Doppler-based UVP–PD rheometry
568 method to concentrated model and industrial suspensions. *Flow Measurement and*
569 *Instrumentation*, 19(3–4), 171-179. doi:
570 <http://dx.doi.org/10.1016/j.flowmeasinst.2007.11.002>

571 Wikström, K., & Bohlin, L. (1999a). Extensional flow studies of wheat flour dough. I. Experimental
572 method for measurements in contraction flow geometry and application to flours varying in
573 breadmaking performance. *Journal of Cereal Science*, 29(3), 217-226.

574 Yang, M.-H. (2001). The rheological behavior of polyacrylamide solution II. Yield stress. *Polymer*
575 *Testing*, 20(6), 635-642. doi: [http://dx.doi.org/10.1016/S0142-9418\(00\)00084-2](http://dx.doi.org/10.1016/S0142-9418(00)00084-2)

576 Zargaraan, A., Rastmanesh, R., Fadavi, G., Zayeri, F., & Mohammadifar, M. A. (2013). Rheological
577 aspects of dysphagia-oriented food products: A mini review. *Food Science and Human*
578 *Wellness*, 2(3–4), 173-178. doi: <http://dx.doi.org/10.1016/j.fshw.2013.11.002>

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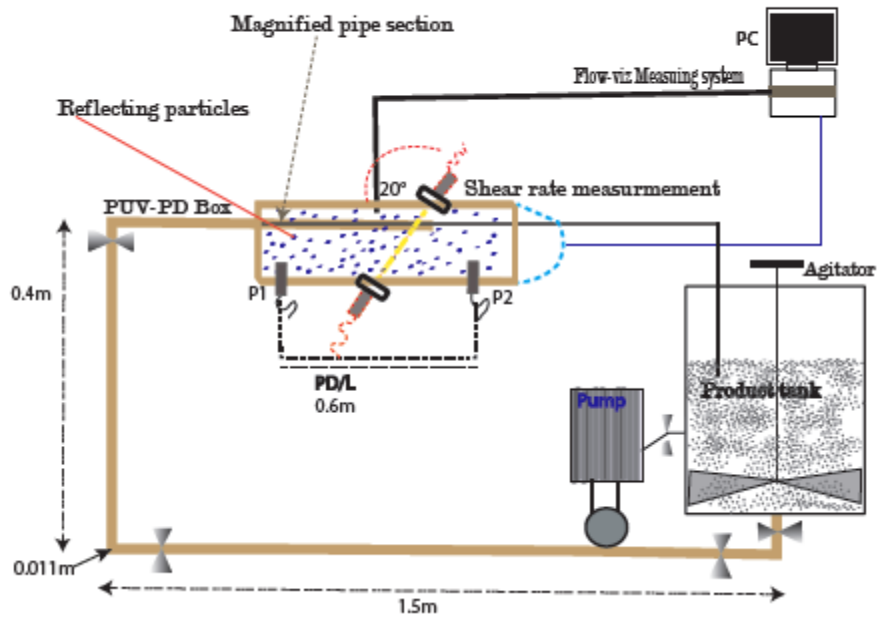
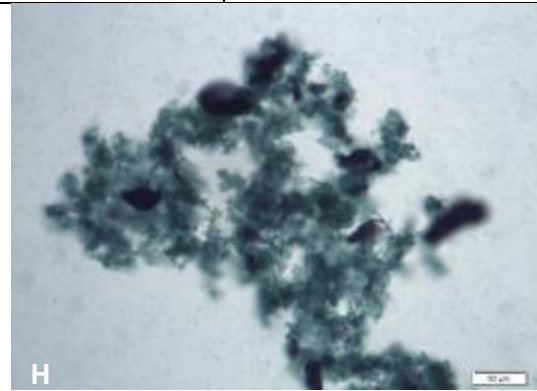
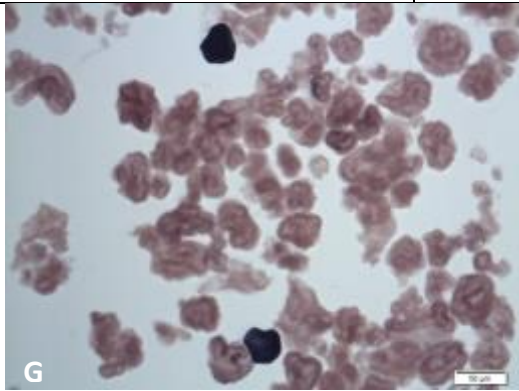
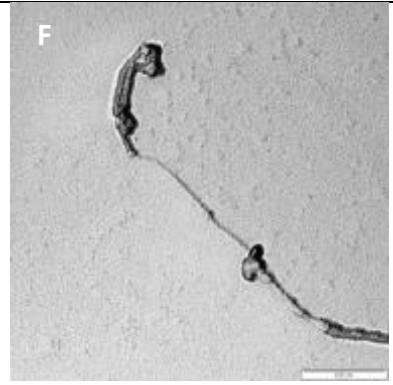
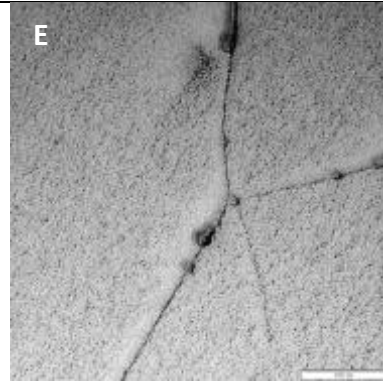
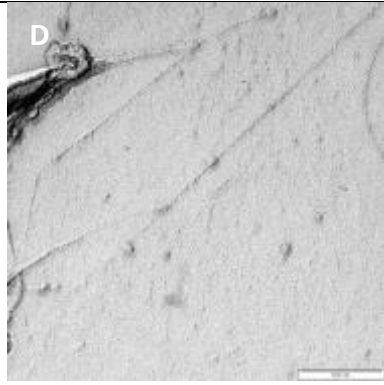
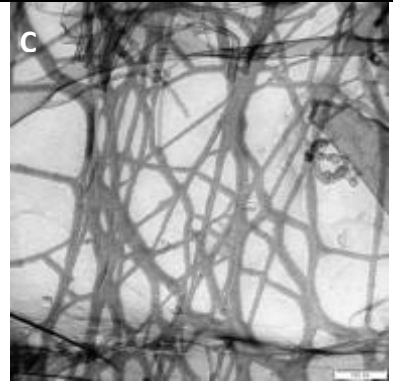
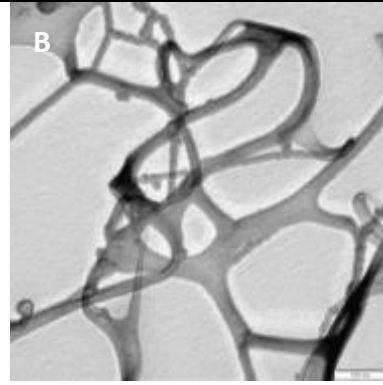
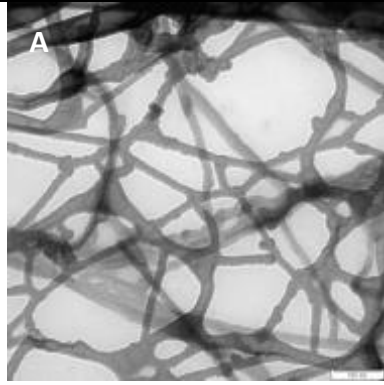


Figure 1: Schematic illustration of how the inline shear viscosity was measured using PUV+PD method



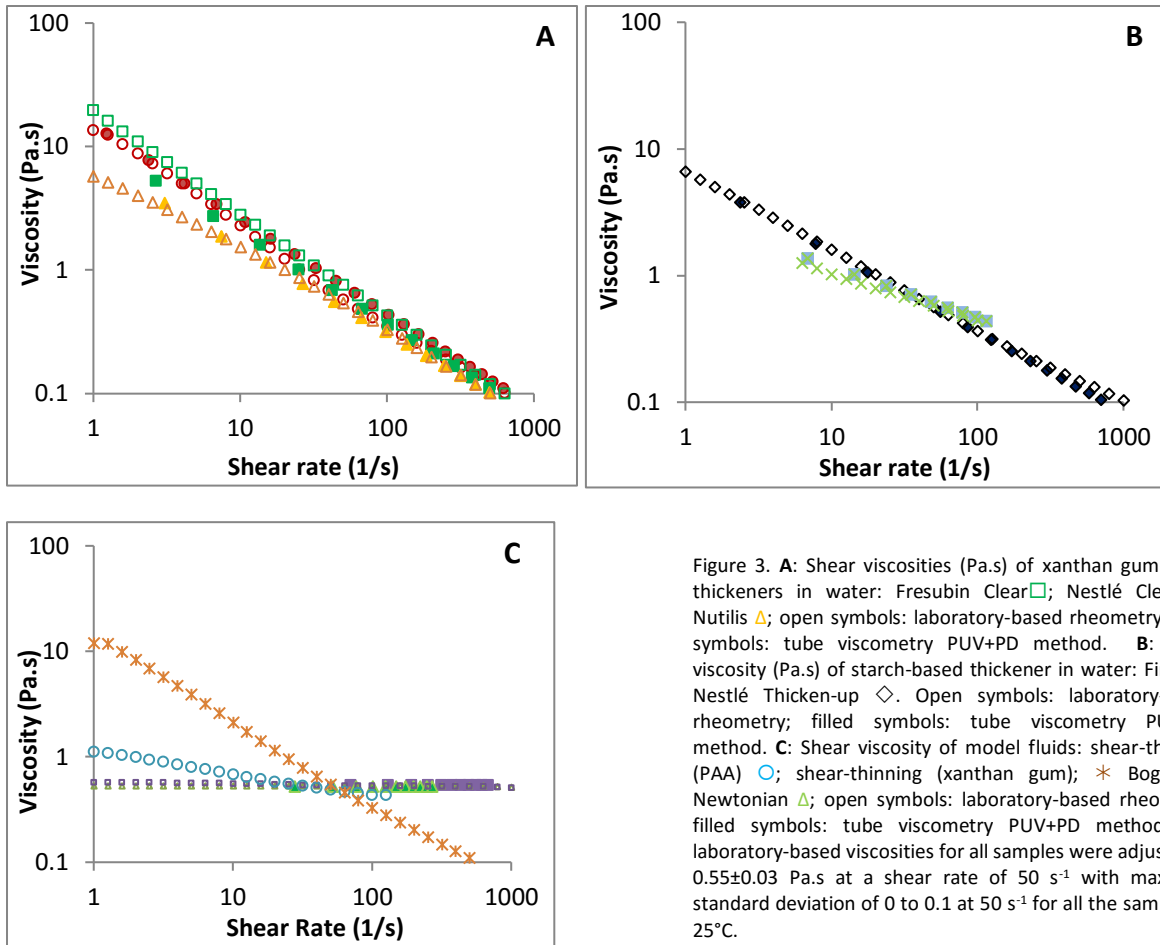


Figure 3. **A:** Shear viscosities (Pa.s) of xanthan gum-based thickeners in water: Fresubin Clear \square ; Nestlé Clear \circ ; Nutilis Δ ; open symbols: laboratory-based rheometry; filled symbols: tube viscometry PUV+PD method. **B:** Shear viscosity (Pa.s) of starch-based thickener in water: Findus \times Nestlé Thicken-up \diamond . Open symbols: laboratory-based rheometry; filled symbols: tube viscometry PUV+PD method. **C:** Shear viscosity of model fluids: shear-thinning (PAA) \circ ; shear-thinning (xanthan gum); \times Boger \square ; Newtonian Δ ; open symbols: laboratory-based rheometry; filled symbols: tube viscometry PUV+PD method. The laboratory-based viscosities for all samples were adjusted to 0.55 ± 0.03 Pa.s at a shear rate of 50 s^{-1} with maximum standard deviation of 0 to 0.1 at 50 s^{-1} for all the samples at 25°C .

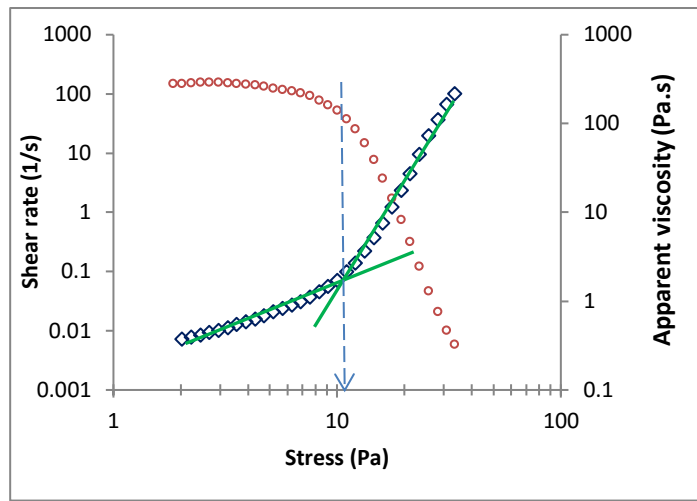


Figure 4: Flow curve showing the apparent viscosity \circ and shear rate \diamond a function of increasing stress. The decrease in apparent viscosity causes a sudden jump of the shear rate curve. The stress at which this change occurs is the yield stress and it was calculated by the intersection of two linear fitting curves.

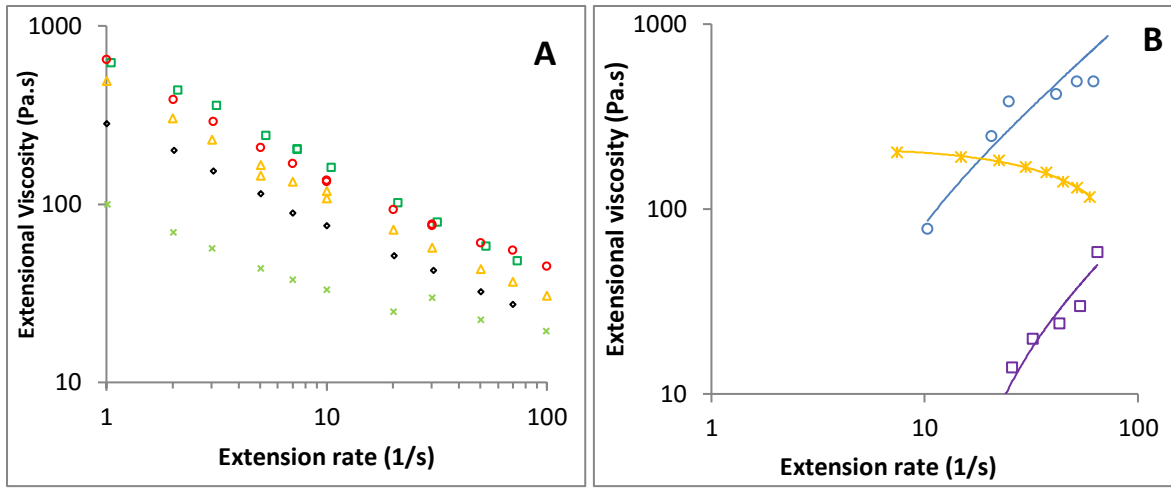


Figure 5. **A**: Extensional viscosities (Pa.s) of thickener-based dysphagia fluids in water: Fresubin Clear \square ; Nestlé Clear \circ ; Nutilis Δ ; Nestlé Thicken-up \diamond ; Findus \times ; and the model fluids, **B**: Boger \square (0.015% PAA in syrup), 2% xanthan gum in water (shear thinning) $*$; and 0.2% PAA in syrup (shear thinning) \circ .