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Agar-collagen fluid gels as a dysphagia food: Rheological characterization *vs* fiberoptic endoscopic evaluation of swallowing

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ABSTRACT

The completely different structuring approach of the fluid gels makes them rheologically-tunable systems with a high underexplored potential for application in dysphagia diets. The aim of this study was to characterize the swallowing behavior of high-protein collagen (20 %*w*/w) fluid gels structured with various agar concentrations (0.5 to 2.5 %w/w) using different analytical methods. The oral performance during different phases of dynamic swallowing processes was characterized by rheological methods (shear yield stress, shear-dependent viscosity, strain and frequency-dependent viscoelasticity, shear recovery behavior), International Dysphagia Diet Standardization Initiative tests (IDDSI) and fiberoptic endoscopic evaluation of swallowing. In addition, the stability and particle size of the systems were characterized.

All samples were stable and the polydispersity increased (span from 1.7 to 3.2) with increasing agar concentration. Fluid gels displayed low yield stress values (<2.0 Pa), good shear flowability and high shear recovery behavior which is well-matched with the facilitated swallowing process. The IDDSI tests showed that varying the agar concentration yields fluid gels with different thickness levels, suitable for mild and moderate dysphagia. The results of the endoscopic swallowing assessment test correlated with the results of the rheological analysis and confirmed the suitability of these systems for safe swallowing.

1. Introduction

Swallowing of food is a complex process involving many muscle groups, the weakening or damage to which can cause dysphagia. Dysphagia is a swallowing disorder that leads to impaired food bolus formation and disruption of the normal closure of the epiglottis, resulting malnutrition, dehydration, pulmonary aspiration, and even choking [1]. This disorder can occur in all age groups, but is most commonly observed among older people [2]. Rheologically-tunable food has become an effective approach to meet the needs of populations with dysphagia. Thicker products reduce the flow of the food by prolonging the muscular reactions and reflexes that facilitate swallowing and enhance safety [3,4]. Commercial food thickeners are successfully used for this purpose, but they have a number of disadvantages, such as difficulty in dosing causing the possibility of errors, some of them impart off-flavours as well as different rheological and structural behavior in different food matrices [5,38], which makes it necessary to look for alternative solutions for modifying food texture. The psychological discomfort experienced during eating leads to chronic deficiencies in protein and energy intake, resulting in weight loss, developing sarcopenia and even death [6,7]. This becomes the main reason that protein-rich diets are beneficial for maintaining human health. It is still a challenging task to develop foods with the proper rheological properties (texture) while maintaining or restoring good nutritional status. The consistency of novel fluid gel systems seems to be well-matched with swallowing dynamics, which is important for swallowing ease and safety. Fluid gels are defined as a suspension of gelled particles distributed in a non-gelled continuous medium and are obtained by applying shearing during the gelation process [8]. Rheological properties of these gels could be controlled by adjusting the concentration of gel-forming materials as well as the shear-broken power during gelation kinetics [9,10]. An agar structured fluid-gel prepared by pipe wall laminar shear, as well as oleogel-in-hydrogel Pickering emulsion fluid gels were previously fabricated for the dysphagia diet [10,11]. Agar appeared attractive to us for the production of such systems, as beyond its well-known gelling and stabilizing capabilities, it demonstrates antioxidant, anti-inflammatory, antidiabetic, anti-obesity, and anti-atherogenic properties attributed to the soluble fibers, which

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constitute 80 % of agar [12]. Scientists have also found that consumption of agar can increase appetite, most likely due to delayed gastric emptying, which is particularly beneficial in cases of long-term consumption (Clegg and Shafat, 2014) [13]. In addition, the structural uniqueness of fluid-gel systems is expected to allow them to be supplemented with higher quantities of proteins compared to other regular dysphagia-oriented systems. Among all, protein collagen attracted our attention due to health benefits such as improving joint health and bone strength [14]. It is highly believed, that such innovative and not previously studied high protein agar-structured fluid gel systems could be more palatable options for dysphagia patients.

The classic nomenclature (based on the viscosity of the food at 25 °C and a shear rate of 50 s^{-1}) currently available in the National Dysphagia Dietary Guidelines is used to categorize texture-modified, thickened foods [15]. However, characterization of dysphagia-oriented food is challenging, as the high viscosity of the food system does not automatically imply safe swallowing [16]. It is extremely important to properly characterize the fabricated systems during the complex dynamic swallowing process, in particularly their behavior in the oral and pharyngeal phases [17]. This can be achieved by using a wider range of rheological techniques to measure swallow-related shear rheological properties. By measuring shear yield stress, shear-dependent viscosity, strain/frequency-dependent viscoelasticity, and shear recovery behavior, it is possible to evaluate how the food bolus behaves under reduced tongue and palate muscle strength in the oral phase and whether it maintains structural integrity during transit in the pharyngeal phase [10,18]. The selection of methods to monitor the swallowing process of foods is crucial for the development of dysphagia-related foods that provide improved swallowing safety.

Another group of methods, such as video-fluoroscopic swallowing study (VFSS) and fiberoptic endoscopic evaluation of swallowing (FEES) may be used to assess swallowing function and to detect aspiration. In comparison with the VFSS, fiberoptic endoscopic instruments are much easier to access in public healthcare services, allowing real-time analysis of the anatomy and sensitivity of the pharynx, supraglottis and glottis [19,20]. While this instrument is commonly used to diagnose dysphagia and to guide therapeutic and rehabilitative interventions, our aim is to use it as one of the characterization tools for dysphagia-oriented food. There are limited studies on applying VFSS for swallowing safety for supramolecular structures composed of konjac glucomannan and xanthan gum [21], drum-dried thickeners [22], and an agar-structured fluid prepared by pipe wall shear [11]. Meanwhile, we could not find any studies on the use of FEES techniques for this purpose.

Thus, the aim of this study was twofold: a) to characterize the rheological properties of the novel high-protein (20 *w*/w% of collagen) fluid gels with various agar concentrations (0.5, 1.0, 1.5, 2.0, 2.5 % *w*/w) and establish their correlation according to IDDSI; b) to compare and contrast two products that are classified as slightly thick and mildly thick on IDDSI testing as well as to evaluate aspiration and residue through fiberoptic endoscopic evaluation of swallowing.

2. Materials and methods

2.1. Materials

Agar powder (containing 0.3 % fat, 3 % carbohydrates, 0.4 % protein and 0.1 % sodium chloride) was purchased from a local supermarket (Alvo, Kaunas, Lithuania) and used without further purification. Collagen hydrolysate (bovine, type I and III mixture (50:50), 90 % protein) was received from My Protein (Manchester, UK). The degree of hydrolysis was 20 %. The commercial thickener Nutilis Clear (Nutricia, Utrecht, the Netherlands) was used for the water thickening in the fiberoptic endoscopic test. Its composition includes dried glucose syrup, xanthan gum, and guar gum.

2.2. Methods

2.2.1. Agar-collagen fluid gel preparation

Different concentrations of agar (0.5, 1.0, 1.5, 2.0, 2.5 % *w*/w) were used for the preparation of the agar-collagen fluid gels, while the concentration of the protein collagen was kept constant at 20 % w/w. Accordingly, different quantities of agar powder and collagen were dissolved in distilled water until fully hydrated and then heated in a water bath at 90 °C for 20 min. The heating was followed by a cooling period (gelation step) during which the samples were sheared for 60 min at room temperature (25 °C) and for 60 min in an ice-water bath (0–2 °C) at 300 rpm with an overhead stirrer (AT-M, Matest, Treviolo, Italy) (AT-M, Matest, Treviolo, Italy). A control sample was prepared with 0.5 % agar concentration, and no shearing was applied during the gelation phase. Prior to analysis, all samples were stored in a fridge at +4 °C for 24 h.

2.2.2. Fluid-gel structure confirmation

To confirm that the fluid-gel system had actually formed, the samples were poured into special bottles and left at +4 °C for 24 h. After the fluid-gel samples have been inverted and a visual assessment has been made to confirm whether the gel samples are holding their shape or starting to flow.

2.2.3. Particle size

The particle size analysis of prepared samples was conducted using a laser diffraction particle size analyzer Mastersizer 2000 (Malvern, Worcestershire, UK), with the following parameters: speed of 1400 rpm, refractive indexes - 1.42 (dispersion phase) and 1.33 (solvent) respectively, droplet absorption value 0.01; obscuration 10–15 %. The results were expressed as mean diameter d_{43}, span factor, and droplet size distribution.

2.2.4. Rheological properties

Swallow-related rheological properties of the agar-based fluid gels were performed as previously described [9] by using a MCR92 rheometer (Anton Paar, Graz, Austria) equipped with parallel plate PP25 geometry (diameter: 25 mm;) set to a 1 mm gap. All rheological measurements were carried out at a temperature of 25 °C.

Viscosity at 50 s^{-1}: is used as a reference method based on the National Dysphagia Diet Task Force (NDD) [12]. The viscosity was measured at a constant shear rate of 50 s^{-1} and, based on the results, classified into one of four consistency levels: thin (1–50 mPa·s), nectar-like (51–350 mPa·s), honey-like (351–1750 mPa·s), and spoon-thick/pudding-like (>1750 mPa·s).

Shear yield stress: the shear yield stress of the samples was determined by conducting flow sweep tests within a shear rate of 0.001 to 0.1 s^{-1}. During the test, the shear rate was gradually increased, and the initial peak in shear stress was identified as the shear yield stress.

Shear-dependent viscosity: the flow behavior of samples was measured in a shear rate range from 0.1 to 100 s^{-1}. Data were analyzed according to the Ostwald-de Waele regression model:.

$$\sigma = \kappa \gamma^{n} \quad (1)$$

where: κ is the consistency coefficient and n is the flow behavior index.

Shear recovery behavior: a three-step "low-high-low" flow sweep protocol was employed to evaluate the shear recovery behavior of the samples. The test involved three sequential shear rate holds: 0.1 s^{-1} for 3 min, followed by 50 s^{-1} for 1 min, and returning to 0.1 s^{-1} for an additional 3 min.

Frequency sweep of the samples was managed within a linear viscoelastic region at a constant strain of 0.05 %, with oscillation frequency ranging from 1 to 100 rad/s.

Large amplitude oscillatory shear (LAOS) rheology: strain sweep of the samples was performed within a linear viscoelastic region at a fixed frequency (1 Hz) and a strain range of 0.01–1000 %.

2.2.5. International dysphagia diet standardization initiative (IDDSI) tests

Based on the IDDSI framework (2019), the consistency and suitability of the resulting fluid-gels for patients with dysphagia were characterized by a syringe test. For this purpose a reference syringe (Amedi+plusLuer slip 21Gx1 ½", Jiangyin Nanquan Macromolecule Product CO., LTD. Jiangsu Province, China) with a measured length of 61.5 mm from the zero line to the 10 ml line and a Luer-Lok tip was used. Description of the method: the tip of the syringe was covered with a finger until the syringe was filled with 10 ml of the sample. The nozzle was opened by allowing the sample to flow for 10 s and then recapped. The amount of remaining fluid-gel was checked, and the level of dysphagia was determined according to IDDSI: thin (less than 1 ml remaining), slightly thick (1–4 ml remaining), mildly thick (4–8 ml remaining), moderately thick (8–10 ml remaining), extremely thick (10 ml remaining).

2.2.6. Fiberoptic endoscopic evaluation in the case study

Three patients suffering from mild-moderate oropharyngeal dysphagia, were selected for this test: 87 years female with OD due to stroke, 72 years male with OD due to Parkinson's disease (duration of illness 8 years), and 80 years women, whose cause of oropharyngeal dysphagia has not yet been determined. The sensations experienced by all three patients during and after swallowing were very similar, therefore the data of one patient was selected to be shown in the manuscript as an example, as it best visually illustrates the performed swallowing process. 87 years, female was consulted for worsening oropharyngeal dysphagia (OD). OD due to stroke was diagnosed a year earlier, and complex treatment, consisting of a modified diet, transcutaneous neuromuscular electrostimulation, and swallowing exercises, was applied. Recently, the patient has not been exercising at home and has mostly stopped drinking thickened fluids. Fiberoptic endoscopic swallowing examination (FEES) was performed using a 3.7 mm HD Video Rhino-Pharyngo-Laryngoscope (STORZ, Tuttlingen, Germany). To enhance comfort during swallowing, a 2 % lidocaine topical anesthetic aerosol (1–2 sprays) was used. Bioethics authorisation was approved by the Lithuanian University of Health Science (Lithuania) no. P1-BE-2-59/2018 (11-10-2022).

The severity of oropharyngeal dysphagia, aspiration risk (AR) and pharyngeal residue were assessed endoscopically using water with a commercial thickener (to achieve different levels of liquid thickening). The 8-point Penetration-aspiration scale, PAS (Rosenbek, 1996), was used for the evaluation of AR. Pharyngeal residue was evaluated by using 5 points the Yale Pharyngeal Residue Severity rating Scale, YPRSRS [23], and severity of oropharyngeal dysphagia – by using the Dysphagia Outcome and Severity Scale, DOSS.

Two different fluid-gel samples were selected for this study, representing different categories of dysphagic diets in terms of consistency (agar 1.0 %slightly thick and agar 2.0 %mildly thick).

2.3. Statistical analysis

All experiments were repeated at least three times, and results are expressed as the mean ± standard deviation. A *p*-value of <0.05 was used to represent significant differences in means determined by analysis of variance (ANOVA) using Statistica 12.0 (StatSoft Inc., Oklahoma, AK, USA, 2013).

3. Results and discussion

3.1. Visual appearance and structure confirmation test

To confirm the formation of a fluid-gel structure, the bakers containing the prepared systems were inverted and visually observed. After inverting the baker, all types of gels started to flow, which is clearly visible in the photos (Fig. 1). In order to be sure that the structure formed was due to the preparation method (mixing during the cooling stage), a control hydrogel (with the same concentration of collagen (20 %) and the lowest concentration of agar (0.5 %) was prepared by traditional heating-cooling stages. It can be seen that the control gel is a rigid gel that retains its shape even when the container is inverted (Fig. 1), similar to systems with higher agar concentrations (data not shown). It can also be visually observed that the collagen used in the production of the gels resulted in an intense yellow color. In contrast, increasing concentration of agar resulted in a more opaque appearance.

The mechanism of structure formation of agar-based gels has already been addressed in several studies. During the heating phase, the agarose molecules in the solution in a random coil conformation. When the temperature is lowered, they transform into a helix, resulting in the aggregation of the helical domains through junction zones [11,24]. During the gel formation phase, a stable, fine-stranded hydrogel forms in the absence of any trigger. While in the case of the fluid-gel preparation agar solutions are sheared during the sol-gel transition (during the cooling step), inducing kinetic competition between the gel network formation process and shear-induced network disruption [25]. For this reason, structured fluid is formed through the "hairy" structure of smaller domains into a multidomain structure stabilized by weak domain-domain interactions [25]. Unlike other studies, our gels contained high quantities (20 % *w*/w) of the partly hydrolyzed (~20 %) protein collagen, which interpenetrated the agar network. We believe that collagen molecules act as inactive fillers in the agar structure, with limited interaction with the gel matrix. Some authors attribute a unique fluid gel structure to the lubricity that potentially could have a positive effect on the oral performance for the dysphagia patients [10,11].

3.2. Particle size

Fig. 2 shows the particle size distributions for agar-collagen fluid gels prepared with different agar concentrations. At the lowest agar concentration (0.5 %), a monomodal droplet size distribution was obtained, with an average droplet size of 174.69 μm, which is much smaller than the droplet size (677.70 μm) in the agar fluid gel (0.5 %) prepared using the pipe wall shear method [11]. A bigger droplet size is considered to be an undesirable phenomenon in terms of stability and a possible unpleasant swallowing sensation. Various studies present different methods of reducing particle size in polysaccharide-proteins systems designed for dysphagia diets which are different compared to our systems in terms of lower total protein content. The reduction in particle size in soy protein isolate-xanthan gum composite gels was achieved by increasing xanthan gum concentration, thereby preventing excessive protein aggregation at high temperatures [26]. While in high internal phase Pickering emulsions structured with xanthan gum complex particles and *Rana chensinensis* ovum protein isolate particles, reduction was achieved by changing the mass ratio between biopolymers [27]. In our study, upon addition of a higher amount of agar, a shift towards smaller particles and polydispersity of the system (higher span index) was observed (Fig. 2). The results are in line with previous studies showing that particle size decreases and a broader particle size distribution appears with increasing concentrations of biopolymers such as agarose, agar, alginate, and whey proteins [25,28,29]. This could be related to the simultaneous enlargement of nucleation and growth of the gel particles and irregular decomposition of the nucleating particles by the shear forces applied [30]. After agar concentration has been increased to 2.0 and 2.5 % polydispersity of the system increased by two times (span index from 1.035 to 3.124–3.021) with appearance of a bimodal particle size distribution (Fig. 2). A higher concentration of agar (>2.0 %) caused an increase in the number of junction zones, promoting both the formation and aggregation of helical domains, which directly affects the formation of larger particles. Another reason for the increase in particle size is that, due to the higher concentration of agar molecules (random close packing fraction), the gelation process starts to dominate

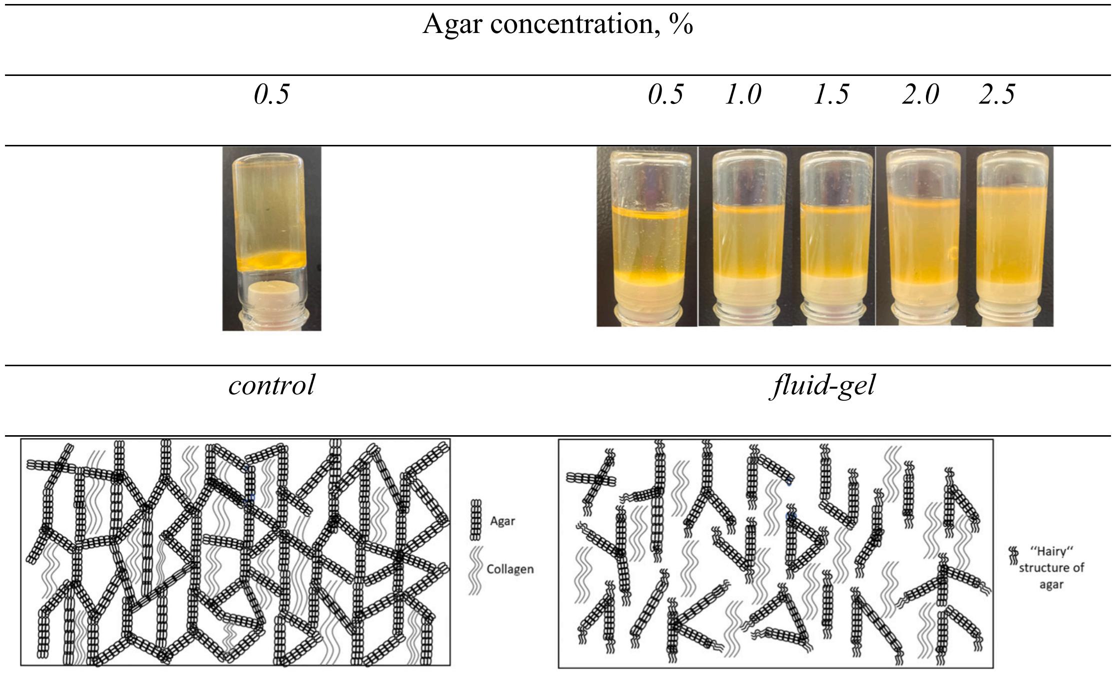


Fig. 1. Visual appearance of the manufactured control agar-collagen gel with 0.5 % agar concentration and agar-collagen fluid gel systems with different agar concentrations (0.5-2.5 %) and schematic representation of the control and fluid gel structure (below).

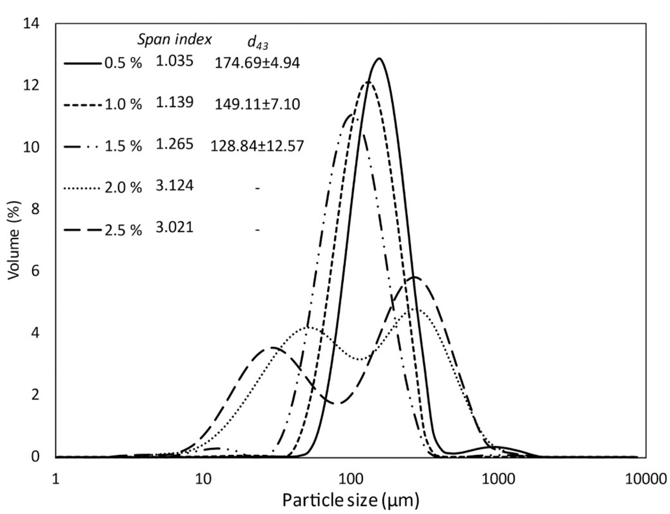


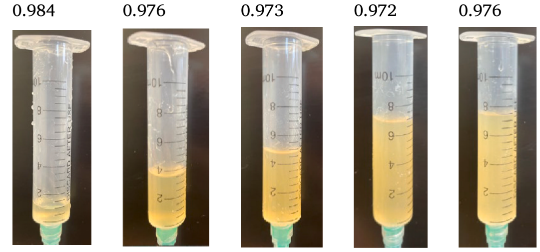
Fig. 2. Particle size distribution in the agar-collagen fluid gels with different agar concentrations (0.5-2.5 %). Mean droplet sizes are not presented in the samples with the bimodal droplet size distribution (2.0% and 2.5 %).

over the applied shear rate in the kinetic competition during fluid gel formation, as was previously reported by other authors [24]. It is claimed that, compared to a monomodal particle size distribution, the transition to a bimodal particle size distribution should reduce the apparent viscosity, as long as the same particle concentration is maintained in the system [31]. However, in our case, the changes were related to a higher agar concentration, which results in a higher concentration of the dispersed phase (particles), which can have the opposite effect on the thickness of the system.

3.3. Shear-dependent viscosity

Shear rate-dependent viscosity profiles show shear thinning behavior (viscosity decreases with increasing shear rate), which is characteristic of regularly arranged polysaccharides and has been previously reported for agarose [25], low acyl gellan gum [32] and gellan gum [33] fluid-gels. In addition, when the agar concentration is increased to 2.0 %, a

Table 1
Rheological characteristics and IDDSI test results in agar-collagen fluid gels prepared with different agar concentrations.

	Agar concentration, %				
	0.5	1.0	1.5	2.0	2.5
η_{10}(mPa·s)	115.90 ± 5.37^{a}	338.85 ± 16.33^{b}	588.90 ± 1.84^{c}	915.80 ± 2.97^{d}	928.80 ± 38.89^{de}
η_{50}(mPa·s)	70.54 ± 0.47^{a}	170.28 ± 0.98^{b}	351.46 ± 1.54^{c}	640.41 ± 19.00^{d}	794.50 ± 36.75^{e}
Category based on η_{50}	Nectar-thick (51–350 mPa·s)		Honey-thick (351–1750 mPa·s)		
κ (Pa·s^{n})	0.412 ± 0.022^{a}	1.531 ± 0.028^{b}	2.901 ± 0.165^{c}	4.930 ± 0.030^{e}	4.592 ± 0.232^{d}
n	0.495 ± 0.004^{d}	0.388 ± 0.015^{c}	0.345 ± 0.029^{b}	0.302 ± 0.006^{a}	0.342 ± 0.004^{b}
R^2	0.984	0.976	0.973	0.972	0.976
IDDSI syringe test					
Category based on IDDSI	Level 1 (1–4 ml) (slightly thick)		Level 2 (4–8 ml) (mildly thick)		

Values are reported as means±standard deviation; $^{a\text{-}e}$ different number superscripts represent significant ($p < 0.05$) differences in rheological characteristics between agar-collagen fluid gels with different agar concentrations.

noticeable increase in the consistency index (κ) and a decrease in the flow index (n) are observed (Table 1), indicating a higher volume fraction caused by a higher degree of inter particle interaction. Although the apparent viscosity values η_{10} and η_{50} are noticeably increased across the entire range of agar concentrations tested (from 0.5 to 2.5 %). Increased viscosity slowed the passage of the formed food bolus through the mouth and pharynx and prolonged the opening of the upper esophageal sphincter and pharyngeal peristalsis [34]. This phenomenon positively affects swallowing safety by preventing choking and even reducing the risk of mid-stage pneumonia [35]. The same trend of increasing viscosity was observed when measuring viscosity at a shear rate of 50 s^{-1} (η_{50}), which is used to classify food products according to their thickness levels [15]. It was found that fluid gels with agar concentrations of 0.5 and 1.0 % corresponded to level 2 (nectar-like; viscosity 70.54–170.28 mPa·s), while the rest were more viscous and corresponded to level 3 (honey-like; viscosity 351.46–794.50 mPa·s). It is also very important to mention that the bimodal particle size in samples 2.0 % and 2.5 % could have contributed to the higher viscosity values (η_{10} and η_{50}), indicating relation of apparent viscosity with the higher total volume fraction of the particles. The obtained thickness level of agar-collagen fluid gels shows their eligibility for the mild and moderate dysphagia [36]. It was also previously reported that samples having a viscosity η_{50} lower than 10 Pa·s are considered beneficial for improving swallowing ability [37,38].

3.4. IDDSI syringe test

The fluid gels were additionally tested by IDDSI and the samples were classified accordingly and summarized in Table 1. As could be expected, increasing the agar concentration from 0.5 % to 2.5 % increased the thickness levels, transitioning the fluid gels from a slightly thick (Level 1) to a mildly thick (Level 2) category, corresponding to a consistency slightly thicker than water and similar to nectar. The same IDDSI levels were achieved in dysphagia-oriented thickened liquids prepared by various blending ratios of guar gum and xanthan gum (0:10 and 5:5) but with a lower final concentration of the two colloids 0.4 % (*w/v*) and no additional proteins. When the amount of guar gum in the system exceeded that of xanthan gum (while maintaining the same total amount of biopolymers), the IDDSI level increased from 2 to 3 [39]. It is clear that the agar concentrations tested (0.5–2.5 %) are insufficient to achieve this level of consistency, and it can only be theoretically assumed that a higher agar concentration or additional thickeners are required for this result.

The obtained results show the discrepancies between tests conducted according to the IDDSI methodology and the apparent viscosity measurements of the samples. Similar discrepancies between the two categories were also reported in the previous studies [36]. It should be noted that IDDSI is a subjective methodology for the fluid thickness evaluation and does not use objective metrics. These tests are specifically designed for end users, such as nurses, patients or carers, having no access to instrumental analysis, and are therefore not suitable for research purposes [40]. However, it must be emphasized that in both cases, agar gels of the same concentrations fell into different categories (0.5–1.0 and 1.5–2.5 % respectively), showing a clear difference between in the thickness of these samples.

3.5. Shear yield stress and shear recovery behavior

The yield stress is one of the critical factors that affect food swallowing, indicating the minimum stress that is required for the fluid to flow. Higher yield stress values were obtained by increasing the agar concentration up to 2.0 % (Fig. 3A), indicating that such samples require a higher pressure at which the tongue initiates the swallowing. It is evident that when the agar concentration is increased to 2.5 %, the yield stress value no longer increases, as the curves for samples 2.0 % and 2.5 % overlap. Higher yield stress values as well as higher viscosity could be attributed to the higher inter particle interactions formed in the gel structure [24]. If patients need to use more tongue and oral palate muscles to initiate the swallow of a food bolus, this can make swallowing more complicated [41], especially considering that dysphagia patients tend to have reduced tongue strength. However, the overall yield stress values for all samples were quite low (<2.0 Pa) compared to those of other authors who have studied systems for dysphagia patients (10.7–200.8 Pa) [10].

A three-step flow sweep was further performed in order to evaluate how fast the structure recovers after a simulated oral shearing process (at a shear rate of 50 s^{-1}). The viscosity dropped considerably when the samples were subjected to a high shear regime, with subsequent structure recovery when the shear rate was adjusted to a low strain rate (0.1 s^{-1}). This phenomenon may be related to the rearrangement of the weak "hairy" structure (in the pharyngeal phase) through the formation of the new domain-domain interactions [25]. This is partially confirmed by the percentage recovery of the structure, which increases from 112 % to 134 % when the agar concentration is increased to 2.0 %.After agar concentration was increased up to 2.5 %, the same trend as in the shear yield stress was observed - the degree of structure recovery no longer depended on the agar concentration and was the same as in 2.0 % sample. Evaluating the structure's ability to recover, other studies present results that contradict our findings, as structures created as dysphagia food were deformed and did not fully recover [42,43]. It is expected that the higher number of weak interactions between the agar particles results in a more rapid rearrangement of the links between the

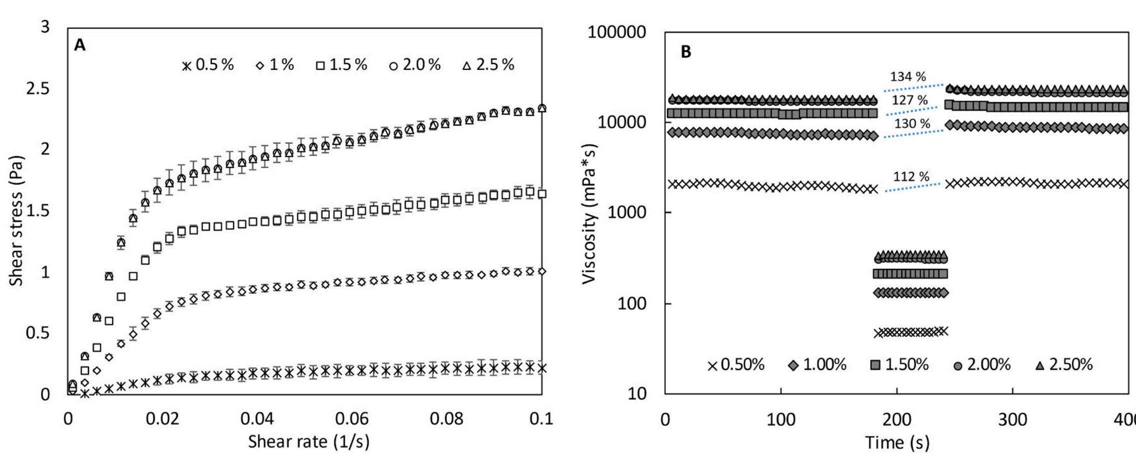


Fig. 3. Shear yield stress (A) and the thixotropic recovery, evaluated by the shear viscosity as a function of time (B) in the agar-collagen fluid gels, prepared with different agar concentrations (0.5-2.5 %). The data presented are the mean ± standard deviation(n = 3).

particles and the formation of new interactions after the deformation has been removed. Regarding safe swallowing, maintaining the structural integrity of the system is essential for safe pharyngeal swallowing as it prevents individual fragments and residue of the sample being restrained in the pharynx, risking inhalation into the lungs [10].

3.6. Frequency sweep test

Dynamic frequency scanning was conducted within the linear viscoelastic regime (LVR) to characterize the viscoelastic behavior of the fluid gels. All the samples exhibited solid-like gel properties with G′ greater than G″ over the given frequency range (Fig. 4), which is indicated as beneficial for people with dysphagia ([44]) The storage and loss moduli values increased with the increasing agar concentration, which is in agreement with other authors studies on fluid gels and other dysphagia-oriented food systems [11,26,45]. Both moduli values increased with increasing frequency (most clearly visible in the sample with an agar concentration of 0.5 %), which could be related to relaxation time linked to structural reorganization. At higher frequencies, structures have less time to completely reorganize during measurement, making them more resistant to deformation (higher moduli), whereas at low frequencies the process is reversed [46]. A lower frequency dependence and more stable structure were found in systems where the agar concentration was >0.5 % (in 1.0 %, 1.5 % and 2.0 % samples), contributing to more predictable and controllable swallowing for people with dysphagia [45]. The sample with a 2.5 % agar concentration exhibited exceptional behavior, as a decrease in G' module and a very small gap between both moduli was observed at the highest frequency, indicating structural disruption. Under the same conditions, in other samples, for example, 0.5 % 1.0 % and 2.0 %, the decrease in G" modulus was also found; however difference is that G' modulus was increasing or remained almost the same, with a much larger gap between moduli (which indicates not a structural disintegration, but rather a more characteristic elastic behavior). Notably, the tanδ value was lower than 1 in all tested samples (data not shown) which is the recommended limit for ensuring a safe swallowing process. When tanδ exceeds 1, food becomes liquid in consistency, with a faster flow rate, and can accidentally enter the respiratory tract, causing coughing and choking [46].

3.7. Amplitude sweep

As it was previously reported food bolus is subjected to mechanical processing in the mouth, which often exceeds the linear viscoelastic regime; therefore, it is essential to perform rheological analysis in the non-linear conditions [47]. To describe the structural properties of the agar-collagen fluid gels obtained, amplitude scanning (strain 0.01–1000 %) at a constant frequency (1 Hz) was performed. All samples displayed an elastically dominated response (G' > G"; tanδ<1) within the LVR of the strain sweeps. When the critical strain was exceeded, the fluid gel structure began to be destroyed, where G' decreases and tanδ is followed by an increase (Fig. 4). The fluid gel sample structured with the lowest agar concentration (0.5 %) indicates the formation of a weaker physically entangled network structure (lower gap between G' and G") with reduced mechanical resistance to strain and much smaller strain necessary to start solid-liquid transformation (Fig. 5 A).

It should be noted that fluid gels have an advantage over conventional gel systems developed for patients suffering from dysphagia. First of all, a much lower (0.01–0.1 %) critical strain (G' and G" intersection) is required for the sample to be deformed and passed smoothly through the esophagus which is more advantageous for people with decreased strength of the tongue [48,49]. Additionally, brittleness of the gels is avoided, which leads to a structurally inhomogeneous bolus of food during chewing, increasing the risk of choking [50].

3.8. Fiberoptic endoscopic evaluation of swallowing (FEES)

Rheological methods enable the measurement of specific food properties, but their impact on the safety of the swallowing process can only be hypothesized. After testing the samples using rheological methods, it can be assumed that the manufactured fluid gels are suitable

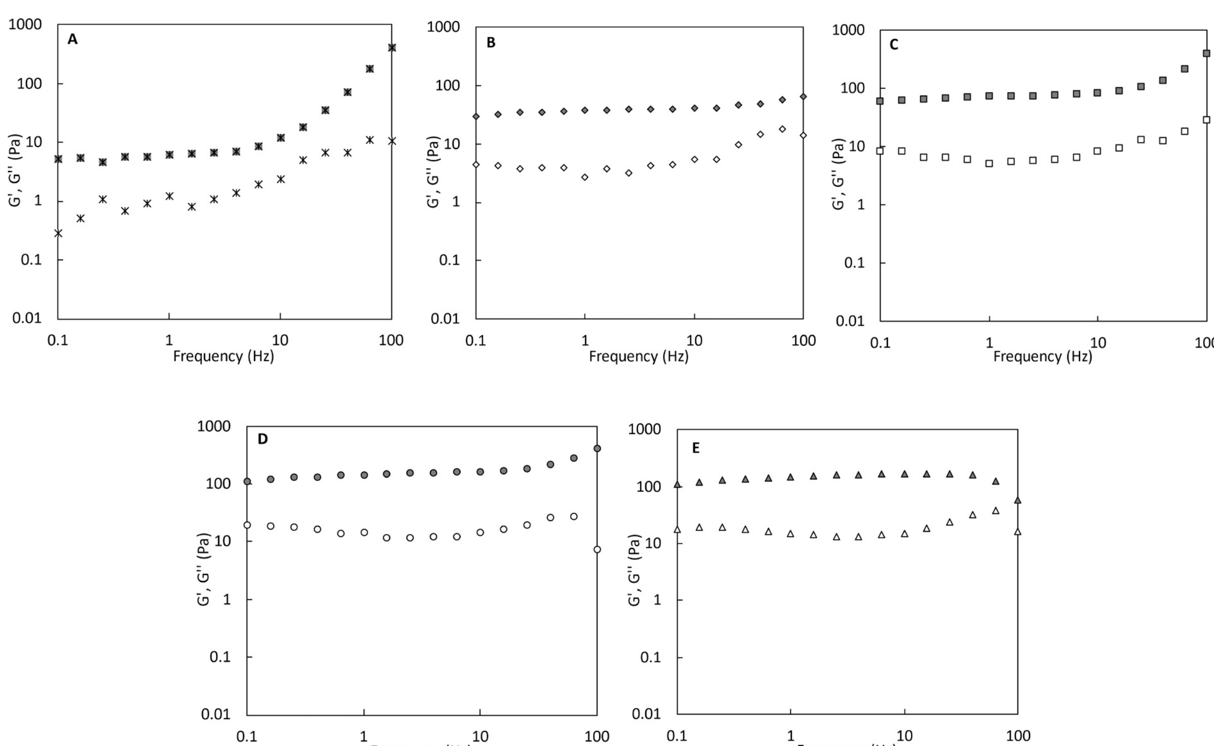


Fig. 4. Frequency dependent G′ and G″ of agar-collagen fluid gels prepared with a different agar concentrations (A – 0.5 %, B – 1.0 %, C – 1.5 %; D – 2.0 %; E – 2.5 %) at a constant strain of 0.1 %. The data presented are the mean ± standard deviation(n = 3). Where the solid represents G′ and the open represents G″.

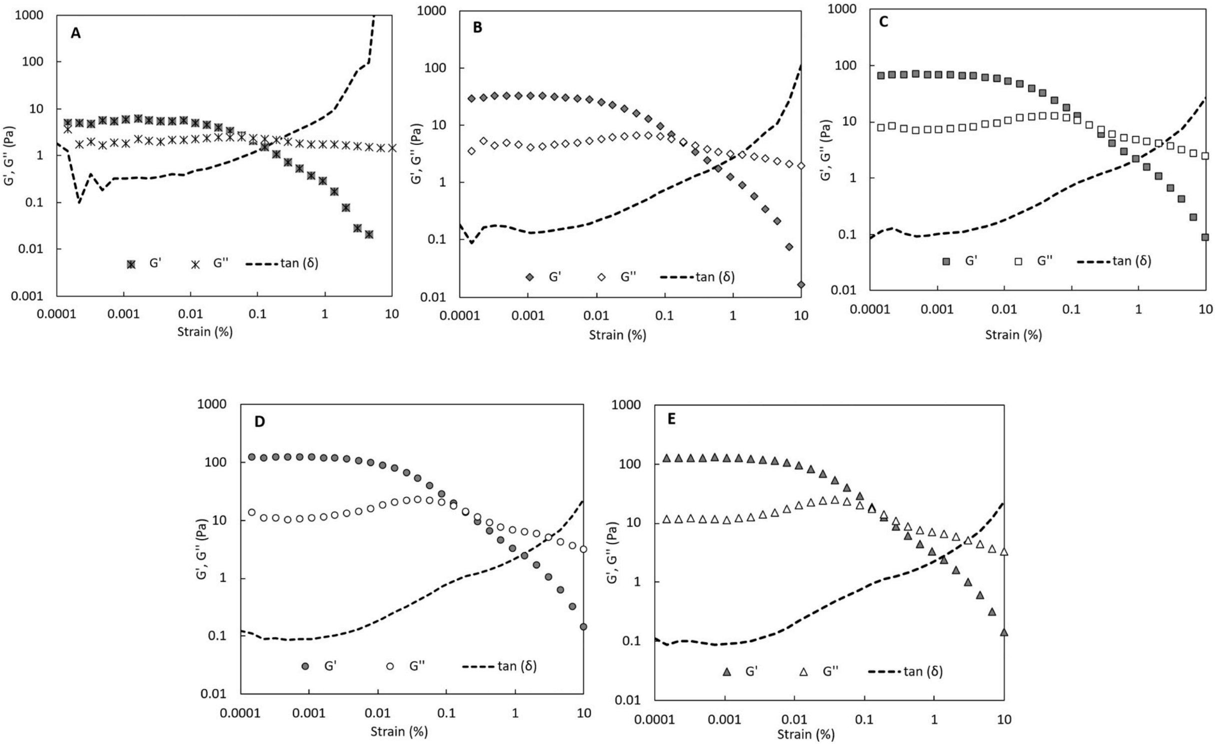


Fig. 5. Strain dependence of storage modulus (G′), loss modulus (G″) and loss tangent tan (δ) for fluid gels prepared with a different agar concentration (A – 0.5 %, B – 1.0 %, C – 1.5 %; D – 2.0 %; E – 2.5 %) with a frequency of 1 Hz. The data presented are the mean ± standard deviation (n = 3). Where the solid represents G′ and the open represents G″, dashed line - tangent tan (δ).

for safe use by patients with mild and moderate dysphagia levels. To confirm or deny this, the fiberoptic endoscopic method was chosen to be used, helping to assess the actual risk and providing a realistic assessment of whether the product's properties are compatible with a safe swallowing mechanism, which demonstrates its advantage over rheological methods. It is essential to highlight that FEES is an invasive procedure and can cause discomfort, epistaxis as well as hypotension. Additionally, visualization may be hindered by pharyngeal constriction, which covers the endoscope tip, resulting in a "white-out" to appear. However, unlike videofluoroscopy, this methodology addresses shortcomings such as non-mobile aspect and Ionising Radiation exposure risks [51].

Two different samples (with 1.0 and 2.0 % agar concentration) were selected for this study, representing different categories of thickened fluids in terms of consistency (in accordance with the IDDSI and NDD tests). Based on the results of rheological tests, systems in this category are safe for use by dysphagia patients, which must be additionally verified by endoscopy. Selected patient for the case analysis has mild-moderate oropharyngeal dysphagia. High aspiration risk for water, medium aspiration risk for nectar, and no aspiration risk for honey during FEES was determined. Recommendations for patients were: MM5 diet (Minced and Moist food), moderately thick fluids (Level 3 by IDDSI), bolus size up to 10 ml, active re-swallowing (3–4 times), and coughing after swallowing is required. Transcutaneous neuromuscular electrostimulation, swallowing exercises were also recommended.

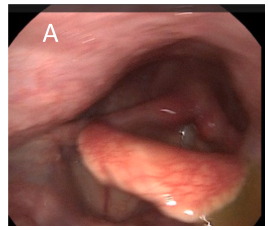
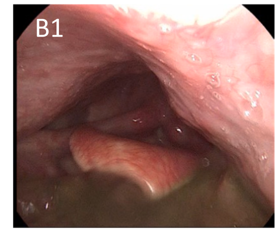
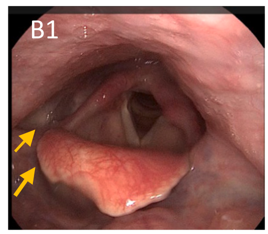
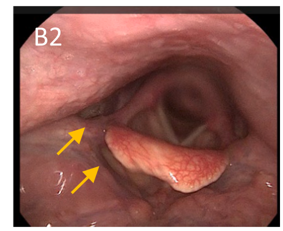
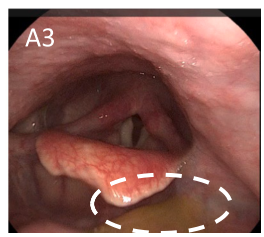
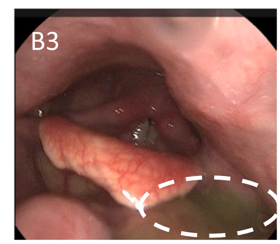
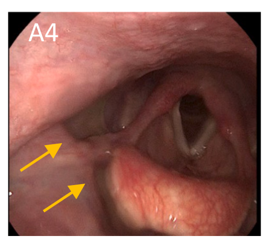
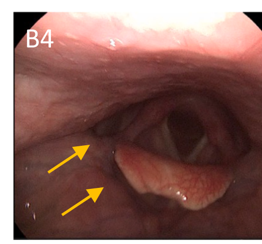
It was found that when the patient drank 10 ml of 1.0 % agar fluid gel, the sample was seen to enter directly onto the edge of the epiglottis and there was no "white-out" (Table 2 A1). The signs of penetration were observed – sample residue on the vocal cords (Table 2 A2, A4) and there was no patient reaction (cough). Fluid gel sample enters the airway, contacts the vocal folds and is not ejected from the airway.

After the patient swallowed 10 ml of 2.0 % agar fluid-gel, it deposited on the epiglottis, and after swallowing, small residues were observed in the valleculae and the pyriform sinuses (Table 2 B2). Although the patient had to swallow one portion 3 times, patient swallowed spontaneously, and no residue remained after swallowing. It is also relevant to highlight that there were no signs of aspiration and no residue at the airway (Table 2 B4). In this way, both fluid gel samples were safe to swallow with medium or no risk of aspiration, which correlates with the conclusions obtained using rheological methods. It should be additionally emphasized that, the use of FEES provides an additional opportunity to assess the sensation of visually visible particles in the mouth and throat, which could not be covered by other methods. No particle sensations (throat scratching, residual dryness, difficulty swallowing) were observed during swallowing, which is a positive result for the further development of these systems (adding taste, color, and aroma). In the previously reported studies agar fluid gel prepared by pipe wall shear at the video-fluorography analysis (providing real-time visualization of the anatomy/ physiology of swallowing) was performed as a xanthan gum thickened liquid showing the potential of reducing the risk of aspiration in patients with dysphagia by controlling bolus rheology [11]. It was also highlighted that the use of such pre-thickened products would help to reduce the number of incidents of incorrect viscosity preparation (caused by human error or malfunction of the dosing equipment), which is often the case with powdered thickeners [52].The limitations of this study is that physiological differences between individuals may also affect the results obtained. However, despite this, using methods of this nature provides a unique opportunity to realistically assess the safety of the manufactured formulation, which was previously almost never done and can be considered a strength of this study.

4. Conclusions

In this study, high-protein (20 w/w% collagen) agar-collagen fluid gels were produced for dietary use in patients with dysphagia. A comprehensive evaluation, including rheological properties (shear yield stress, shear-dependent viscosity, strain- and frequency-dependent

Table 2
Fiberoptic endoscopic swallowing examination of the agar-collagen fluid gels prepared with 1.0 % and 2.0 % agar concentration.

	Agar concentration, %	
	1.0	2.0
Initiation of the swallowing		
After the swallowing		
Re-swallowing - the remains of the content runs down[a].		
After re-swallowing		
PAS	3	1
YPRSRS points	3	1

[a] The dashed line in A3 and B3 indicate the remains of the fluid gel in the left vallecula that runs down during the re-swallowing process.

viscoelasticity, shear recovery behavior), IDDSI tests, stability, and particle size analysis, has led to an improved understanding of the effect of different agar concentrations (0.5 to 2.5 %) on the swallow-related behavior of high protein agar-collagen fluid gels. Rheological analysis showed that fluid gels have good shear recovery, viscoelastic properties, yield stress, and flow characteristics that potentially contribute to safer and easier swallowing for dysphagia patients. Varying the concentration of agar has led to significant changes in the properties of the fluid gels, which allows for modifying their properties according to the severity of dysphagia, offering improved dysphagia management. The clinical experiment confirmed that the agar collagen fluid gels obtained were safe to swallow, especially the one containing higher agar concentration, as there was no risk of aspiration. The FEES method used in this study may open up new opportunities for research into the properties of safer foods for patients with dysphagia. Although our study provides valuable insights into the rheological properties of high-protein agar-collagen fluid gels, *in vitro* digestion analyses as well as the long-term stability of these systems are still currently lacking, delving deeper into the possible interaction between agar and collagen.

Compliance with ethics requirements

All procedures were performed in compliance with relevant laws and the guidelines of Lithuanian University of Health Science. Bioethics authorisation was approved by Lithuanian University of Health Science (Lithuania) no. P1-BE-2-59/2018 (11-10-2022). The privacy rights of patient have been observed and informed consent was obtained for experimentation with human subjects.

CRediT authorship contribution statement

Gintarė Dyglė: Writing – original draft, Investigation, Formal analysis. **Viktorija Eisinaitė:** Writing – review & editing, Data curation. **Gytė Damulevičienė:** Methodology, Investigation. **Daiva Leskauskaitė:** Supervision.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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