Universitat Autònoma de Barcelona

Rheological in vitro and ex vivo characterisation of thickening products used for compensatory treatment of swallow dysfunction in patients with oropharyngeal dysphagia Study of the rheological parameters affecting their therapeutic effect and the optimal doses for different phenotypes of patients with swallowing disorders

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Doctoral thesis presented by **MIREIA BOLÍVAR PRADOS** to obtain the PhD degree

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CONSORCI SANITARI DEL MARESME

A mis yayos,
A mi tata y pino,

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Bueno, después de hacer mil copias de esta tesis (por si cualquier cosa pasara), llega la parte que más ansiaba escribir: los agradecimientos. Y es que, me había propuesto, no escribirlos hasta redactar la última coma de este documento, y así ha sido. Así que ahora sí: aquí se cierra una de las etapas (no sé si la más importante) pero sí una de las más difíciles de mi vida.

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Y aquí se acaba mi Tesis.

Incluso un camino sinuoso, difícil, nos puede conducir a la meta si no lo abandonamos hasta el final – Paulo Coelho

LIST OF ABBREVIATIONS

AE: adverse events

AI: artificial intelligence

AIMS-OD: artificial intelligence massive screening for oropharyngeal dysphagia

ATP: adenosine triphosphate

BIA: bioelectrical impedance analysis

BMI: body mass index

BUD: break up diameter

CaBER: capillary break-up rheometer

CGPM: general conference on weights and measures

CGRP: calcitonin gene-related peptide

CNS: central nervous system

CT: clinical trials

DC: daily conditions

EAT-10: eating assessment tool-10

EC: Ethics Committee

ESSD: European society for swallowing disorders

EU: european

FCT: Fresubin clear thickener

FEES: fiberoptic evaluation of swallowing

FSMP: food for special medical purposes

GPJ: glossopalatal junction

HNC: head and neck cancer

HRM: high resolution manometry

HV: healthy volunteers

ICMJE: international committee of medical journal editors

IDDSI: international dysphagia diet standardization initiative

JDD: japanese dysphagia diet classification

JSDR: Japanese society for dysphagia research

KE: kinematic energy

LES: lower esophageal sphincter

LV: Laryngeal vestibule

LVC: laryngeal vestibule closure

MNA-sf: mini nutritional assessment short form

MS: modified starch

Mx: mixtures

NDD: national dysphagia diet

NIHSS: National Institute of Health Stroke Scale

OD: oropharyngeal dysphagia

OSR: oral swallowing response

pSEP: pharyngeal sensory evoked potential

PSOD: poststroke oropharyngeal dysphagia

QoL: quality of life

SI: international system

ScR: scoping review

SR: systematic review

TA: thickening agents

TF: thickening fluid

TMD: texture modified diets

TMS: transcranial magnetic stimulation

TP: thickening products

UES: upper esophageal sphincter

UESO: upper esophageal sphincter opening

VAS: visual analogue scale

VFS: videofluoroscopy

V-VST: volume-viscosity test

XG: xanthan gum

INDEX

ABSTRACT

Oropharyngeal dysphagia (OD) is a motility disorder characterized by the difficulty to form or move a bolus from the mouth to the esophagus. Thickening products (TP) are a widely used compensatory strategy to manage patients with OD by increasing fluid viscosity. Viscosity is a rheological parameter and can be quantitatively assessed in the international system of units (Pa·s). Therapeutic effect (TE) of TP can be analyzed by clinical trials (CT), and optimal viscosity levels can be established. Nevertheless, TP are currently commercialized describing viscosity with qualitative descriptors and recommending arbitrary ranges based on consensus and not scientific evidence. Viscosity can be affected during swallowing by two main rheological factors: salivary amylase and shear rate. Both factors can decrease viscosity and thus, the TE of TP. Very few studies have examined rheological factors other than shear viscosity which might have an impact on the therapeutic effect. In consequence, this thesis has the following aims: 1) To describe the effect on safety of swallow of two xanthan-gum-based (XG) TP and a mixture (Mx) at several levels of viscosity; 2) To determine the therapeutic range and optimal viscosity doses for each OD phenotype; 3) To describe the effect of shear viscosity on efficacy of swallow; 4)To describe the rheological factors (salivary amylase and shear rate) involved in TP's TE and to determine whether this information is included on the label. To assess the risks of the use of qualitative descriptors instead of objective viscosity levels; 5) To validate in several international laboratories a scientific protocol to measure shear viscosity and the rheological swallowing factors affecting TP; 6) To assess shear viscosity, extensional deformation, maximal force, adhesiveness and cohesiveness for varying doses of TP and determine their relationship with safety of swallow assessed by videofluoroscopy.

This research project includes 4 Studys with the following methodology and results: Study 1 – Three CT including data on 327 patients studied the TE of 3 TP for several viscosity levels (100–2000 mPa·s). Results showed that TE presented a viscosity-dependent behavior for safety of swallow and that only two viscosity levels were needed to cover more than 90% of the population. Oral residue increased with the increment of viscosity; Study 2 – The analysis of 10 TP showed that manufacturers recommend the use of 3 viscosity levels randomly selected without any evidence supporting their TE. No description on the amylase effect or shear rate was identified; Study 3 – design and validation of a scientific protocol to measure shear viscosity of TP in 4 international laboratories, including the simulation of swallowing factors with a laboratory-variability below 10%; Study 4 – analysis of 4 different rheological properties for different doses of TP and their relationship with the TE on safety of swallow assessed by videofluroscopy in 267 patients. Results confirmed that shear viscosity is the main parameter associated with the safety of swallow.

Main conclusions from this thesis are: 1) TP improve the safety of swallow by the increment of shear viscosity in a dose-response manner in OD patients. 2)A therapeutic range can be established between 100 and 1000 mPa·s for TP to manage OD patients. Increasing viscosity above 800-1000 mPa·s did not cause any further significant improvement in safety of swallow; 3) The increment of shear viscosity is related to an increase in oral residue. 4) The use of qualitative viscosity descriptors for labeling TP leads to several risks and contradictions, jeopardizing the safety of patients; 5) A rheological protocol to measure shear viscosity in experimental conditions reproducing TP behavior during swallowing can be applied worldwide;

6) Shear viscosity is the main property causing the TE of TP with a strong dose-dependent effect on safety of swallow.

RESUM

La disfàgia orofaríngia (DO) és un trastorn de la motilitat caracteritzat per la dificultat per formar o moure el bol de la boca a l'esòfag. Els espessidors, són una estratègia utilitzada per la DO, augmentant la viscositat del fluid. La viscositat, és un paràmetre reològic i es pot avaluar quantitativament. L'efecte terapèutic (ET) dels espessidors, es pot analitzar mitjançant estudis clínics (EC) i es poden establir nivells òptims de viscositat. Tanmateix, els espessidors, es comercialitzen descrivint la viscositat mitjançant descriptors qualitatius i recomanant intervals de viscositat arbitraris. La viscositat, es pot veure afectada durant la deglució per dos factors reològics: l'amilasa salival i la velocitat de cisalla. Tots dos factors, poden disminuir la viscositat i per tant, l'ET dels espessidors. Pocs estudis han examinat altres factors reològics diferents de la viscositat. En conseqüència, aquesta tesi, té els següents objectius: 1)Descriure l'efecte sobre la seguretat de la deglució de dos espessidors: un de goma xantana (XG) i un de barreja, a diversos nivells de viscositat; 2)Determinar el rang terapèutic i les dosis de viscositat òptimes per a cada fenotip de DO; 3)Descriure l'efecte de la viscositat de cisalla sobre l'eficàcia de deglució; 4)Descriure els factors reològics implicats en l'ET dels espessidors i determinar si aquesta informació s'inclou a l'etiquetat. Avaluar els riscos de l'ús de descriptors qualitatius; 5)Validar en diversos laboratoris internacionals un protocol científic per mesurar la viscositat i els factors reològics de deglució que l'afecten; 6)Avaluar la viscositat de cisalla, deformació extensional, força màxima, adhesivitat i cohesió per a dosis variables d'espessidors i determinar la seva relació amb la seguretat de la deglució avaluada per videofluoroscòpia.

Aquest projecte de recerca inclou 4 capítols amb la següent metodologia i resultats: Capítol 1 - Tres EC amb dades de 327 pacients on s'analitza l'ET de 3 espessidors per a diversos nivells de viscositat (100–2000 mPa·s). Els resultats van mostrar, que l'ET presentava un comportament dependent de la viscositat, i que només es necessitaven dos nivells per cobrir més del 90% de la població. El residu oral augmenta amb l'augment de la viscositat; Capítol 2: l'anàlisi de 10 espessidors va mostrar que els fabricants recomanen l'ús de 3 nivells de viscositat seleccionats aleatòriament. No es va identificar cap descripció sobre l'efecte de l'amilasa o la velocitat de cisalla; Capítol 3: disseny i validació d'un protocol científic per mesurar la viscositat dels espessidors en 4 laboratoris internacionals, incloent la simulació de factors de deglució amb una variabilitat entre laboratoris inferior al 10%; Capítol 4: anàlisi de 4 propietats reològiques per a diferents dosis i la seva relació amb l'ET sobre la seguretat de la deglució, avaluada en 267 pacients. Els resultats van confirmar que la viscositat de cisalla és el principal paràmetre associat a la seguretat de la deglució.

Les principals conclusions són: 1) Els espessidors, milloren la seguretat de la deglució mitjançant l'augment de la viscositat de cisalla d'una manera dosi-depenent. 2) Es pot establir un rang terapèutic entre 100 i 1000 mPa·s per a tractar pacients amb DO. L'augment de la viscositat per sobre de 800-1000 mPa·s no provoca cap millora significativa en la seguretat de la deglució; 3) L'augment de la viscositat de cisalla, està relacionat amb un augment del residu oral. 4) L'ús de descriptors qualitatius de viscositat en l'etiquetat dels espessidors comporta diversos riscos i contradiccions, que posen en perill la seguretat dels pacients; 5) Es pot aplicar a tot el món, un protocol reològic per mesurar la viscositat de cisalla en condicions experimentals que reprodueixen el comportament dels espessidors durant la deglució; 6) La viscositat de cisalla és la principal variable causant l'ET dels espessidors amb un fort efecte dosi-resposta.

1. INTRODUCTION

1.1 Swallowing

The process of swallowing or deglutition is the physiological event involving the transfer of the alimentary bolus from the oral cavity to the stomach via the pharynx and is performed with a combination of voluntary and reflexive responses [1]. It is a complex and fast process which needs the coordinated action of more than 30 pairs of muscles in the mouth, pharynx, larynx and stomach, sharing part of the anatomy with the airway and protecting it [1].

1.1.1 Anatomy of swallowing

Oral cavity

The oral cavity is the place where the bolus is prepared and formed. This phase includes salivation, taste, mastication and ingestion of food. The oral cavity is divided into the vestibule and the central or buccal cavity [2].

The vestibule is the anterolateral space positioned between the oral mucosa and the external surface of the teeth and gums. The labial seal including superior and inferior lips ensures the union of the two lips to form the buccal orifice.

The buccal cavity (mouth) is the space containing the tongue, teeth and gums. The roof of this cavity, he palatal vault, separates the nasopharynx from the oropharynx and is composed by the soft palate, a muscle-membranous septum located in the posterior part, and the hard palate, composed by maxillary bone, palate bones and some glandular and mucosal layers, occupying the anterior two-thirds of the vault. On the posterior border of the palatal vault hangs the uvula. The floor of the buccal cavity is limited by the lower gums and is composed by the mylohyoid, digastric and geniohyoid muscles and is delimited by the maxillary bone and the hyoid bone. The isthmus of the fauces which is delimited by the anterior pillars of the soft palate and the upper surface of the tongue base, marks the beginning of the oropharynx [1], [2].

The tongue is a hydrostatic muscle located in the medial part of the floor of the mouth. It is an organ with great capacity of movement, which can be differentiated into retraction, projection and articulation, allowing the formation and propulsion of the alimentary bolus. The terminal sulcus divides the tongue in two parts: 1) the root, constituting the anterior part of the oropharynx attached to the soft palate and the epiglottis through the palatoglossal arches and the epiglottis glossoepiglottic folds, respectively. It contains food channels in both lateral sites; and 2) the body, constituting the mobile part of the tongue and occupying almost the entire buccal cavity. The dorsal part of the tongue is covered by specialized mucosa that contain the taste buds. The taste buds can be found in fungiform papillae, circumvallate papillae and foliate papillae which are responsible for taste perception (sweet, salty, sour, bitter and umami) [2].

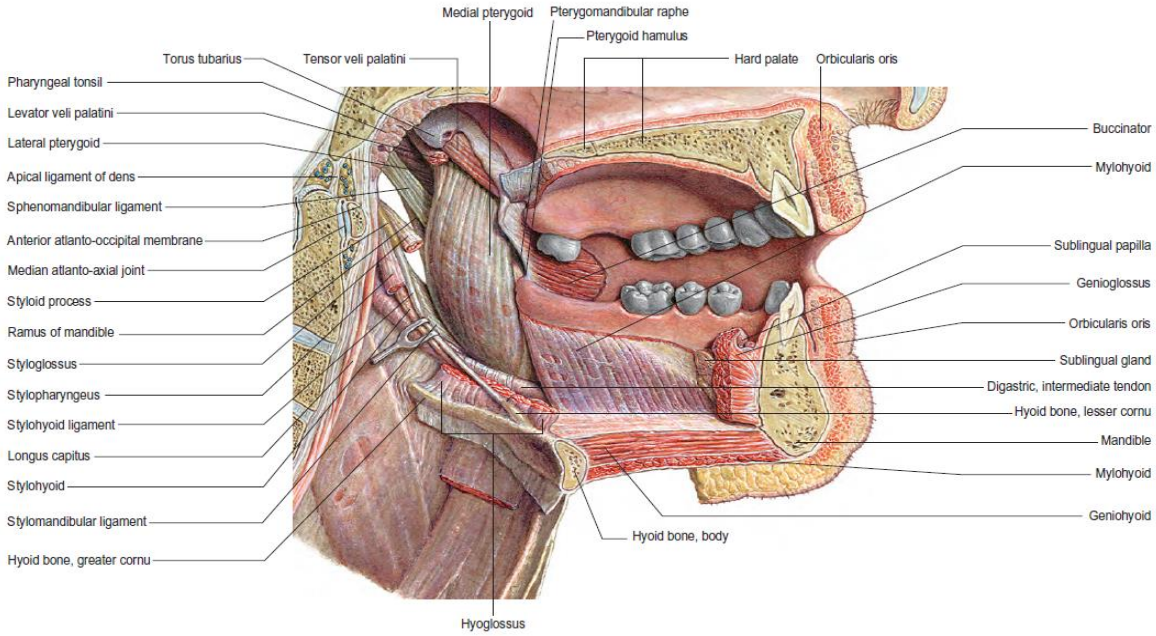

Figure 1. Oral cavity anatomy. Reproduction from Standring et al (2008) [2].

Pharynx

The pharynx is a muscular tube which connects the nasal and oral cavity to the larynx, trachea and esophagus and therefore shares the respiratory and digestive systems. The pharynx is comprised of three parts, from superior to inferior (Figure 2 and 3) [3]:

The nasopharynx extends from the base of the skull to the soft palate (Figure 2). It communicates with the nostril through the coanes and to the oral cavity through the isthmus of the pharynx. Its main function is respiratory and phonatory and therefore, in order to prevent nasal regurgitation during swallowing, the communication between the oropharynx and nasopharynx closes at the velopharyngeal junction (palate veil rises and contacts the posterior wall of the pharynx) [3], [4].

The oropharynx is located between the isthmus of the pharynx and the hyoid bone (Figure 2). The isthmus of the fauces establish the communication with the oral cavity. This specific space combines function from the respiratory (breathing) and digestive (feeding) systems by allowing the transfer of air through the larynx and the alimentary bolus through the pharynx [3], [4].

The laryngopharynx or hypopharynx composes the lowest part of the pharynx (figure 2). It is located behind and parallel to the larynx and extends from the hyoid bone to the cricoid cartilage at the height of the sixth cervical vertebra (C6) where the esophagus begins. The laryngeal orifice, which has an elliptic or rhomboid shape, is found in the anterior wall between the edges of the epiglottis at the top and the aryepiglottic folds at the bottom. The pyriform sinus is found below the aryepiglottic folds, which are an extension of the hypopharynx. The pharynx is formed by three histological layers: the inner mucosa, the pharyngobasilar fascia (slow-twitch muscles that participate in breathing and the phonation process) and an external muscular layer which is formed by fast-twitch muscles that participate in the swallowing process: three pairs of constrictor muscles (upper, middle and lower) which narrow the the pharynx through peristaltic

movements when the bolus passes; and three lift muscles (palatopharyngeal, stylopharyngeus and salpingopharyngeal) with the function of elevating and shortening the pharynx during swallowing [3].

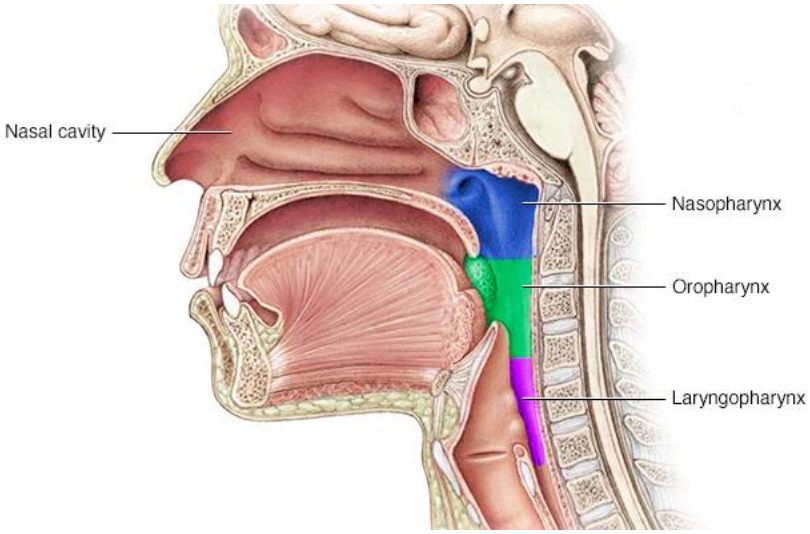

Figure 2. Pharyngeal parts. Reproduction from Mayo Foundation [5].

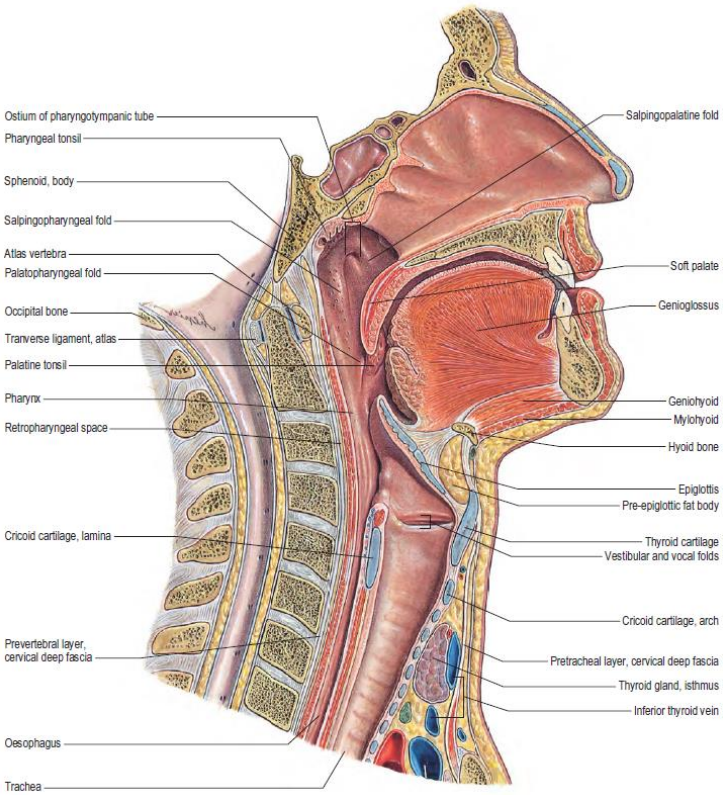

Figure 3. Pharynx anatomy. Reproduction from Standring et al (2008) [3].

Larynx

The larynx is a structure of the respiratory tract which contains the phonation organ. It is located inferior to the pharynx and the hyoid bone and its superior part is communicated with the trachea. It is composed of 11 cartilages linked by joints and fibroelastic structures. The main cartilages are the thyroid, the cricoid, the arytenoid and the epiglottis (Figure 4). The epiglottis anterior face is covered by lingual mucosa and forms the three gloss-epiglottic folds delimiting the valves. The main function of the epiglottis during swallowing is to close the airway tract to avoid respiratory complications during feeding. This protection occurs by the epiglottis rising and moving forward through the contraction of the aryepiglottic muscle, pressure from the base of the tongue and the displacement of the hyoid bone upward and forward. Internally, the larynx can be divided in three different parts: Laryngeal vestibule, laryngeal ventricle and infraglottic cavity (Figure 4) [6].

The laryngeal vestibule (LV) is the beginning of the larynx and ends in the vestibular folds of false vocal cords. The laryngeal ventricle is the following part. It is delimited by the vestibular folds and by the vocal cords. The last part is the infraglottic cavity and is the space between the vocal cords and the trachea [6].

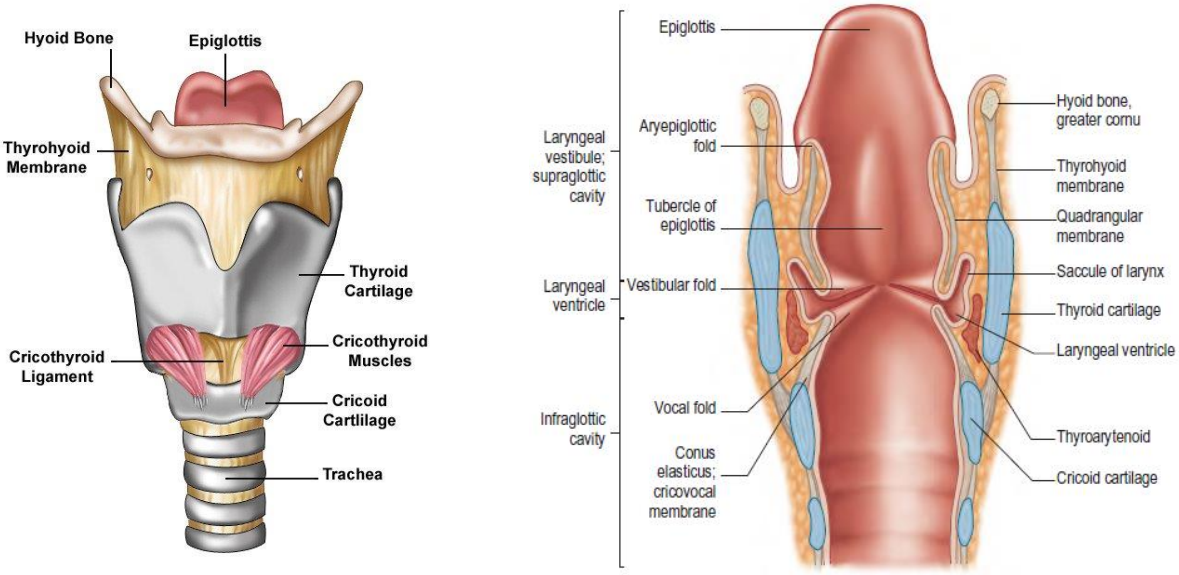

Figure 4. On the right, anterior vision of the larynx, reproduction from Intechopen Studys (2013) [7]; On the left, internal larynx anatomy, reproduction from Standring et al (2008) [6].

Esophagus

The esophagus is a muscular tube, typically 25 cm long, which connects the pharynx with the stomach. The esophagus is divided in three sections: cervical, thoracic and abdominal. The upper part is composed by striated muscles of involuntary activation and as it advances to the lower part, the proportion of smooth muscle increases until it becomes the only type (mid and distal

part). Peristaltic waves are produced due to muscle contraction that helps to move the alimentary bolus from the pharynx to the stomach.

The esophagus has two well-differentiated valves: the upper esophageal sphincter (UES) and the lower esophageal sphincter (LES). The UES separates the pharynx from the esophagus and its length is between 2 and 4 cm. This valve is formed by the cricopharyngeal muscle, the upper part of the cervical esophagus muscle and the lower part of the pharyngeal constrictor muscle. Its function is to prevent the passage of air into the digestive tract and to avoid reflux from the esophagus to the pharynx. When swallowing, this valve is opened to allow the pass of the bolus. In contrast, the LES is a specialized zone composed by circular smooth muscles surrounding the esophagus with semicircles of fibres: sling muscle fibres (at the greater stomach curvature) and short clasp fibres (at the lesser stomach curvature). This valve has a basal pressure which maintains contraction at physiological conditions and relaxes when a peristaltic wave occurs in the esophagus [8]–[11].

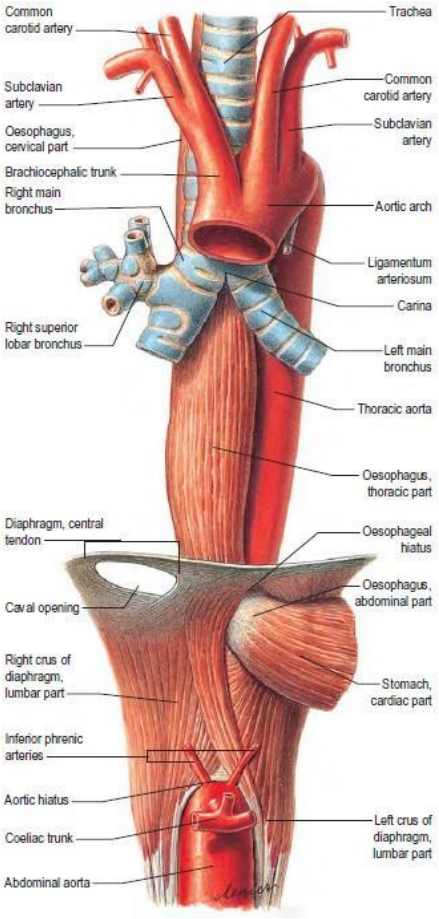

Figure 4: Anterior vision of the esophagus, reproduction from Sobotta, 2006 [12].

1.1.2 The swallow response in healthy subjects

The process of swallowing is a highly coordinated action which is activated when food stimulates the sensory nerves in the oral cavity. It is defined as the course of safely translating the food from the oral cavity through the pharynx and esophagus to the stomach. For this purpose, the aerodigestive tract reconfigures from the respiratory to the digestive tract during swallowing

and then recovers its respiratory configuration again after the bolus reaches the esophagus [13], [14].

This complex process has been divided into four sequential phases [15], [16]: oral (preparatory and propulsive), pharyngeal and esophageal (Figure 5).

Oral preparatory phase

The main aim of this phase is the formation of the alimentary bolus by the action of masticating. The mastication process is performed by cyclic mandibular and rotatory tongue movements which bring the food to the teeth to be ground. Saliva has a main role in this phase by preparing (hydration and lubrication) and starting the digestion (enzyme break-up) of the food bolus [17]. The final part of this specific phase is characterized by the tongue holding the formed bolus against the hard palate in preparation for translating it to the posterior oral cavity.

Oral propulsive or transport phase

This phase aims to transport the bolus formed in the preparatory phase from the oral cavity to the oropharynx. While the tongue presses the hard palate, the posterior part of it forms the glossopalatal junction (GPJ) with the soft palate which prevents the early entrance of the bolus to the oropharynx. The pressure of the tongue against the hard palate causes a wave that propels the bolus to the oropharynx. In order to close the nasopharynx cavity and open the GPJ, the soft palate is elevated. Once the bolus has already been transferred to the oropharynx, the tongue returns to its previous position to close the GPJ [18].

Pharyngeal phase

The pharyngeal phase has a critical role during swallowing that involves the reconfiguration of the pharynx from the air tract to the food tract completed in approximately 1 second [3]. All these changes are produced through the coordinated opening and closing of the four main valves involved in deglutition: the GPJ, the velopharyngeal junction, the LV and the UES (Figure 5).

The arrival of the bolus in the oropharynx triggers the beginning of the pharyngeal phase which sends the sensory information via the central nervous system (CNS) to start the oral swallowing response (OSR). It starts with the elevation of the soft palate to allow the opening of the GPJ and the upward movement of the posterior pharyngeal wall to close the nasopharynx (velopharyngeal junction closure) to avoid the regurgitation of the bolus through the nose. At the same time, the vocal cords and arytenoids are adducted, closing the airway. In addition, the arytenoids bring the base of the epiglottis closer. Then, a retroflexion of the epiglottis occurs in response to passive pressure from the base of the tongue and the active contraction of the aryepiglottic muscles, completing the closure of the LV (LVC) and avoiding the entrance of the bolus to the larynx. At the same time, the hyoid and the larynx move upward and anteriorly, positioning the entrance of the larynx below the base of the tongue in order to increase the protection of the airway system. These processes shorten and expand the hypopharyngeal space at the same time as the UES opening (UESO) allows the transfer of the bolus to the esophagus. Another relevant action is the pharynx propagated contraction to propulse the bolus which

facilitates its clearance working in collaboration with pharyngeal shortening to reduce the hypopharyngeal residue [19]–[22]. This phase ends with the exit of the bolus through the UES.

Esophageal phase

This last phase begins with the UESO and the passage of the bolus through it. The UESO occurs by four mechanisms: a) relaxation of the cricopharyngeal muscle by interrupting the vagal tone of the muscle which keep it closed; 2) the traction on the anterior side of the sphincter by the suprahyoid muscle contraction; 3) the pressure on the sphincter exerted by the alimentary bolus propulsion forces which depend on the lingual propulsion force exerted and; 4) the sphincter compliance that allows its complete relaxation, with low residual pressures and limited resistance during the passage of the bolus [23].

Peristalsis is controlled by several neurons along the esophagus. The upper third is controlled by the motor neurons in the CNS while the mid part including LES relaxation depend on enteric motor neurons in the myenteric plexus. There are several neurotransmitters with a potential effect in this peristalsis. The main excitatory neurotransmitters are acetylcholine and tachykinins; the main inhibitory neurotransmitters are nitric oxide, vasoactive intestinal peptide, adenosine triphosphate (ATP), pituitary adenylate cyclase-activating polypeptide and calcitonin gene-related peptide (CGRP) [24].

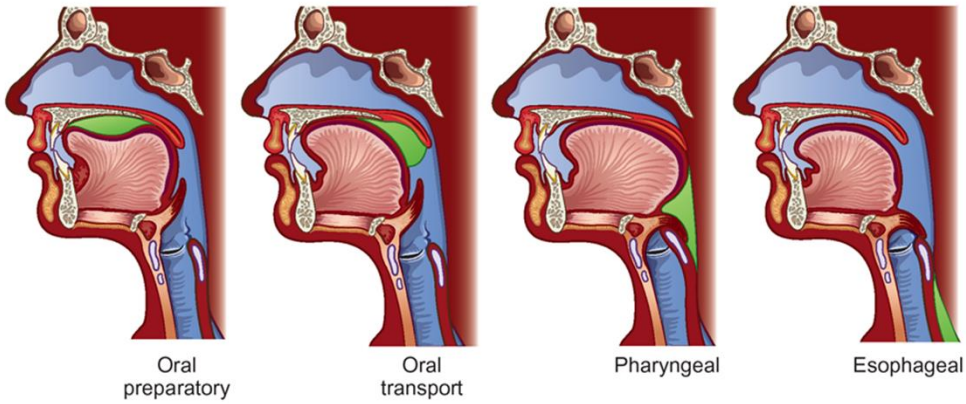

Figure 5: Lateral view of the oral and pharyngeal cavity during swallowing. Reproduction from Fastest otolaryngology & Ophthalmology insight engine [25].

Measurements of OSR can be determined by a radiographic technique explained in section 2.6 (*diagnosis*) called videofluoroscopy (VFS) [26] (Figure 6): *"i) Oropharyngeal reconfiguration, timing of the opening (O) or closing (C) events at the glossopalatal junction (GPJ), velopharyngeal junction (VPJ), LV, and upper esophageal sphincter (UES) are measured, GPJ opening being given the time value 0; ii) hyoid motion (vertical and anterior movement) determined in a X–Y coordinate system; iii) anteroposterior diameter of UES opening (mm); and; iv) bolus propulsion force of the tongue measured by means of Newton's second law of motion and expressed in mN; mean and maximal velocity (ms^{-1}) and kinetic energy (mJ) acquired by the bolus prior to entering the UES"*

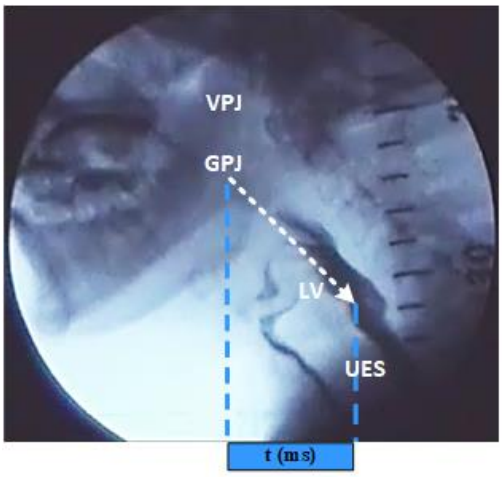

Figure 6. Patient with oropharyngeal dysphagia and aspiration. All temporal measurements were referenced to glossopalatal junction opening (GPJO) as time 0. The white point depicts time to bolus entering the laryngeal vestibule (penetration) and blue points depicts time to bolus passing below the vocal folds (aspiration). *t: time (ms), GPJ:* glossopalatal junction*; VPJ: velopharyngeal junction; LV: laryngeal vestibule; UES: upper esophageal sphincter.*

The swallowing process in healthy people ranges between 600 and 1000 ms. It includes a rapid response of submentalis muscles [27] and a short OSR (<740 ms). OSR includes: a) a fast time to LVC measured from GPJO to LVC (<160 ms); b) fast time to UESO (<220 ms) measured from GPJO to UESO; c) high bolus velocity (>35 cm/s) and; d) a lingual propulsion force above 0.33 mJ (Figure 7) [28].

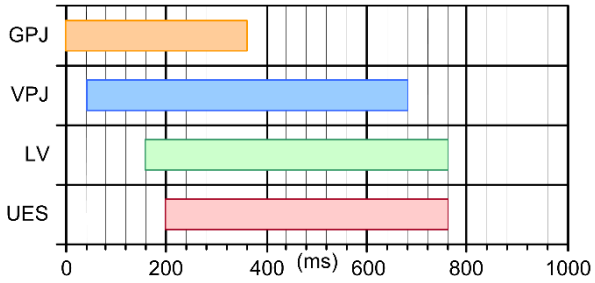

Figure 7: Normal oropharyngeal swallow response chronogram. *GPJ: glossopalatal junction; VPJ: velopharyngeal junction; LV: laryngeal vestibule; UES: upper esophageal sphincter.*

1.2 Oropharyngeal dysphagia

1.2.1 Prevalence

Prevalence of oropharyngeal dysphagia (OD) is extremely high among older people. However, it is difficult to determine its exact value as it depends on the patient phenotype, its origin and the diagnostic method used (Table 1). According to clinical (volume viscosity test (V-VST) and water swallow test) and instrumental explorations (VFS, fiberoptic evaluation of swallowing (FEES)), prevalence for the main phenotypes ranges between: 23-92% for older, 25-81% for post-stroke, 34-86% for neurodegenerative diseases and 50-86% for structural causes (Table 1).

It is relevant to mention the underdiagnoses of OD suggesting the real prevalence is probably higher and it has been estimated to be similar to that of diabetes: up to 30 million EU, 16 million USA and 7 million Japanese citizens present OD [22], [29].

1.2.2 Causes and phenotypes

OD can develop from several causes including neurological (i.e. cranial trauma, multiple sclerosis and Parkinson's disease), myopathic, structural (i.e dermatomyositis, myotonic dystrophy and sarcoidosis), metabolic, infectious and iatrogenic. OD phenotypes are mainly divided into four types according to the cause: older, post-stroke, neurodegenerative disease and patients with structural dysphagia.

Older patients

Several processes related to aging (anatomical changes or modifications in neurological mechanisms) can cause functional impairment which affects the swallowing process, causing OD. When these changes do not affect the safety of deglutition it is called presbyphagia. Several factors including a decrease in cortical plasticity, olfaction, taste, dental status, muscle function, saliva secretion and tissue elasticity are associated with a higher risk of OD [30]. In addition, co-morbidities and polymedication in frail older people suggest that this population is at high risk of developing dysphagia [26], [31], [32]. However, the characteristics of physiologically normal deglutition in healthy and robust older people are difficult to determine and it has not been established at what point the naturally slowing OSR can be considered dysphagia [33]–[36].

OD is considered to be a geriatric syndrome by two European societies [37] as it meets the following criteria: has high prevalence in older patients, is related to multiple risk factors (aging, frailty, sarcopenia, neurogenic disorders, stroke, drugs that affect the oropharyngeal motor response, and head and neck cancer), and contributes to the development of various geriatric syndromes with poor prognosis and complications such as disability and frailty, functional impairment, malnutrition, hospital readmissions and morbidity and mortality [121].

Post-stroke patients

Unihemispheric stroke in the dominant hemisphere for swallowing is the main cause of developing OD in this phenotype of patients due to the inter-hemispheric asymmetry of cortical activation during swallowing [38]. OD is highly prevalent in this phenotype of patients, ranging between 40 and 45% during patient admission, and is an independent risk factor for prolonged hospitalization, institutionalization after discharge, and poorer functional capacity, increasing mortality rates three month after stroke [39].

It is relevant to mention that 30% of patients with ineffective swallows and 42% with unsafe swallows reverse their swallowing impairment during the first week after the stroke. However, in patients who do not experience this improvement, the risk of nutritional and respiratory complications increases as a consequence of the poor functionality [40]–[42].

Patients with neurodegenerative diseases

Several diseases are classified as neurodegenerative diseases including Alzheimer's, Parkinson's, multiple sclerosis and amyotrophic lateral sclerosis. In this specific phenotype of patients, the damage occurs in both central and peripheral sensory nerves [43], [44]. Prevalence of OD ranges between 50 and 82% according to the neurodegenerative disease which affects the patient.

However, more than half the patients suffering from OD as a consequence of a neurodegenerative disease present safety impairments causing aspiration pneumonia [45]–[48].

Patients with structural dysphagia

As shown in Table 1, prevalence of OD in this phenotype of patients ranges between 39% and 86% when evaluated with clinical or instrumental tests, respectively.

Dysphagia is a main symptom in patients suffering with head and neck cancer as well as a main complication related to the treatment applied [49]. The most widely used treatment for this type of patients is surgery, radiotherapy and chemotherapy. Surgical treatment is usually related to anatomical modifications (partial or total resections) of the organs which can affect the swallowing path and thus, deglutition can be altered. Radiotherapy is an aggressive treatment which can affect critical swallowing structures and can contribute to the appearance of OD due to several causes such as hypogeusia, thyroid dysfunction, and dental decay. Chemotherapy also increments the risk of OD causing dysfunction of the oropharynx and larynx.

Other causes inducing dysphagia are osteophytes and Zenkers' diverticulum. Osteophytes are bony spurs on the cortical bone of the vertebral body due to age-related changes in the structure of bone lead [50]. They are usually asymptomatic but can also compress part of the bolus path and interrupt the swallowing process. Zenkers' diverticulum is an anatomical pouch posterior to the UES located in the Killian's dehiscence (weakness area of the cricopharyngeus muscle formed by both oblique and fundiform parts of it which are relatively unsupported by pharyngeal muscles). This pouch may trap food bolus or part of it, interrupting the correct bolus path during deglutition. Symptoms can include food regurgitation, globus sensation, aspiration pneumonia, dysphagia, halitosis and weight loss [3].

Table 1. Prevalence of oropharyngeal dysphagia according to patients' phenotype. Reproduced and odified from Clavé et al (2015) [29].

Phenotype	Target population	Evaluation method	Prevalence (%)	Reference
Older people	**Independently-living**	Screening (questionnaires)	11-38	Holland et al. 2011 [51], Roy et al. 2007 [52] Bloem et al. 1990 [53], Kawashima et al. 2004 [54], Yang et al. 2013 [55]
		Clinical exploration (V-VST)	23	Serra-Prat et al. 2011 [56]
	Hospitalized in an acute geriatric unit	Not specified/Clinical exploration (water swallow test or V-VST)	29.4–47.0	Lee et al. 1999 [57], Cabré et al. 2014 [58]
	Hospitalized with community-acquired pneumonia	Clinical exploration (water swallow test or V-VST)	55.0–91.7	Cabré et al. 2010 [59], Almirall et al. 2012 [60]
		Instrumental exploration	75	Almirall et al. 2012 [60]
	Institutionalized	Screening (questionnaires)	40	Nogueira & Reis 2013 [61]
		Clinical exploration (water swallow test)	38	
		Screening and clinical exploration	51	Lin et al. 2002 [62]
Stroke	**Acute phase**	Screening (questionnaires)	37-45	Martino et al. 2005 [63]
		Clinical exploration	51-55	
		Instrumental exploration	64-78	
	Chronic phase	Clinical exploration	25-45	
		Instrumental exploration	40-81	
Neurodegenerative disease	**Parkinson's disease**	Reported by patients	35	Kalf et al 2012 [64]
		Instrumental exploration	82	
	Alzheimer's disease	Instrumental exploration	57-84	Langmore et al. 2007 [65], Horner et al. 1994 [66]
	Dementia	Reported by patients	19-30	Langmore et al. 2007 [65], Ikeda et al. 2002 [67]
		Instrumental exploration	57-84	Suh et al. 2009 [68], Langmore et al. 2007 [65], Horner et al. 1994 [66]
	Multiple Sclerosis	Screening (questionnaires)	24	De Pauw et al. 2002 [69]
		Instrumental exploration	34.3	Calcagno et al. 2002 [70]
	Amyotrophic lateral sclerosis	Clinical and Instrumental exploration	47-86	Chen & Garrett 2005 [71], Ruoppolo et al. 2013 [72]
Structural	**Head and neck cancer**	Clinical exploration	50.6	García-Peris 2007 [73]
		Instrumental exploration	38.5	Caudell et al. 2009 [74]
	Zenker's diverticulum	Instrumental exploration	86	Valenza et al. 2003 [75]
	Osteophytes	Screening	17-28	Utsinger et al. 1976 [76]

1.2.3 Pathophysiology of impaired biomechanics and neurophysiology of the swallow response

The pathophysiology varies according to the phenotypes of patients. In the case of structural causes, the pathophysiology is related to anatomical changes that push the bolus to the incorrect places or block its pass through the pharynx or esophagus. The other three phenotypes (older, post-stroke and neurodegenerative diseases) are usually characterized by impairment in both biomechanics and neurophysiology of swallowing.

Biomechanics

The impairment in biomechanics of swallowing in patients with OD is usually characterized by a delay in the pharyngeal phase, specifically an increase in the duration of the OSR in the reconfiguration from the respiratory to the digestive tract [21], [27], [28]. The main changes include a delay in the time to LVC which causes an increment in the prevalence of penetrations and in the time to UESO, increasing the risk of regurgitation of the bolus [21], [28]. In addition, bolus velocity and kinetic energy is also decreased causing an increment in oropharyngeal residue [28], [77].

Total duration of swallowing in older people is 1013±53 ms with a time to LVC and to UESO of 476±48 ms and 403±45 ms, respectively [26], [28], [78]. In contrast, patients with post-stroke, dementia and Parkinson's disease with OD presented a unchanged time of the total duration of the swallowing process and of the time to UESO but not for time to LVC which was similar to that of older people (increased): 416±129 ms, 398±117 ms, 293±90 ms, for post-stroke, dementia and Parkinson's diseases, respectively [47], [77], [79]. As mentioned above, the LVC is the main process that protects the airway during deglutition, and a LVC cutoff of 340 ms in older and post-stroke patients and those with dementia [47], [48], [77] and 260 ms in patients with Parkinson's disease [79] predicts unsafe swallows in these phenotypes of patients (Figure 8).

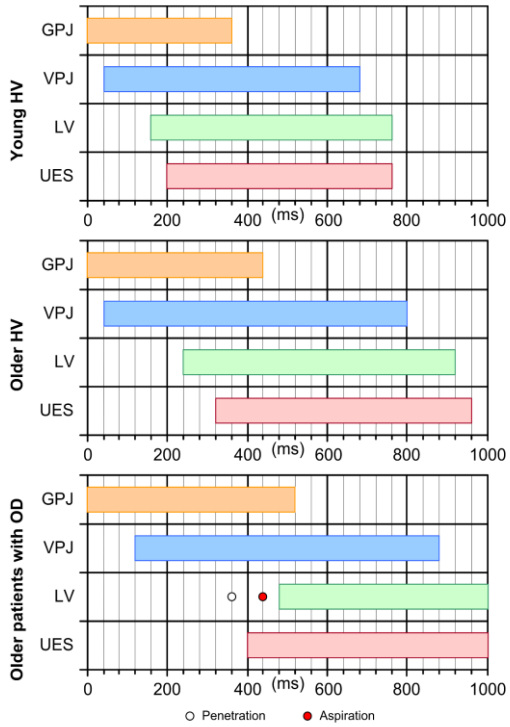

Figure 8: Biomechanical chronogram comparison between a young healthy volunteer, older healthy volunteer and older patient with oropharyngeal dysphagia. Adapted from Rofes et al. 2010 [26]. *HV: healthy volunteer.*

Kinematics

In addition, other main alterations observed in patients with OD correspond to kinematics impairments such as a decrease in the lingual propulsion force, bolus velocity and kinetic energy. Those impairments increase the prevalence of oropharyngeal residue [28], [77].

Neurophysiology

The neurophysiological swallow response also plays an important role in swallowing impairments. However, it has been much less studied than biomechanics. Recently, our research group has characterized the neuropsychological impairments in both the sensory and motor pathways in older patients with OD (Figure 9). Neurophysiological tools to assess OD are explained in section 2.6 (*diagnosis*).

Our research group found the aging process impairs the afferent (or sensory) pathway of neurophysiological swallowing response, more so in older people with OD in which the pharyngeal sensitivity and the conduction and integration latencies of sensory inputs are significantly impaired when compared to young healthy people [80], [81]. Similar results have also been found in patients with chronic post-stroke OD. These patients not only show greater latencies but also a loss in the symmetry of the sensory neurophysiological response and their cortical representation and a reduction in the excitability of the cortical swallow motor areas [82], [83].

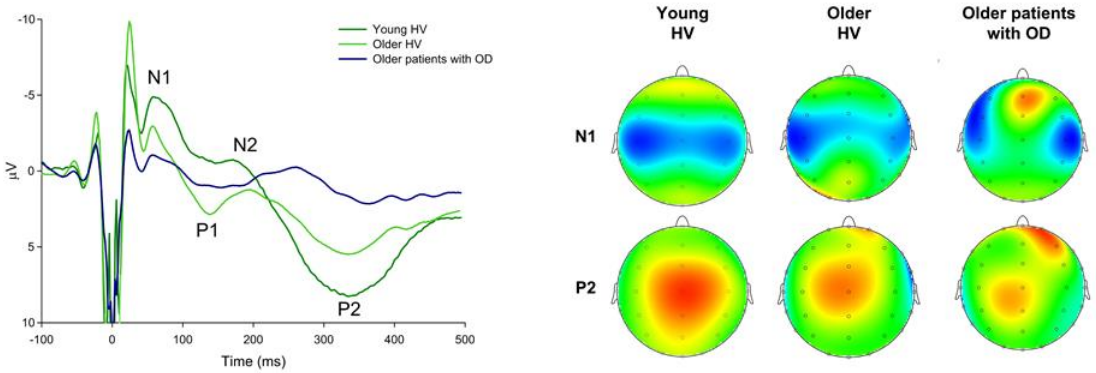

Figure 9: On the left, pharyngeal sensory evoked potentials (pSEP) obtained at Cz electrode for young healthy volunteers (green solid line), older healthy volunteers (green dashed line), and patients with dysphagia (blue line) after pharyngeal electrical stimulation. On the right, current scalp density maps at each event related potential peak time point for young and older healthy volunteers and patients with dysphagia. Adapted from Rofes et al. 2016 [80]. *HV: healthy volunteers; OD: oropharyngeal dysphagia.*

1.2.4 Clinical complications

The main dysphagia complications can be divided into two groups: efficacy and safety impairments.

Efficacy impairments

These are characterized by the inability or difficulty of preparing or moving the bolus. They can be due to several causes such as reduced or weak tongue propulsion force, poor control of

oropharyngeal muscles and labial seal impairment. The most common sign of efficacy impairment is the oropharyngeal residue and main complications are malnutrition and dehydration which can lead to low functional status and several comorbidities such as sarcopenia [59], [84]–[89].

Residue severity can be classified according to Robbins Scale which divides oropharyngeal residue according to location and severity (Table 2).

Table 2. Residue analysis according to Robbins scale for oropharyngeal residue. Adapted from Robbins et al. 2007.

Severity of residue on VFS		**Location**	
Score	**Event**	**Oral residue**	**Pharyngeal residue**
0	No residue	Oral cavity	Vallecula Posterior Pharyngeal wall Pyriform sinuses Upper Esophageal Sphincter
1	Residue coating		
2	Residue pooling		

Safety impairments

Safety impairments are characterized by the entrance of part or all the alimentary bolus into the respiratory tract due to a delay in the time to LVC as commented above. Safety impairments include penetrations and aspirations. Penetrations are described as the entrance of part of the bolus into the LV above the vocal cords. If the bolus passes through the vocal cords, then it is defined as an aspiration. These safety impairments are classified following the Penetration Aspiration Scale (PAS) developed by Rosenbek at al. according to their severity [90]: 1-2 correspond to safe swallowing, 3-5 are penetrations, while 6-8 are aspirations (Table 3).

Aspirations can include the arrival of pathogenic microorganisms from the oral and pharyngeal cavity (due to poor oral hygiene) into the lungs leading to aspiration pneumonia which is the first cause of death among patients with OD [29], [91], [92].

Table 3. Penetration-Aspiration Scale scores and their interpretation. Adapted from Rosenbek et al (1996) [90].

Swallowing	**Score**	**Visuoperceptual sign**
Safe swallows	**1**	Material does not enter the airway
	2	Material enters the airway but remains above vocal cords and is ejected from the airway
Penetrations	**3**	Material is above vocal cords and is not ejected from the airway
	4	Material enters the airway, contacts vocal cords and is ejected from the airway
	5	Material contacts the vocal cords and is not ejected from the airway
Aspirations	**6**	Material passes below the vocal cords and is ejected into larynx or out of the airway
	7	Material passes below the vocal cords and is not ejected from the trachea despite effort
	8	Material enters the airway, passes below the vocal cords and no effort is made to eject the material

1.2.5 Diagnosis

Screening tools

The main aim of these tools is the early detection of patients at risk of unsafe swallows [93]. Two of the most commonly used specific tests are the Eating Assessment Tool-10 (EAT-10) [94], [95], and the Sydney Swallow Questionnaire [96]. Screening tools should be characterized by being fast and easy to perform to be applied systematically to screen the maximum number of patients as possible to detect those at risk of OD. Recent studies from our laboratory developed a massive screening system using artificial intelligence (AI) with high accuracy to detect patients at risk of suffering OD: AI massive screening for OD (AIMS-OD). AIMS-OD is an AI expert system which provides quick, automatic and real time risk OD value based on medical records. AIMS-OD presents a sensitivity of 0.940, specificity of 0.416, PPV of 0.834, and NPP of 0.690 and an AUCROC of 0.840 (95% CI: 0.829 - 0.867). As an artificial intelligence tool, it improves its predictive capacity with each new case, allowing rapid adaptation to new populations. It uses anonymous and encrypted data and international codes, which enables it to be used in any country [97].

Clinical assessment tools

The aim of these specific tools is to perform a clinical diagnosis in order to detect the patients who need further evaluation and those who cannot be submitted to instrumental assessment to be able to prescribe an optimal treatment. The Volume-Viscosity Swallow Test (V-VST) is one of the best clinical tools to identify signs of both impaired efficacy and safety with a high sensitivity (93.17%) and specificity (81.39%) [98], [99]. The V-VST is an effort test consisting of a series of bolus of different volumes and viscosities administered in a specific algorithm.

The aim of the V-VST assessment is to identify clinical signs of impaired efficacy of swallow, such as impaired labial seal, oral or pharyngeal residue, piecemeal deglutition (multiple swallows per bolus), and clinical signs of impaired safety during swallow such as changes in voice quality (including wet voice), cough or a decrease in oxygen saturation ≥3% measured with a finger pulse-oximeter. The probe of the pulse-oximeter is placed on the index finger of the right hand and baseline readings are obtained 2 min prior to starting the test. Cough, voice changes and/or fall in oxygen saturation ≥3% are considered major clinical signs of safety impairments of swallowing. The V-VST is designed to protect patients from aspiration by starting with nectar viscosity and increasing volumes from 5 mL, to 10 mL and 20 mL boluses in a progression of increasing difficulty. When patients have completed the nectar series without major symptoms of aspiration (cough, voice changes and/or fall in oxygen saturation ≥3%), a liquid viscosity series will be assessed also with boluses of increasing difficulty (5 mL to 20 mL). Finally, a safer pudding viscosity series (5 mL to 20 mL) will be assessed in the same way. If the patient presents signs of impaired safety at nectar viscosity, the series will be interrupted, the liquid series will be omitted, and a safer pudding viscosity series will be assessed. If the patient presents signs of impaired safety at liquid viscosity, the liquid series will be interrupted, and the pudding series will be assessed (Figure 1).

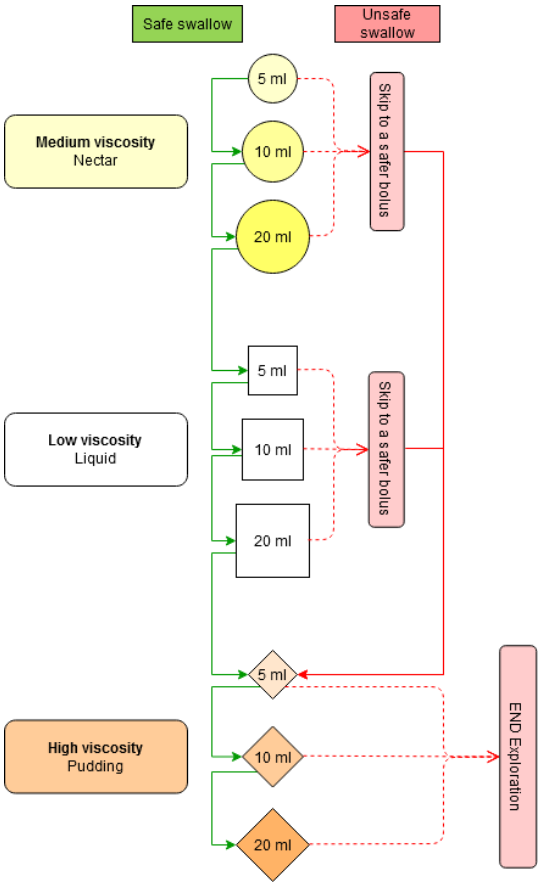

Figure 10: Volume-viscosity swallow test algorithm. Reproduced from Riera et al (2021) [98].

Instrumental diagnostic tools

Instrumental tools evaluate swallowing structures and their function as well as the role of volume and viscosity, swallowing maneuvers and postural changes on the respiratory tract. These tools make it possible to confirm the diagnosis and select the optimal treatment for each individual. The main instrumental tools widely used for OD exploration are: VFS, FEES and high-resolution manometry (HRM).

VFS is the gold standard tool to diagnose and explore dysphagia and other swallowing disorders. It is a dynamic radiological exploration in which the patient is evaluated in a lateral projection while swallowing boluses of varying viscosity and with radiological contrast. The image obtained includes the lips, oral cavity, pharynx, larynx, spine and esophagus. Subsequent image-by-image analysis of the VFS recording enables qualitative (prevalence of safety and efficacy signs) and quantitative (timing of the OSR, kinematics of the bolus and hyoid and larynx movements) studies to be made of the swallow response [26], [77] as explained above. Safety impairments are classified according to the degree of penetration or aspiration following the PAS [90]; efficacy impairments are also measured OD severity according to the Robbins scale [100].

As commented above, VFS also enables us to measure the timing of the OSR while swallowing. Oropharyngeal reconfiguration is measured through the opening and closure times of the GPJ, the VPJ, the LV and UES. The time to GPJ opening is considered the time-point 0 (Figure 10) [26]. The kinematics of the bolus can also be determined by the mean bolus velocity (time taken for the bolus from the entrance at GPJ to the arrival at the UES divided by the distance between both locations), final bolus velocity (at the arrival at the UES) and the tongue propulsion force

which is calculated according Newtons' second law formula (F=ma where 'm' is the bolus mass and 'a' the bolus acceleration).

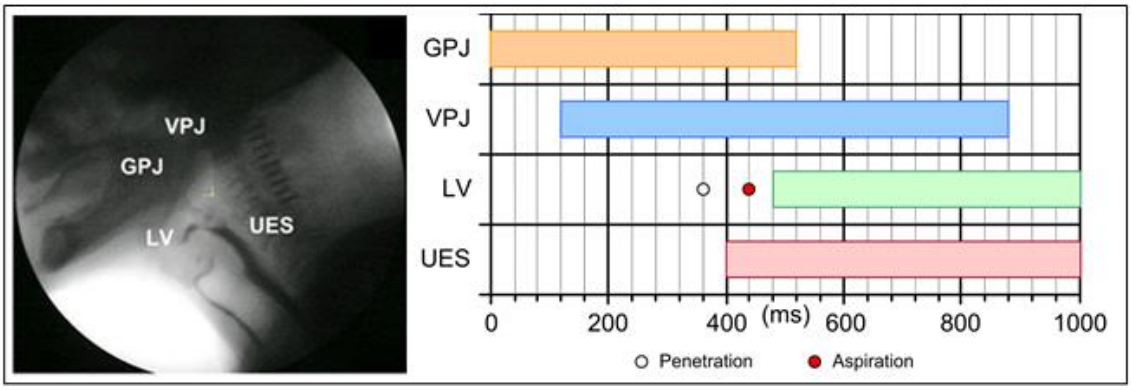

Figure 10: Chronogram of the timing of oropharyngeal swallow response in a patient with an aspiration caused by a delay in time to laryngeal vestibule closure. *GPJ: glossopalatal junction; VPJ: velopharyngeal junction; LV: laryngeal vestibule; UES: upper esophageal sphincter.*

FEES is used to assess the swallowing process by visualizing the pharyngeal and laryngeal structures. The equipment consists of a flexible fiberscope with light connected to a video device that records the sequence of images during the scan. It is well tolerated, repeatable and can be performed at the patient's bedside. With this specific technique the efficacy and safety impairment signs can be determined while the patient swallows boluses of varying viscosity and volume as with the VFS. The main limitation associated with FEES is that the oral phase cannot be visualized [101]–[103].

HRM (HRM) is used to assess and quantify the propulsive forces of the pharynx, the relaxation of the UES and the restrictive capacity of the UES through intravalvular pressure at the level of the hypopharynx. This equipment uses a catheter with an array of solid-state pressure sensors which straddles the pharynx, UES and the body of the esophagus. The measure of the different pressures observed is distinguished using a contour color plot. While swallowing, the velopharynx, pharyngeal stripping wave, UES movement/relaxation and proximal esophageal contraction can be determined (Figure 11). Three phenomena can be defined with HRM: tongue and pharyngeal propulsion, UES relaxation and pharyngeal intrabolus pressure [104].

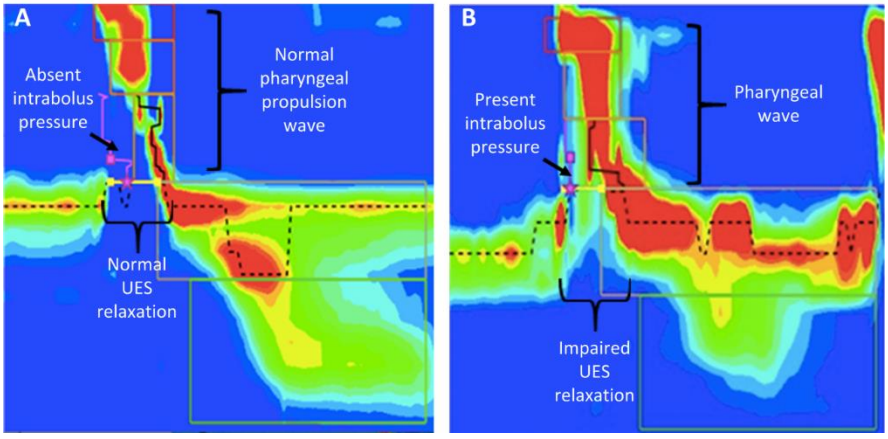

Figure 11: High-resolution manometry tracings of 5 ml swallow. Figure A shows a normal UES relaxation and a normal pharyngeal propulsion wave. Figure B shows an incomplete UES relaxation that causes

increased resistance to bolus leading to a high pharyngeal wave and increased intrabolus pressure at the hypopharynx level. *UES: upper esophageal sphincter.*

Neurophysiological tools

Pharyngeal Sensory Evoked Potentials (pSEPs) is the neurophysiological technique used to assess the afferent or sensory pathway. Electrical stimuli are given to the pharynx by a nasopharyngeal probe during swallow and electroencephalogram (EEG) and conduction and integration of sensory inputs are measured. In contrast, Pharyngeal Motor Evoked Potentials (pMEPs) can assess the integrity of the efferent or motor pathway. The cortex areas in charge of the pharyngeal movements control are stimulated using a transcranial magnetic stimulation (TMS) by the use of a nasopharyngeal probe.

1.3 Compensatory treatment

The main treatment used to manage OD is compensatory treatment which consists of a texture adaptation for solids [105], [106] to maintain the nutritional status of dysphagic patients and bolus viscosity modification for alimentary fluids to maintain the hydration status [107]–[109].

Texture Modified Diets (TMD) aim to avoid aspirations, improve nutritional status and increase quality of life of patients with OD. A nutritional TMD based on the Mediterranean diet was created by our group including more than 200 recipes which are reproducible at home [106]. It is called the Triple Adaptation according to the three aspects it covers: 1) viscosity adaptation for fluids; 2) nutritional adaptation to meet the necessary requirements according to the nutritional status of the patient and; 3) organoleptic adaptation to improve the taste, smell, presentation and palatability of the dishes offered to the patients [106] [194]. Nonetheless, there is a need to further explore this field as TMD are currently being prescribed and tested by qualitative descriptors which can jeopardize the safety of patients. The rheological and textural laboratory at the Physiology Digestive Department of Hospital de Mataró is working on the textural characterization and standardization of the diets in a scientific and objective manner by assessing the hardness, adhesiveness and cohesiveness of each meal served to patients admitted to the hospital.

For fluids, viscosity is usually increased by the use of thickening products (TP) which is a widely accepted and effective strategy to reduce the risk of airway invasion in OD as stated in a review by the European Society for Swallowing Disorders (ESSD) [107]. The main conclusions of this review are that: a) TP are an effective strategy to reduce penetrations and aspirations in patients with OD; b) there is a need to develop new TP with less residue and higher palatability to improve compliance to this specific treatment and; c) Clinical trials (CT) must be performed to establish the optimal viscosity levels for each OD phenotype. However, other authors question the use of thickening fluids to prevent aspiration in patients with OD, arguing limited scientific evidence on its therapeutic effect on clinical outcomes mainly the hydration status of the patients and the prevention of aspiration pneumonia [110].

A recent systematic review (SR) consisting in 2 SR and 2 scoping reviews (ScR) performed by our group shows that dehydration is a highly prevalent complication in several phenotypes of

patients with OD [109]. Results from SR-1 showed that prevalence of dehydration in OD assessed by objective bioimpedance electrical analysis (BIA) or biochemical methods ranged from 19–100%. Although the exact prevalence of dehydration in OD is not clear, most studies suggested OD patients were at higher risk for this complication. However, studies also showed that prevalence of dehydration was also very high in older non-dysphagic patients. Therefore, some of the studies have described no significant differences between OD and non-OD patients with respect to these high rates of poor hydration status. Studies included in the ScR also highlighted the need to standardize the biochemical and/or bioelectrical impedance analysis (BIA) markers to assess and monitor the hydration status of patients with dysphagia. In SR-2, scientific evidence on the positive effect of thickening fluid (TF) therapy on the hydration status of patients with OD was also found, with high quality studies including a large number of patients with dysphagia. Most studies reported low consumption of TF in patients with OD, so strict monitoring of fluid volume intake is essential to improve hydration status of patients with OD.

1.3.1 Thickening Products

TP are composed of different types of hydrocolloids called thickening agents (TA). TP can be divided into three categories according to the main TA: modified starch (MS), xanthan gum (XG) and mixtures (Mx). Starch is composed of a mixture of amylose and amylopectin and is a chemically modified hydrocolloid [111]. It is generally achieved by derivatizations such as etherification and esterification or by weakening the starch using physical treatments (such as heat and moisture). It is composed of more than 300 molecules arranged in linear chains with O-glycosidic bonds. It works by absorbing water and swelling which presents some limitations such as instability over time (progressive increase of viscosity) and the appearance of lumps [112]. In addition, MS presents α 1→4 bonds when mixed with water which are susceptible to be broken by oral enzymes such as salivary amylase. In contrast, XG is a long chain polysaccharide composed of glucose, mannose and glucuronic acid, forming a tridimensional structure when mixed with water with internal β 1→4 bonds [113].

Regulations and legal framework

TP's are included in the category of Food for Special Medical Purposes (FSMP) and are a group of alimentary products intended for the dietary management of dysphagia and need to be used under medical supervision [114]. FSMP must fulfill the General Food Law 2002/178 [115], [116], and European (EU) regulation Nº 1169/2011 on food information to consumers [117] of the EU Parliament and of the Council and regulations for this specific group of products: EU Nº 609/2013 [118] and the supplementing EU regulation 2016/128 [119]. These regulations also include the minimum information to be displayed on the label which is the main way to inform the client on the properties of the product.

FSMP labels should contain the minimal information on the health characteristics of the product to decide and compare whether it is the best option for the patient. Information should be given in a homogeneous manner for all the products by using the International System (SI) of units [120]. However, TP are commercialized with labeling recommendations on how to prepare arbitrary viscosity levels based on qualitative classifications without scientific evidence [111].

The SI units, is the international standard for measurement adopted by the general conference on weights and measures (CGPM). This system was developed to avoid overlaps between the

several different measures used by scientists to accomplish their practical needs. According to the uniform requirements for manuscripts submitted to biomedical journals [121] developed by the international committee of medical journal editors (ICMJE), units of measurements must be reported in the SI units and additionally, local units (non-SI units) can be included. Nonetheless, in each Member State, mandatory provisions regulate technical characteristics of measuring instruments and methodology for metrological control. In Spain, it is the Royal Decree 2032/2009 [122] which establishes the legal units of measure including viscosity which should be reported in Pa·s.

Viscosity classifications

There are several fluid classifications being used around the world to prescribe and manage patients with OD. NDD is the most common classification, it uses 4 viscosity levels with very wide viscosity ranges measured at 25ºC and $50s^{-1}$ [123]; while the international diet standardization initiative (IDDSI) uses up to 5 levels according to a gravity flow test through 10 ml syringe [124], defined by some authors as an empirical test [125]. In Australia, viscosity is tested by the evaluation of the fluid flow through a fork and divided into four levels [126]. In contrast, the Japanese Society for Dysphagia Research (JSDR) developed a classification called Japanese dysphagia diet classification (JDD2013) based on viscosity at 25ºC and $50s^{-1}$ and bolus flow with the Line Spread Test with just 3 viscosity levels only available for those TP composed by xanthan gum [127]. Table 4 shows the viscosity classifications used around the world.

Table 4. Main viscosity classifications used around the world to prescribe thickening products to patients with dysphagia.

VISCOSITY CLASSIFICATIONS					
NDD (Viscosity $50s^{-1}$)	Thin liquid (<50)	Nectar (51-350)	Honey (351-1750)	Pudding (>1750)	
Australia (Level)	Regular Fluid	Mildly thick (150)	Moderately thick (400)	Extremely thick (900)	
IDDSI (Level)	Thin (0)	Slightly thick (1)	Mildly thick (2)	Moderately thick (3)	Extremely thick (4)
JDD2013 (Viscosity $50s^{-1}$)	Mildly thick (50-150)		Moderately thick (150-300)	Extremely thick (300-500)	

NDD: National Dysphagia Diet; IDDSI: International Dysphagia Diet Standardization Initiative; JDD: Japanese Dysphagia Diet

It is also important to include the therapeutic information of the product in the label not only for the patient but also for the healthcare professional. Viscosity plays a major role in the therapeutic effect produced by TP. The increment of viscosity with TP has been associated with the increment of safe swallows in different phenotypes of patients with OD [28], [107], [108]. However, few viscosity levels have been studied, and the optimal viscosity levels for patients with OD have not yet been determined. In addition, other factors such as the composition, the preparation mode or viscosity behaviour when submitted to the rheological swallowing factors should also be considered.

1.4 Rheology

Rheology is the science which describes the flow behaviour of materials when they are submitted to external forces [128]. Viscosity is the rheological parameter to measure the resistance of a fluid to flow and as commented above, is the main factor related to safe swallowing [107]. However, viscosity can be modified during swallowing due to two rheological factors: the effect of salivary amylase during the oral phase and shear the effect of rate during the pharyngeal phase [129].

1.4.1 Swallowing factors affecting the rheology of alimentary fluids

Salivary amylase is an oral enzyme which breaks A-glycosidic bonds during the oral phase. This enzyme strongly affects MS-based TP due to its linear disposition and the high prevalence of those bonds.

Shear rate is defined as the rate at which a fluid is sheared when passing between two adjacent layers and affects velocity. In the rheological field, fluids can be divided in two categories according to viscosity behaviour when an increment of shear rate is applied (Figure 12). Newtonian fluids are also described as the ideally viscous fluids because shear rate does not produce any viscosity modification. Some examples of Newtonian fluids are water, oil or sugar [111]. In contrast, non-Newtonian fluids can increase (shear thickening) or decrease (shear thinning) their viscosity when they are submitted to an increment of shear rate.

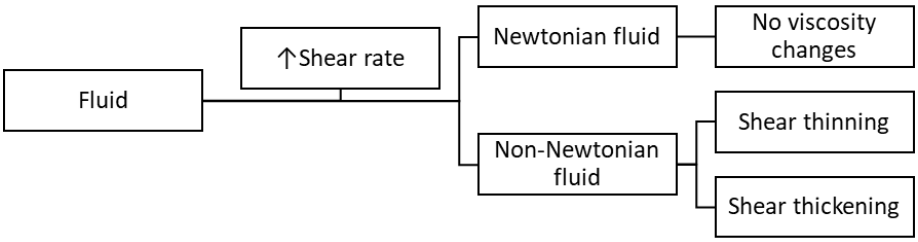

Figure 12: Diagram of fluid behaviour when submitted to an increment of shear rate.

Shear thinning is the most common behaviour for alimentary fluids and TP. When swallowing, shear rate varies according to the increment of bolus velocity [130], therefore, the viscosity behaviour of TP should be tested before being commercialized. The shear rate spectrum for the swallowing process ranges from 1 to 1000 s^{-1} but two main shear rates are considered when swallowing in patients with OD: 50s^{-1} in the oral phase [123], [131] and 300s^{-1} in the mesopharynx [130] by different acquired bolus velocities at each phase [26], [130]. Shear rate in the oral phase is suggested to range between 10 s^{-1}, value described by Shama&Sherman [132] and 50 s^{-1} described by Wood [131]. Both studies estimated the values by intersecting flow curves from pairwise comparison [133] 50 s^{-1} was later selected as the representative shear rate value in the oral cavity by professional consensus [123]. In contrast, pharyngeal shear rate was calculated at 262 s^{-1} by mathematics formula including the swallowing anatomy and bolus velocity [130] by using data on healthy volunteers. Later, a study performed by Clavé et al. [134], determined that the bolus velocity of patients with dysphagia (<10 cm/s) was similar to the

bolus' tail velocity in healthy volunteers (10.3 cm/s). This correlation was used to estimate the share rate value for patients with OD (262 s^{-1}) and was rounded up to 300 s^{-1} (Table 5).

Table 5. Bolus velocity and estimated shear rate extracted from studies in healthy volunteers and patients with dysphagia.

Bolus	Bolus vel. (cm/s)	Estimated shear rate (s^{-1})	Publication
Healthy volunteers			
Bolus' head	37.6[1]	990[2]	[1]Bardan 2006 [135] [2]Brito de la Fuente 2017 [130]
Bolus' tail	10.3[1]	**262[2]**	[1]Bardan et al. [135] [2]Brito de la Fuente 2017 [130]
Bolus' head	>35[1]	990*	[1]Clavé 2006 [28]
Dysphagia patients			
Bolus' head	<10[1]	**262***	[1]Clavé 2006 [28]

In summary, to assess the impact of rheological swallowing factors of TP on viscosity, there are two parameters which need to be considered according to the swallowing phase where the bolus travels: in the oral cavity a shear rate of 50 s^{-1} is considered with an important effect of salivary amylase amylase (especially in MS-based TP); in the pharyngeal phase, a shear rate of 300 s^{-1} is estimated and thus, a shear thinning behaviour is observed.

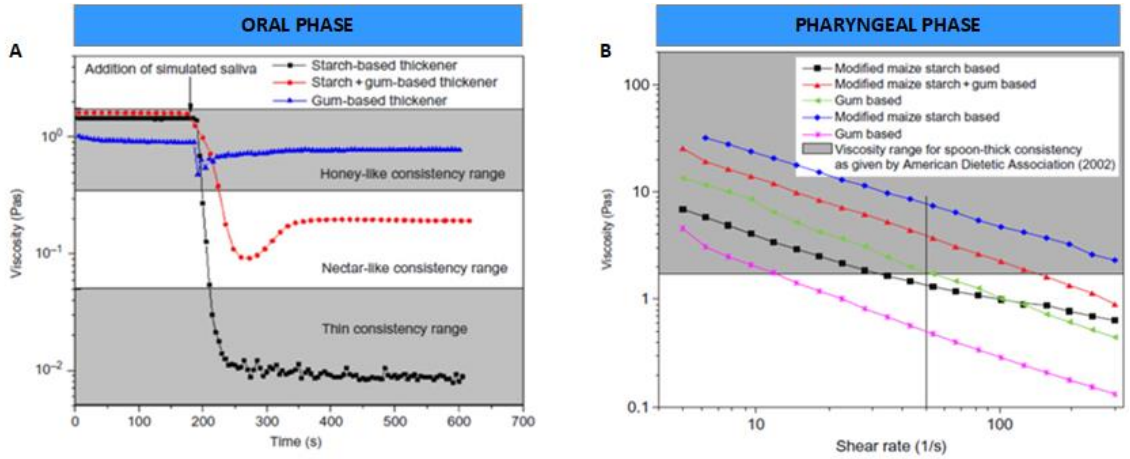

Figure 13. Swallowing factors affecting the rheology of alimentary fluids during oral (A) and pharyngeal (B) phases. Adapted from Gallegos et al. 2017 [129].

Very few researchers have studied the link between viscosity, rheology and swallowing both in healthy subjects and patients with swallowing disorders. In order to protect patient safety, these changes in viscosity must be taken into account in thickened fluids used for patients with dysphagia. Nowadays, there are several in vitro rheological techniques to accurately assess these factors affecting shear viscosity which should be characterized by manufacturers prior to their commercialisation due to their potential impact on patients' health.

2. HYPOTHESIS

2.1 Main hypothesis

Shear viscosity is one of the main properties related to the therapeutic effect of TP and it is possible to obtain an optimal dose to manage each OD phenotype. Viscosity is altered by the oral enzyme salivary α-amylase and the effect of the shear rate at the level of the mesopharynx. Manufacturers commercialise various TP's levels of viscosities according to a qualitative classification, without scientific evidence of the therapeutic effect.

2.2 Specific hypothesis:

H1. Increasing viscosity of alimentary fluids with TP improves the safety of swallow in patients with OD without major changes in the physiology of the swallow response and increases significantly oral and pharyngeal residue.

H2. Qualitative descriptors for the classification of thickening products are heterogeneous and can overlap compared with objective viscosity measurements in the IS of units (mPa·s). Shear rate and salivary amylase have distinct effects according to the composition of the TP.

H3. A common scientific rheological protocol to assess the effect on viscosity of the main rheological factors (oral salivary amylase and shear rate during pharyngeal transit) can be established in separate rheological laboratories around the world with very low variability.

H4. The combination of the rheological characterisation of TP using different *in vitro* and *ex vivo* studies in the rheology laboratory with the therapeutic effect assessed by VFS in previous CT can help to determine the rheological properties associated with safety of swallow. Properties other than shear viscosity including extensional rheology might have a major impact on the therapeutic effect of TP.

3. AIMS

3.1 Main aim

Main aim of this thesis is to determine the relationship between shear viscosity, other rheological properties and the therapeutic effect of TP at different viscosity levels for the main OD phenotypes.

3.2 Specific aims

A1. To describe and assess the effect of two xanthan-gum-based TP and a mixture (xanthan gum and modified starch) on safety, efficacy and physiology of swallow at several levels of viscosity and to determine the therapeutic range and optimal viscosity doses for each OD phenotype.

A2. To describe the rheological factors (salivary amylase and shear rate) involved in the therapeutic effect of TP and to determine whether this information is included in the labelling of these products. To determine the risks of over TP commercialized with qualitative descriptors instead of objective viscosity levels.

A3. To validate a scientific and standardized protocol to measure shear viscosity and the rheological swallowing factors affecting TP (salivary amylase and shear rate) in different international laboratories.

A4. To assess the following rheological properties: shear viscosity, extensional deformation and maximal force, adhesiveness and cohesiveness for several TP doses. To determine the relationship between these rheological properties and the therapeutic effect on safety of swallow.

4. METHODOLOGY

This doctoral thesis is composed of four studies:

- Study 1: Evaluation of the therapeutic effect of different TP and the establishment of the optimal viscosity doses to treat OD patients. Includes the three following CT:
    - ✓ Sub-Sub-study 1.1 – Effect of a gum-based thickener on the safety of swallowing in patients with poststroke oropharyngeal dysphagia
    - ✓ Sub-Sub-study 1.2 – Therapeutic effect, rheological properties and alpha salivarly amylase resistance of a new mixed starch and xanthan gum thickener on four different phenotypes of patients with oropharyngeal dysphagia
    - ✓ Sub-Sub-study 1.3 – Shear viscosity dependent effect of a gum-based thickening product on safety of swallow in older patients with oropharyngeal dysphagia
- Study 2: Analysis of the risks of using qualitative descriptors for viscosity levels in TP labelling
- Study 3: Design and validation of a rheological protocol to standardize shear viscosity measurements
- Study 4: The rheological properties of TP that have a therapeutic effect on safety of swallow in patients with post-stroke oropharyngeal dysphagia

All the studies included in this doctoral thesis performed with the recruitment of patients and volunteers were reviewed and approved by the Ethics Committee (EC) of the Consorci Sanitari del Maresme: 41/15 (Sub-study 1.1), 57/15 (Sub-study 1.2); 58/19 (Sub-study 1.3 and 3) and; 12/19 (Study 2 and 4). In addition, the clinical trials included are registered in Clinical Trials registers: NTR5628 (The Netherland Trial register; Sub-study 1.1); NCT04565587 (Clinical Trials Gov; Sub-study 1.3).

All the procedures were conducted in full conformance with the principles of the 'World Medical Association Declaration of Helsinki' (64th WMA General Assembly, Fortaleza, Brazil, October 2013), International Conference on Harmonisation (ICH) guidelines for Good Clinical Practice (GCP, September 1997) as appropriate for nutritional products and local legislation of the country in which the research is conducted, whichever affords the greater protection to the participants.

Data has been managed according to the Regulation (EU) 2016/679 of the European Parliament and of the Council and the Organic Law 03/2018 of Protection of Personal Data and Guarantee of Digital Rights.

4.1 Patients

4.1.1 Study 1

Sub-study 1.1

This study included 120 PSOD patients who were consecutively recruited from March 2016 to December 2017 at the GI Physiology Lab of the Hospital de Mataró, Barcelona. Main inclusion criteria were patients older than 18 years, minim of 28 days since diagnosis of stroke, clinical signs or symptoms of swallowing dysfunction in the V-VST [99] or referral by physician for VFS or current use of thickened products, no alteration in consciousness, and written informed consent. Main exclusion criteria were need of oxygen therapy, OD not related to stroke, history of other neurological disorders or head and neck cancer, xerostomia induced by drugs, severe cognitive disorder, incapability to perform VFS, pregnancy or lactation, participation in another research study, and allergy to any ingredient tested. In addition, for the description of study population, we collected demographic parameters such as age, sex, weight, height, type of stroke, time after stroke, severity of dysphagia, nutritional status, comorbidities, medication and stroke severity according to the National Institute of Health Stroke Scale (NIHSS) [136].

Assuming discordant proportions of 7.5% (safe swallow on thin liquid, and unsafe swallow on main viscosities), and 30% (unsafe swallow on thin liquid, safe swallow on main viscosities), a sample size of 95 patients would be sufficient to have 90% power to detect statistically significant differences in safe swallowing between each of the three main viscosities and thin liquid, using a two-sided Mcnemar's test with an α of 0.5, assuming 20% of patients do not complete the measurements.

Sub-study 1.2

Study population was prospectively recruited from the Gastrointestinal Physiology Unit of the Hospital de Mataró (Spain) between February 2016 and February 2018. Inclusion criteria were: patients over 18 years old, presenting OD according to the V-VST caused by any of the following etiologies: aging (>70 years), following treatment for head and neck cancer, stroke and Parkinson's disease. Exclusion criteria were: pregnancy or lactation, unstable cardiopulmonary status, unstable medical conditions or major respiratory disease needing oxygen therapy. All participants were informed about the study and signed the informed consent form. The following data were also collected: sociodemographic characteristics of the population, functional capacity according to the Barthel Index [137], force with a hand dynamometer (Takei Analogue Dynamometer 5001, Takei Scientific Instruments, Japan), quality of life according to the EUROQoL-5D [138] and nutritional status according to body mass index (BMI) and the short form of the Mini Nutritional Assessment short form (MNA-sf) test [139].

Accepting an alpha risk of 0.05 and a beta risk of 0.1 in a two-sided test, 30 subjects are needed (in each group) to recognize as a statistically significant difference greater than or equal to 1 points in the PAS score. The standard deviation is assumed to be 1.5, and it has been anticipated a drop-out rate of 20%.

Sub-study 1.3

This clinical trial included 85 patients who were recruited from August 2020 to August 2022 at the Gastrointestinal Physiology Laboratory of the Hospital de Mataró, Consorci Sanitari del Maresme, Catalonia, Spain. The study recruitment process was delayed due to COVID-19 pandemics. Inclusion criteria were: older than 70 years old, clinical signs or symptoms of OD / swallowing dysfunction, impaired safety of swallow at VFS (PAS>3) and written informed consent. Exclusion criteria were: patients with safe swallow on VFS (PAS 1.2), OD associated to structural alterations (ie osteophytes, Zenker diverticulum, bars, etc), dementia and severe cognitive disorders or inability to comply with the protocol requirements, being pregnant or lactating and present allergy to any study ingredient.

Accepting an alpha risk of 0.05 and a beta risk of 0.1 in a two-sided test, 83 subjects are necessary to recognize as statistically significant a difference in safe swallowing prevalence consisting in an initial proportion of 0.15 and a final proportion of 0.62. It has been calculated to have a mean drop-out rate of 62.50%.

4.1.2 Study 2

Five healthy young volunteers were recruited at the Hospital de Mataró, Catalonia, Spain, to analyse the effect of α-salivary amylase during oral incubation of each viscosity level recommended by the TPs manufacturers. Main inclusion criteria were older than 18 years, younger than 40 years, being able to hold the bolus for 30 seconds and ability to sign the informed consent to participate into the study. Exclusion criteria were not accomplishing the inclusion criteria, not testing the three viscosities of the 10 thickening agents selected, and suffering from Sjögren syndrome or sialorrhea. Young volunteers were chosen from those presenting abundant saliva in order to analyse the strongest affection at which thickeners can be submitted to (worst scenario).

The number of participants were selected to detect a difference equal to or higher than 200 mPa·s, accepting an alpha risk of 0.05 and a beta risk <0.2 with a bilateral contrast and assuming a standard deviation of 100 units.

4.1.3 Study 3

Eight healthy volunteers were recruited at the Hospital de Mataró, Catalonia, Spain to participate in the study to assess the effect of α-salivary amylase during oral incubation of each viscosity level assessed. The participants had to incubate two viscosity boluses per day: a viscosity level per day corresponding to two boluses (firstly, bolus without radiological contrast; in second place, bolus with radiological contrast). This part lasted 5 days for each participant. Main inclusion criteria were to be older than 18 years and able to sign the informed consent. Main exclusion criteria were not accomplishing the inclusion criteria, suffering from Sjögren syndrome, taking drugs which affect salivation and irritation or inflammation of the oral cavity.

Number of participants was calculated to detect a difference equal or higher than 160 mPa·s, accepting an alpha risk of 0.05 and a beta risk of 0.1 in bilateral contrast, so 8 participants were needed. A standard deviation of 100 is assumed with loss of 0.2.

4.1.4 Study 4

Participants data included in three previous CT were obtained to review the therapeutic effect of TP: Vilardell, 2017 [112]; Bolívar-Prados, 2019 [140] and; Ortega 2020 [141]. All CT were conducted in our healthcare center by our research group and have been selected to avoid any bias in the analysis of the therapeutic effect. Number of participants included, gender, Barthel index time from stroke and type of stroke (ischemic and hemorrhagic) were determined.

4.2 Products, doses and viscosity levels

4.2.1 Study 1

Sub-study 1.1

Nutilis Clear was used for this study. This specific TP is composed of maltodextrin, xanthan gum and guar gum and manufactured by Nutricia N.V., Zoetermeer, the Netherlands. The viscosity levels used in the study were selected according to the descriptors of the NDD [123]: 1-50 mPa·s for thin liquid, 51-350 mPa·s for nectar, 351-1750 mPa·s for honey and >1750 mPa·s for pudding viscosities at 25°C and 50 s^{-1}. For VFS we prepared a total of 7 different viscosities in 10 mL bolus, consisting of liquid X-ray contrast as control versus 6 thickened X-ray contrasts thickened with Nutilis Clear at each viscosity level. To achieve those viscosities, varying amounts of thickener were added to 50 mL solution composed of 1:1 mineral water and the iodine X-ray contrast: 150 and 250 mPa·s viscosities were obtained by adding 0.56g and 0.75g, respectively; 450, 800 and 1400 mPa·s viscosities were obtained by adding 1.27, 2.08 and 3.81 g, respectively; and 2000 mPa·s was obtained with 5.01 g.

Sub-study 1.2

We used the Fresubin Clear Thickener® (FCT) (Fresenius-Kabi Deutschland GmbH, Bad Homburg, Deutschland) composed of xanthan gum, modified starch, maltodextrin, modified cellulose and flavouring. The solutions used for the in vivo study were prepared by adding the thickener to a given volume of water plus an X-ray contrast agent, and stirring thoroughly for 20 s as recommended by the manufacturer. Then, 250, 1000 and 2000 mPa·s shear-viscosities were obtained by adding 0.7, 2.3, and 4.2 g to 50 mL of liquid obtained by mixing 1:1 mineral water and the X-ray contrast Omnipaque® (GE Healthcare, La Florida, Spain) respectively, at room temperature (20ºC). For the in vitro rheological studies to assess the effect of salivary amylase and the effect of shear-thinning, boluses were prepared with 100 mL mineral water and 1.75, 5.25 and 10.5 g respectively of FCT to achieve each level of viscosity (250, 1000 and 2000 mPa·s).

Sub-study 1.3

The TP used for the study was Tsururinko Quickly (TQ) manufactured by Morinaga Milk Industry, Co., ltd, Tokyo, Japan. This TP is composed of xanthan gum, dextrin, calcium lactate and

trisodium citrate. TQ composition contains the following nutrients (per 100 g): 270kcal, 0.5g protein, 88.90g carbohydrates, 21.90g fiber, 960g sodium, 980g potassium, 30mg phosphorus, 4.50g ash and 6.10g water. The X-Ray contrast used for VFS was Omnipaque™ commercialised by GE Healthcare Bio-Sciences, S.A.U, Madrid, Spain.

A total of 6 different shear viscosity levels (<50, 100, 200, 400, 800 and 1600 mPa·s) were tested during VFS. For VFS, the solution was prepared according to 50 ml 1:1 Omnipaque and water and the following doses were used: 0.58, 1, 1.45, 2.45, 4.3 g respectively for each viscosity level commented above. For rheological tests, two viscosities were selected: 200 (2g) and 800 (5.8g) mPa·s and prepared with 100ml mineral water.

4.2.2 Study 2

Ten TPs widely commercialised in Spain were used to perform this study: Fresubin Clear Thickener (A; Fresubin; Fresenius Kabi GmbH, Bad Homburg, Deutschland), Thick & Easy (B; Hormel Foods Sales, LLC, Austin, USA), Bi1 Espesante (C; Adventia Healthcare, S.L. Las Palmas de Gran Canaria, Spain), Nutilis Powder (D; Nutricia N.V., Zoetermeer, The Netherlands), Espesante Wallax (E; Wallax Farma SL Easy Pharma, Córdoba, Spain), Nutavant Espesante (F; Persan Farma Las Palmas de Gran Canaria, Spain), NM Espesante (G; Cantabria Labs Nutrición Médica, S.L., Madrid, Spain), Nutilis Clear (H; Nutricia N.V., Zoetermeer, The Netherlands), Resource Clear Thicken Up (I; Nestle S.A., Barcelona, Spain), Resource Thicken Up (J; Nestle S.A., Barcelona, Spain). Samples to be analysed were prepared according to the manufacturers' recommendations to achieve the different thicknesses stated on the labelling.

Viscosities obtained following manufacturers' instructions were expressed according to three classifications: NDD [123], IDDSI [124] and the JDD2013 (Watanabe et al., 2017). Classifications and descriptors are described below (Table 6). Results are also presented by a scientific system developed in our laboratory at Hospital de Mataró and endorsed by 11 scientific societies which includes important rheological parameters described in SI units. For the NDD and the JDD2013, the viscosity value used to classify each thickener was the viscosity at 25 ºC and 50 s^{-1}, while for IDDSI the syringe flow test was performed.

Table 6. Thickness descriptors, viscosity ranges and flow tests used in each classification assessed in this study.

Viscosity Classification	Thickness descriptor	Viscosity range (mPa·s)	Flow Test (ml)	Line Spread Test (mm)
NDD	Thin	<50	-	-
	Nectar-like	51-350	-	-
	Honey-like	351-1750	-	-
	Spoon-thick	>1750	-	-
IDDSI	Thin (Grade 0)	-	<1	-
	Slightly thick (Grade 1)	-	1-4	-
	Mildly thick (Grade 2)	-	4-8	-
	Moderately thick (Grade 3)	-	>8	-
	Extremely thick (Grade 4)	-	10*	-

JDD2013	Mildly thick	50-150	-	36-43
	Moderately thick	150-300	-	32-36
	Extremely thick	300-500	-	30-32

NDD: national dysphagia diet; IDDSI: international dysphagia diet standardisation initiative; JDD: japanese dysphagia diet.

4.2.3 Study 3

The TP used for the study is TQ (batch 23.03.2021). Mineral water was Font D'Or (Vichy Catalan Corporation, Barcelona, Spain) and the X-Ray contrast used for VFS was Omnipaque™ (GE Healthcare Bio-Sciences, S.A.U, Madrid, Spain). Composition of the product has been described in Sub-study 1.3.

Doses were selected to determine different viscosity levels ranging between 100 and 1600 mPa·s to validate the protocol in a wide range of shear viscosities. 1.25, 2, 3.2, 5.8 and 10.5g of TQ were used in 100 ml mineral water to achieve the following viscosity levels: 100, 200, 400, 800 and 1600 mPa·s.

4.2.4 Study 4

TP were selected according to the CT: Resource Thicken Up (A; Nestle S.A., Barcelona, Spain), Resource Thicken Up Clear (B; Nestle S.A., Barcelona, Spain); Nutilis Clear (C; Nutricia N.V., Zoetermeer, The Netherlands), and Fresubin Thickener Clear (D; Fresenius Kabi GmbH, Bad Homburg, Deutschland). Qualitative TP composition is shown in Table 7. TP were divided in 3 categories according to their main composition (modified starch, xanthan gum and mixtures; [140]). Dose (grams and solvent) for each viscosity level used in the CT are presented in Table 7 according to the VFS performed for each CT. Preparation mode was conducted following the instructions of the TPs' labelling. Product B solutions were prepared 3h prior to the analysis following the instructions of the CT ([112]).

Table 7. Thickening products composition, classification and viscosity levels selected from the clinical trials. Doses are presented by grams and solvent used to prepare each videofluoroscopy viscosity level.

Thickening Product	Qualitative composition (Classification)	CT Viscosity levels	Grams	Solvent (ml)	Bolus volume (ml)
A	Modified Starch (MS)	Thin liquid	0	100ml (1:1) Mineral water : Gastrografin	5
		Nectar	3.5		5
		Pudding	8		5
B	Maltodextrine Xanthan gum (XG)	Thin liquid	0	100ml (1:1) Mineral water : Gastrografin	5
		Nectar	2.4		5
		Extreme spoon thick	5.4		5
C	Maltodextrine Xanthan gum Guar gum (XG)	Thin liquid	0	50ml (1:1) Mineral water : Omnipaque	10
		250	0.85		10
		800	2.31		10
		2000	5.61		10

D	Modified starch Xanthan gum Maltodextrine (Mx)	Thin liquid	0	50ml (1:1) Mineral water : Omnipaque	5
		100	0.7		5
		1000	2.3		5
		2000	4.2		5

4.3 Swallowing evaluation

4.3.1 Study 1

V-VST was used as a clinical examination to screen whether patients presented positive signs of OD. V-VST was adapted to each TP used for each study and followed the algorithm presented in figure 10.

VFS was selected as the diagnostic instrumental tool to analyse the swallowing process and analyse safety, efficacy and OSR parameters accordingly to previous studies [108], [140]. Safety was analysed according to the PAS [90] and efficacy to the Robbins scale [100]. Unsafe swallow was defined as presenting a PAS score greater than two [90]. Timing of OSR and bolus kinematics was assessed for each bolus given to the patient during VFS. Time to LVC and the total duration of the swallow response were also measured. In addition, mean bolus velocity of the bolus between the GPJ and the UES, propulsion forces, and kinetic energy were calculated.

For all the studies presented in this Study, VFS started with thin liquid boluses (<50 mPa·s) to assess the OD prevalence in the population and continued with boluses from the highest viscosity to the lowest. If the patient aspirated any of the thickened boluses, the study was terminated to avoid any further aspiration as a safety measure. VFS procedure was adapted to different volumes and viscosity levels determined for each study.

VFS recordings were obtained with a Super XT-20 Toshiba Intensifier (Toshiba Medical Systems Europe, Zoetermeer, The Netherlands) and recorded at 25 frames/s using a Canon DM-XM2 E video camera (Canon Inc. Japan).

Sub-study 1.1

Patients were clinically screened according to the V-VST using <50, 250 and 800 mPa·s as thin liquid, nectar and pudding, respectively.

Viscosity levels used for VFS for this study corresponded to: <50 (thin liquid), 150, 250, 450, 800 and 2000 mPa·s. During the VFS, 10 mL boluses were given in duplicate to each patient.

Sub-study 1.2

Viscosities used for the V-VST procedure were: <50, 200 and 1000 mPa·s for thin liquid, nectar and pudding according to the V-VST scheme (Figure 14).

For the VFS, viscosity levels selected corresponded to: <50, 200, 1000 and 2000 mPa·s. Two different volumes of each viscosity were administered to the patient: 5 and 20ml. Highest volume was only administered when a safe swallow for 5 ml was obtained. OSR was measured with the 5 mL bolus at each of the studied viscosities.

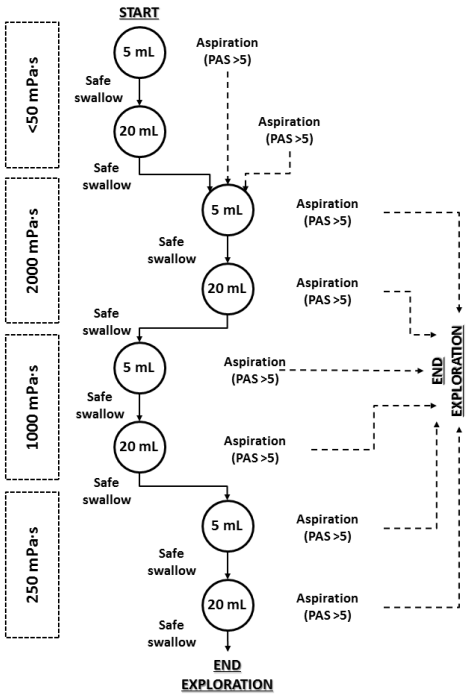

Figure 14. Study algorithm and safety stop rule for the videofluoroscopic exploration of the therapeutic effect of the different levels of viscosity. *PAS: penetration-aspiration scale* [90].

Sub-study 1.3

V-VST was performed on patients who had not been diagnosed with OD. For this specific study, viscosity levels used for the V-VST were <50, 200 and 800 mPa·s.

Viscosity levels used for the VFS corresponded to: <50, 100, 200, 400, 800 and 1600 mPa·s. Patients were asked to swallow 10 ml boluses per duplicate for each viscosity level.

4.3.2 Study 4

Therapeutic effect was determined by the revision of the three CT mentioned above. Safety of swallow, efficacy, time to LVC and kinematics of swallowing was obtained for each study.

4.4 Palatability

4.4.1 Study 1

Sub-study 1.1

During the VFS, patients were asked whether they felt comfortable during the swallowing experience *''I felt comfortable during swallowing this product''* using a 9 point-Likert scale, at each viscosity level. Results are presented by using three categories: 1) strongly agree, agree and moderately agree 2) mildly agree, undecided, mildly disagree; and 3) moderately disagree, disagree and strongly disagree.

Sub-study 1.2

Subjective palatability of the product was measured after each of the swallowed boluses at every volume and viscosity during VFS. Patients were asked to answer the following: "*What is*

your perception about the palatability of the given bolus?" Each bolus was evaluated with a visual-analogue scale (VAS). The VAS scale was presented in a numerical form with values from 0 to 10 presented in a straight horizontal line of fixed length oriented from right to left. The descriptive term used for 0 was "bad" and for 10 was "excellent". No descriptive terms were used for the rest of the numbers.

Sub-study 1.3

During VFS, and after each swallow, study participants were asked for their opinion on the palatability of the product with the 5-point facial Likert hedonic scale with the following question: "*From 0 to 5 rate, how much you liked it?"*

4.5 Adverse events

4.5.1 Study 1

For scoring the severity of the adverse events (AE) the CTCAE Terms and Grades have been used (U.S. Department of Health and Human Services, National Institutes of Health v5.0; Gastrointestinal Disorders). To score the relation with the product of any AE the World Health Organisation (WHO) and the Uppsala Monitoring Centre (UMC) for standardised causality assessment system was used. (ref).

Sub-study 1.1

Recorded all AE occurring during the study and one week after the procedure by a follow-up telephone call.

Sub-study 1.2

Adverse events were reported from the initiation of any study procedure to the end of the study treatment.

Sub-study 1.3

A telephone call 48h after the VFS was made to record if any AE occurred since the beginning of the study.

4.6 Rheological characterisation, equipment, laboratories

To assess shear viscosity the Haake Viscotester®550 (Thermo Fisher Scientific, Haake Viscotester®550, Germany) was used. Briefly, the bolus to be measured (10 ml) is placed in the sensor system's gap. The bolus exerts a resistance to the rotational movement produced by the rotor and results are analysed by the Software RheoWin (Job Manager® and Data Manager®). Two types of sensory system were used depending on the viscosity of the bolus tested: for low viscosities (50 to 300 mPa·s) MV1 (gap: 0.96 mm) was selected, while for high viscosities (>300 mPa·s) SV1 was used (gap: 1.45 mm). Temperature was controlled at 25º C by the ThermoScientific system which consists of 50% water, 50% deionized water and anti-algae.

To assess the effect of shear rate, viscosity at 50 $s^{-1}$and 300 s^{-1} at 25ºC were interpolated from the regression line obtained from the shear rate range from 1 to 1000 s^{-1}. Viscosity flow curves

were fitted to the Ostwald-de Waele model or Power Law (Equation 1) and the index flow (n) and consistency (K) were obtained for each viscosity level to assess the viscosity (η) behaviour of the TP at the shear rate studied (γ̇). The index flow describes the relationship between viscosity and shear rate and divides fluid behaviour into pseudoplastic (<1; shear thinning) or dilatant (>1; shear thickening). The consistency factor indicates the fluid viscosity at the specific shear rate of 1.

Equation 1 $\text{Log } \eta = (n-1) \log (\dot{\gamma}) + K$

Viscosity flow curves are represented by a Cartesian coordinate system where the dependent variable is the shear rate range represented in s^{-1} and the independent variable shows the relative viscosity in mPa.

Shear rate effect was calculated by the variations in apparent viscosity between the value at 50 s^{-1} and at 300 s^{-1} (equation 2). To assess the effect of salivary amylase, viscosity is measured after an oral incubation of a 15ml bolus during 30 seconds in a shear rate range from 1 to 1000 s^{-1}. Amylase effect on was determined by an analysis of the shear viscosity after an oral incubation by calculating the differences in apparent viscosity value between the sample at 50 s^{-1} (reference sample) and the incubated sample for each level of viscosity (equation 3). Tests were always performed in the morning to avoid variations in saliva due to the circadian rhythm. The viscosity affection by both swallowing factors (amylase and shear rate) was also calculated by the difference between the reference sample and the apparent viscosity at 300 s^{-1} after oral incubation (equation 4):

Equation 2 [(viscosity at 50 s^{-1} – viscosity at 300 s^{-1}) / viscosity at 50 s^{-1}] * 100

Equation 3 [(viscosity at 50s^{-1} – viscosity at 50s^{-1} after oral incubation) / viscosity at 50s^{-1}] * 100

Equation 4 [(viscosity at 50 s^{-1} – viscosity at 300 s^{-1} after oral incubation) / viscosity at 50 s^{-1}] * 100

4.7.1 Study 1

Sub-study 1.2

To assess the effect of salivary α-amylase during the oral phase of swallow, 15 mL boluses (250, 1000 and 2000 mPa·s) were given to the patients included in the study in a randomized order.

Sub-study 1.3

The effect of α-salivary amylase on viscosity in the oral phase was determined in healthy young volunteers. All levels of thickness recommended by each manufacturer were assessed before and after oral incubation. Regression lines of viscosity were performed with the mean values obtained from all the participants.

4.7.2 Study 3

Validation of the proposed rheological protocol has been performed in 4 different laboratories. Laboratory 1: Collaborated company with Morinaga, Japan. MCR 302 - Rheometer (Anton Paar); Laboratory 2: Health Care and Nutritional Science Institute Morinaga Milk Industry, Co., Ltd., Kanagawa, Japan. MCR 301 Rheometer (Anton Paar); Laboratory 3: I+D Laboratory on rheology and alimentary texture of Hospital de Mataró, Mataró, Spain. Haake Viscotester 550 (Thermo Fisher); Laboratory 4: Prefectural University of Hiroshima, Hiroshima, Japan. Haake Rheostress 6000 (Thermo Fisher). This study was divided in the following parts:

a) *Harmonization of the preparation protocol*. In order to standardize the preparation method and analysis for the identical rheological protocol to be applied in the four laboratories, the reference laboratory (Lab1) previously assessed the factors that differed which included: i) Stirring conditions: rotations per seconds, stirring speed and time; ii) Stirrer (metallic spatula, 160mm length plastic spoon and 100mm length plastic spoon) for all viscosity levels; iii) Container (glass beaker, white plastic cup, clear plastic cup) and; iv) Standing time before measurement (immediately, 10 and 30 min) for 100, 400 and 1600 mPa·s.

b) Laboratory Measurements Variability. Four different facilities (1, 2, 3 and 4) validated the common rheological protocol to analyze the shear viscosity the selected TP at different doses. The following protocol was established: i) weigh the dissolvent in a clear plastic cup; ii) weigh the TP; iii) add it to the dissolvent over 5s while stirring at 4 rps with a metallic spatula; iv) continue stirring for 30s at the same velocity; v) rest for 10min; vi) analyze viscosity by increasing shear rate from 0 to 1000 s^{-1} in a 10-minute test at 25ºC. Viscosity measurements were performed by triplicate on three samples for each viscosity level. Daily condition (DC) doses (TP with mineral water) have been used for this test.

c) *Rheological characterization.* The rheological characterisation was carried out in Lab3. Oral salivary amylase, shear rate effect and the combined effect of both factors was performed. Time effect on shear viscosity was also assessed for this specific TP, two DC solutions (200 and 800 mPa·s) were analysed at several timings: at the moment when the solution is prepared, and 30, 60, 90 and 120 minutes later. Temperature effect on shear viscosity was analysed in individual experiments by increasing the temperature range of the TP solution in steps of 5ºC from 25 to 40ºC. Effect of X-ray contrast on shear viscosity of TP was also determined: solutions were adapted for the addition of X-ray contrast (Omnipaque™; GE Healthcare Bio-Sciences, S.A.U.) to perform VFS for the diagnosis of OD (VFS doses). The final volume was assessed at 50ml with a 1:1 proportion of water and X-ray contrast. Doses of TQ (g/ 100 ml water) were adjusted to provide the expected viscosity by the reference Laboratory (Lab1) and the Health Care Centre (Lab3) where OD examinations are performed.

4.7.3 Study 4

a) *Shear viscosity.* Shear viscosity was assessed for the viscosity levels selected for each TP explained above in section 3.4 (*Products, doses and viscosity levels*).

b) *Extensional deformation.* Extensional properties of each TP were assessed with a Capillary Breakup Extensional rheometer (HAAKE CaBER, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Briefly, a small quantity of sample (<0.2ml) is placed between two circular plates. The upper plate is separated from the under plate forming a fluid filament. When the stretching finishes, the fluid at the mid-point of the filament is submitted to extensional strain rate according to the extensional fluid properties. A laser monitors the filament diameter and the time at which the filament breaks (filament diameter = 0). The time to Break Up Diameter (BUD) was obtained for each viscosity level for all the TP selected. The parameters selected to carry out the extensional deformation analysis are shown in Table 3. Triplicates were performed for each dose of TP.

Table 8. Parameters selected for the extensional deformation analysis.

Parameter	Value
Temperature (ºC)	25
Initial diameter (mm)	6
Initial height (mm)	1.50
Final height (mm)	12.15

c) *Textural characterization.* A texture profile analysis (TPA) was performed for each dose of TP to assess the maximum force, cohesiveness and adhesiveness at each viscosity level. TPA corresponds to two extrusion forces performed by a Texturometer TA.XTplus (Stable Micro Systems, Haslemere, UK). Each sample was tested by triplicate giving a plot of force vs time (Texture Expert for Windows v. 1.05 software, Stable Micro Systems, Haslemere, UK). Maximum force was determined by the first peak force obtained. Adhesiveness was extracted from the mean assessed by the negative value of the area under the curve (-AUC). Cohesiveness was calculated by Area 2/Area 1. Table 9 presents the texturometer settings assessed for the solutions when TPA was applied. Calibration of the texturometer was performed with 2kg. A quantity of 30g of each TP solution was used to perform the analysis.

Table 9. Probe and texturometer settings

Mode	Texture Profile Analysis
Sample volume	30g
Option	Return To Start
Pre-Test Speed	1 mm/s
Test Speed	1 mm/s
Post-Test Speed	5 mm/s
Distance	5 mm
Trigger Type	Auto 5g
Tare Mode	Auto
Data Acquisition Rate	200 pps

4.8 Labelling and viscosity classifications analysis

4.8.1 Study 2

A summary of the information provided by manufacturers on their labels was made. This information was compiled based on the EU Regulation 1169/2011 [117], EU Regulation

609/2013 [118] and EU Regulation 2016/128 for general food and FSMP labelling [119] and included composition of the TA for the TP, recommended dosage (g/ml, scoops, etc.) and descriptors for each thickness level and preparation method for the thickened fluids.

Viscosity values obtained at 50 s^{-1} were correlated with the 3 textural classifications. In addition, the IDDSI flow test at room temperature was performed with the syringe BD Plastipak™ of 10 ml (Beckton-Dickson model; Ref. 302188) [142] for all TPs as defined by the IDDSI Framework definitions 2.0 2019 [124]. Briefly, the plunger was removed from the syringe, the solution to be measured was filled into the syringe with the nozzle covered, the timer was started and the nozzle uncovered. After 10 seconds, the nozzle was covered again and the millilitres of solution that remained in the syringe were measured. For IDDSI level 4, the fork-drip test was used. Briefly, the flow through the tines of the fork is observed and the level is selected according to the descriptions of each level.

4.8 Data analysis

4.8.1 Study 1

Qualitative data is presented as relative and absolute frequencies and analyzed by the Fisher's exact test (sex, VFS signs of impaired efficacy and safety of swallow) or the Chi-square test (MNA-sf categories, PAS score categories). The viscosity levels were compared with thin liquid by applying the McNemar's test (VFS signs of impaired efficacy and safety of swallow between viscosities). Continuous data is presented as mean ± standard error of the mean (SEM) and compared using the T-test (intergroup comparisons) or Paired T-test (intragroup comparisons); for those variables that did not follow a normal distribution, we used the nonparametric Mann-Whitney U test (intergroup comparisons), the Wilcoxon-paired test (intragroup comparisons) or the Kruskal-Wallis' test for multiple comparisons with Dunn's multiple comparison test. To assess normality, we used the D'Agostino and Pearson omnibus normality test.

The main variable was prevalence of patients with safe swallowing at each one of the viscosities. These data were handled as binary by dividing the patients into two categories: a) patients who could swallow safely (PAS 1-2) vs. patients who could not swallow safely (PAS 3-8, including patients who discontinued the study due to the safety rule). We compared prevalence of patients than could vs. could not swallow safely for each of the thickened viscosities compared with thin liquid. Safety of swallow of each patient for the whole VFS exploration or at a particular viscosity or level was expressed as the worst PAS score. Effect on penetrations (PAS 3-5) and aspirations (PAS 6-8) were also assessed. The efficacy of swallowing was also handled as binary data for oral and pharyngeal residue: if residue was observed in the oral cavity or at any of the three pharyngeal locations (pharyngeal wall, vallecular and pyriform sinus) the residue was present (yes), if no residue was observed at any of the locations the residue was absent (no).

Statistical tests were conducted two-sided with a significance level of 5%. All confidence intervals are presented two-sided with a confidence level of 95%. A resultant probability value of $p<0.05$ was judged as statistically significant. An additional explorative analysis was performed on safety of swallowing and the mean PAS scores to evaluate the therapeutic effects between viscosities. As a post hoc test, the bolus propulsion force was analyzed, and dose-response curves to assess the viscosity-dependent effect of TP on safety and efficacy were

obtained by representing the prevalence of patients with safe swallowing or residue vs viscosity log at each level of viscosity using Graphpad Prism 6 (San Diego, CA, USA).

4.8.2 Study 2

Qualitative data has been used to describe the decrease in viscosity through salivary amylase or shear rate and is presented in percentage as absolute frequencies. Continuous data is presented as mean±standard deviation (SD).

4.8.3 Study 3

Variability between laboratory values has been presented in mean±CV. Quantitative data has been used to describe the decrease in viscosity through salivary amylase or shear rate and is presented as a percentage of absolute frequencies. Significance on the viscosity decrease by those factors has been analysed by a paired t-test. Continuous data is presented as mean±standard deviation (SD). Statistical tests applied to assess temperature and time effect were the non-parametric Anova test (Kruskal Wallis) to compare all the groups and the U-Mann Whitney test to assess 1:1 difference. Significance was considered at $p<0.05$.

4.8.4 Study 4

The correlation between the prevalence of safe swallows with shear viscosity, extensional deformation, MF, adhesiveness and cohesiveness and the correlation between rheological parameters compared with each other were determined with the Spearman correlation coefficient. A linear or a non-linear correlation was applied between variable.

4.9 Experimental design

4.9.1 Study 1 **-** Evaluation of the therapeutic effect of different TP and the establishment of the optimal viscosity doses to treat OD patients

Sub-study 1.1

This was a reference-controlled, multiple-dose, fixed-order, single-blind and single-center study. The study procedure (figure 15) was performed in one single visit. First, the V-VST was performed on each patient to assess clinical signs of OD [28], [136], [143] and those positive for OD were referred for VFS. One week after the completion of the study, a follow-up call was performed to assess potential adverse events.

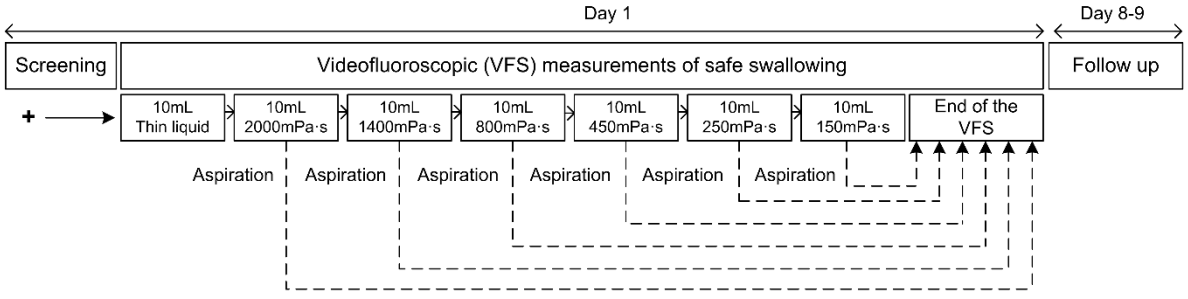

Figure 15. Experimental design from Sub-study 1.1.

Sub-study 1.2

This was a prospective, single-center clinical study on patients with OD caused by the 4 main etiologies (aging, head and neck cancer, stroke and Parkinson's disease) to evaluate and compare the therapeutic effect and rheological characteristics of the TP Fresubin Clear Thickener. First, patients were clinically assessed for OD with the V-VST [99] and if positive they underwent VFS. The general study design is presented in Figure 16. We also designed an in vitro study to assess the effect of α-amylase during the oral phase and the effect of shear-thinning during the oral and pharyngeal phase of this new thickener.

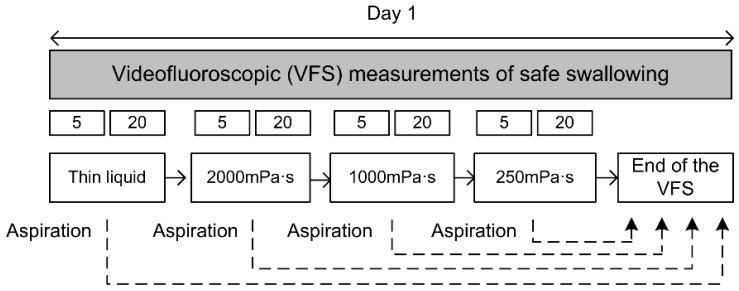

Figure 16. Experimental design from Sub-study 1.2.

Sub-study 1.3

This was an interventional non randomized, multiple dose, fixed order and single-centre study to analyse the therapeutic effect of a TP by VFS on older patients with OD. The overall study procedure was performed in one single visit. Older patients with OD that fulfilled the inclusion criteria were included in the study. Forty-eight hours after the VFS, patients were asked by telephone if they had had any adverse event (AE) after the test.

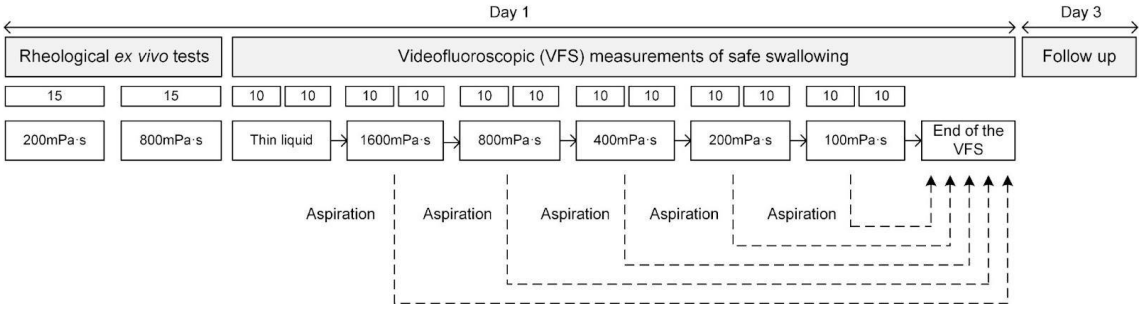

Figure 17. Experimental design from Sub-study 1.3.

4.9.2 Study 2 - Analysis of the risks of using qualitative descriptors for viscosity levels in TP labelling

This was an observational, single-centre study to analyse the information included on each label of TPs and the main rheological factors that can affect the therapeutic effect of prescribed TP.

4.9.3 Study 3 - Design and validation of a rheological protocol to standardize shear viscosity measurements

This study includes three different parts: a) the accuracy, harmonization and validation of a common rheological protocol to assess the shear viscosity of a thickening product in 4 international laboratories; b) the in vitro rheological characterisation (amylase resistance and shear thinning effect) of Tsururinko Quickly (TQ), including the assessment of the effect of fluid temperature and lag time, and; c) the effect of an X-ray contrast on the rheological properties (Figure 18).

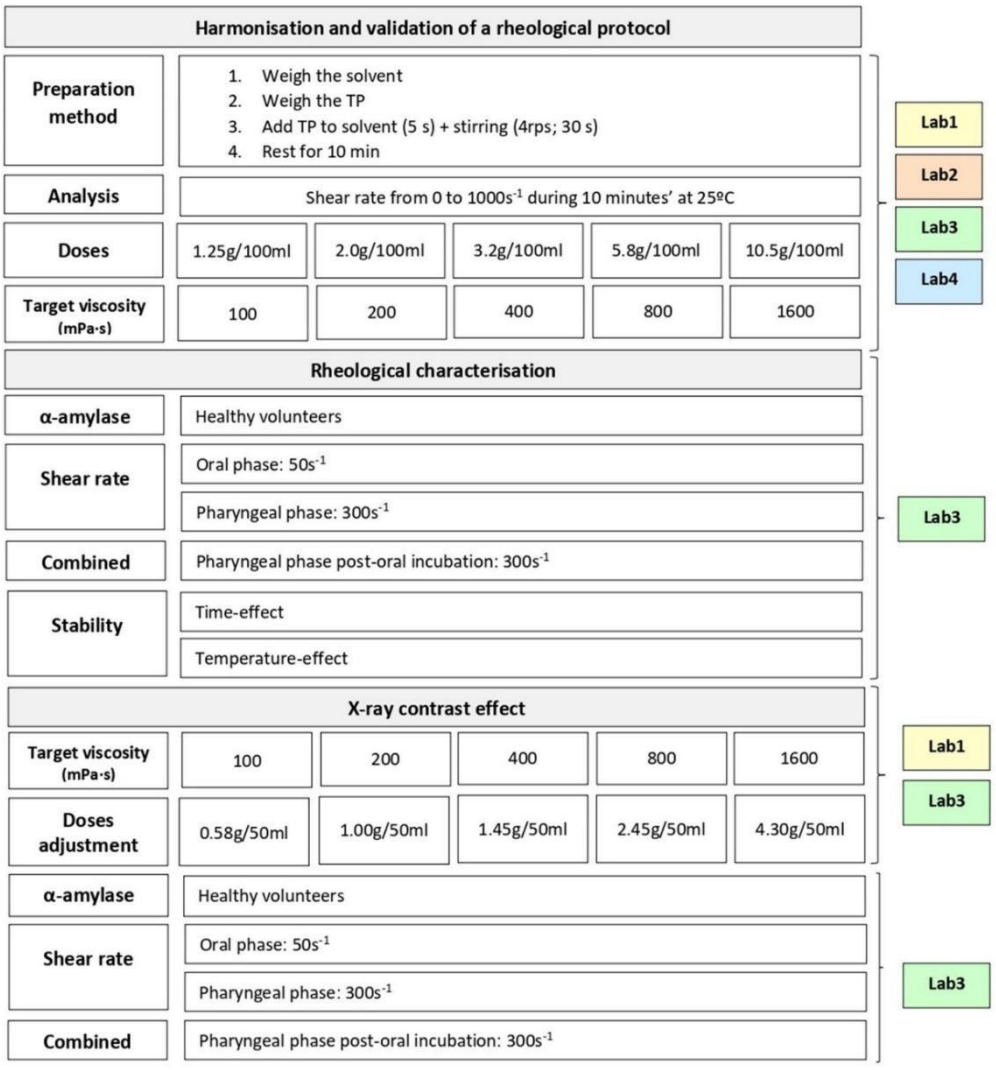

Figure 18. Experimental design from Study's 3 study showing the experiments, the measurements and the laboratories involved in the study. Laboratory 1: Company collaborating with Morinaga, Japan; Laboratory 2: Health Care and Nutritional Science Institute, Morinaga Milk Industry, Co., Ltd., Kanagawa, Japan; Laboratory 3: I+D Laboratory on rheology and alimentary texture at Hospital de Mataró, Mataró, Spain; Laboratory 4: Prefectural University of Hiroshima, Hiroshima, Japan. TP: Thickening Product; OD: oropharyngeal dysphagia.

4.9.4 Study 4 – The rheological properties of TP that have a therapeutic effect on safety of swallow in patients with post-stroke oropharyngeal dysphagia

This was an observational, single-centre study including: a) characterization of the shear viscosity, extensional deformation, maximal force, adhesiveness and cohesiveness and; b) analysis of the therapeutic effect reported by previous CT.

5. RESULTS

5.1 Study 1 - Evaluation of the therapeutic effect of different TP and the establishment of the optimal viscosity doses to treat OD patients

5.1.1 Sub-study 1.1

Study population

Of the 120 patients enrolled, 4 were excluded from the all subjects treated (AST) population because they did not receive any of the thickened viscosities. Additionally, 2 patients were excluded from the per protocol population (PP) because they discontinued due to reasons other than aspiration which was regarded as a protocol deviation. The originally planned analysis was on the intention-to-treat population (ITT). However, because there were 4 patients in this population who did not receive any of the thickened product, it was decided to present the results for the PP population (n=114) (Supplementary Figure 2). The results of the ITT and PP populations were comparable. The majority of our population 76% (N=87) were in the subacute phase (28-180 days after stroke) and 24% (N=27) were chronic (>180 days after stroke). Mean age of the participants was 76.7±8.9 yr. and 54.4% were men. The MNA-SF total score indicated that 54.4% of patients were malnourished or at risk of malnutrition when enrolled in the study. Stroke type was predominantly ischemic 78.1% (n=89) and the prevalent severity of the stroke valued with the NIHSS was scored (mean±SD) 7.5±6.8 on admission and 5.3±5.9 on discharge.

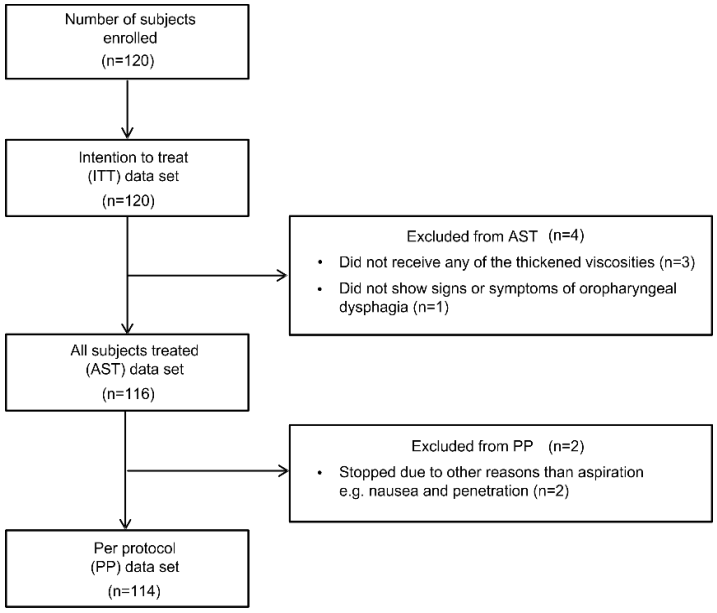

Figure 18. Study flow chart from Sub-study 1.1.

Therapeutic effect

Safe swallowing was observed in only 41.2% (n=47) of the poststroke OD (PSOD) patients at thin liquid but the percentage significantly increased with the main viscosities (all p<0.001 vs. thin liquid) (Fig.2). Similarly, safety of swallowing significantly increased with the explorative viscosities compared to thin liquid (all p<0.001 vs. thin liquid) (Figure 19).

Mean PAS score at thin liquid was 3.7±2.3, and it significantly decreased to 1.9±1.4, 1.8±1.6, 1.7±1.6, 1.4±1.2, 1.2±0.6 and 1.4±1.2 by increasing viscosity from 150 to 2000 mPa·s, (all p<0.001 vs. thin liquid). The distribution of safe swallowing, penetration and aspiration was significantly different at all viscosities compared to thin liquid (all p<0.001 vs. thin liquid). The percentage of patients with penetration and aspiration decreased when viscosity increased. Prevalence of patients with penetrations at thin liquid was 41.2% and ranged between 2.6%-13.2% for the thickened viscosities (figure 20). Prevalence of patients with aspirations showed significant differences (p<0.01) with thin liquid (17.5%) vs. all viscosities (0.0-4.4%) except for 150 mPa·s (2.5%, p=0.180). Among the different viscosity levels, there were significant differences between the therapeutic effect of 250 mPa·s (78.9%) vs. 800 (92.1%), 1400 (95.6 %) and 2000 mPa·s (91.2%), (all p<0.01 vs. 250 mPa·s), but not between 800 and 2000 mPa·s or between 800 and 1400 (p>0.05). The maximal therapeutic effect (ceiling effect) was observed at 800 mPa·s (92.1% of patients with safe swallowing).

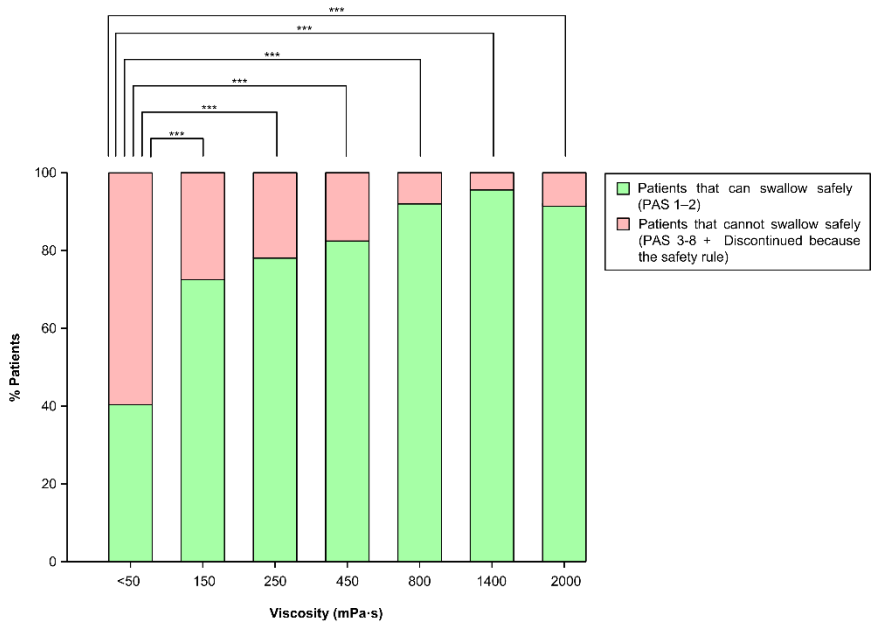

Figure 19. Percentage of poststroke oropharyngeal dysphagia patients with safe/unsafe swallow at each level of viscosity. 'N' represents the number of patients who performed the bolus out of the per protocol population (n=114). The percentage of patients with unsafe swallow includes those with aspirations at the former viscosity who discontinued due to the safety rule. Percentage of patients who discontinued at each viscosity: thin liquid (0.0%), 150 mPa·s (12.3%), 250 mPa·s (8.8%), 450 mPa·s (4.4%), 800 mPa·s (1.8%), 1400 (1.8%), 2000 mPa·s (0.9%)). ****p<0.001 vs. thin liquid.*

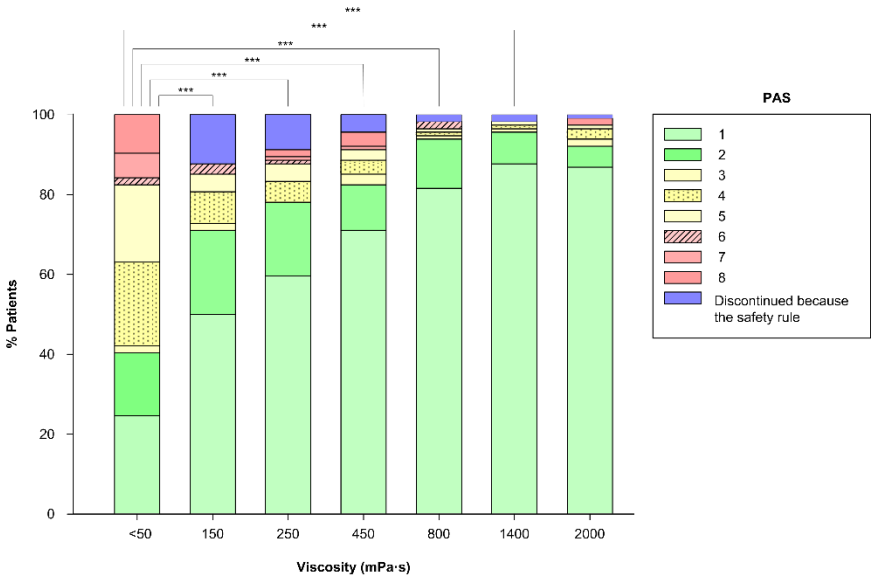

Figure 20. Percentage of poststroke oropharyngeal dysphagia patients at each level of the Penetrations Aspiration Scale [90]. ****p<0.001 vs. thin liquid.*

Regarding the efficacy of swallow, at thin liquid, pharyngeal residue was present in 41.2% (n=47) of patients and it did not increase at any of the tested viscosities (37.7-44.7%, all p>0.05 vs. thin liquid) (Fig. 4). Oral residue was present in 38.6% (n=44) at thin liquid and significantly increased at all thickened viscosities (all p<0.01 vs. thin liquid) (figure 21).

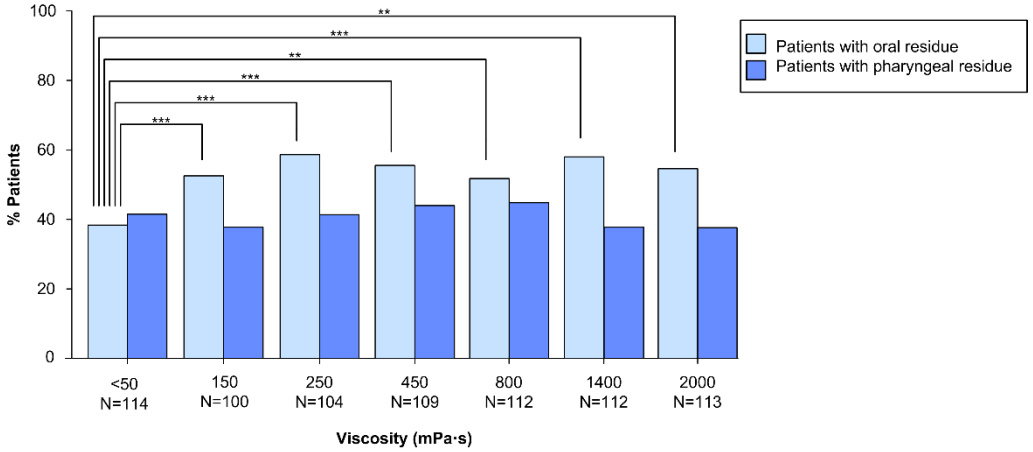

Figure 21. Percentage of poststroke oropharyngeal dysphagia patients of the per protocol population (n=114) with oral and pharyngeal residue at each viscosity level. 'N' represents the population who performed the bolus. ****p<0.001 vs. thin liquid.*

Figure 22 shows the viscosity-dependent therapeutic effect on safety of swallowing for the tested viscosities. 150, 250 and 450 mPa·s offered a protection on safety of swallowing between 71.9% and 82.5% and 800, 1400 and 2000 mPa·s a protection between 91.2% and 95.6%. Safety increased in a viscosity-dependent manner. Pharyngeal residue was not statistically different

compared with thin liquid at any of the tested viscosities. Oral residue slightly, but significantly, increased at all viscosities.

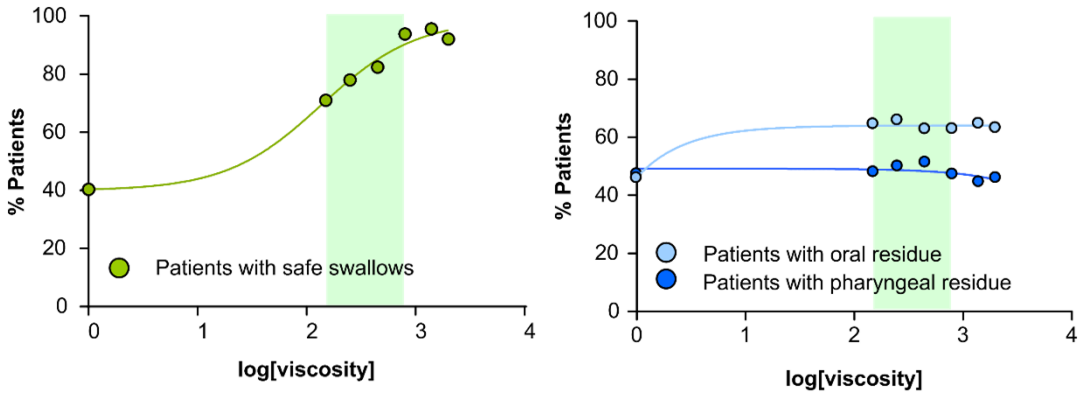

Figure 22. Dose-response curves for the therapeutic effect of the gum-based thickener on safety and efficacy of swallowing in PSOD patients. The upper panel shows the curve of the viscosity-dependent response represented by the percentage of patients with safe swallows vs. the log of the viscosity. The lower panel shows the curve representing the effects on the prevalence of oral and pharyngeal residue vs. the log of the viscosity. The shadowed area represents the therapeutic range (150-800 mPa·s) of the product.

Oral swallow response

Time to LVC at liquid viscosity was severely delayed (382.5±139.1 ms) in PSOD patients. Increasing bolus viscosity ≥150 mPa·s shortened time to LVC for all viscosities (figure 23): mean LVC for each viscosity was 327.3±108.2 (150mPa·s), 330.1±143.4 (250mPa·s), 304.8±109.6 (450mPa·s), 303.3±94.7 (800mPa·s), 300.5±110 (1400mPa·s), 300.4±107.8 (2000mPa·s) ms ($p<0.01$ vs. liquid). Time to LVC was shorter in patients with safe (PAS 1-2) vs. unsafe swallow (PAS 3-8): significant differences were detected in all viscosities except for 2000mPa·s (Fig. 6).

At thin liquid, the total duration of the swallow response was 1020.9±220.8 ms and significantly decreased to 947.1±228.7, 998.8±472.1, 944.1±180.2, 943.1±221.4, 953.5±225.3, and 943.2±234.8 ms at 150, 250, 450, 800, 1400 and 2000 mPa·s, respectively (all $p<0.01$ vs. thin liquid).

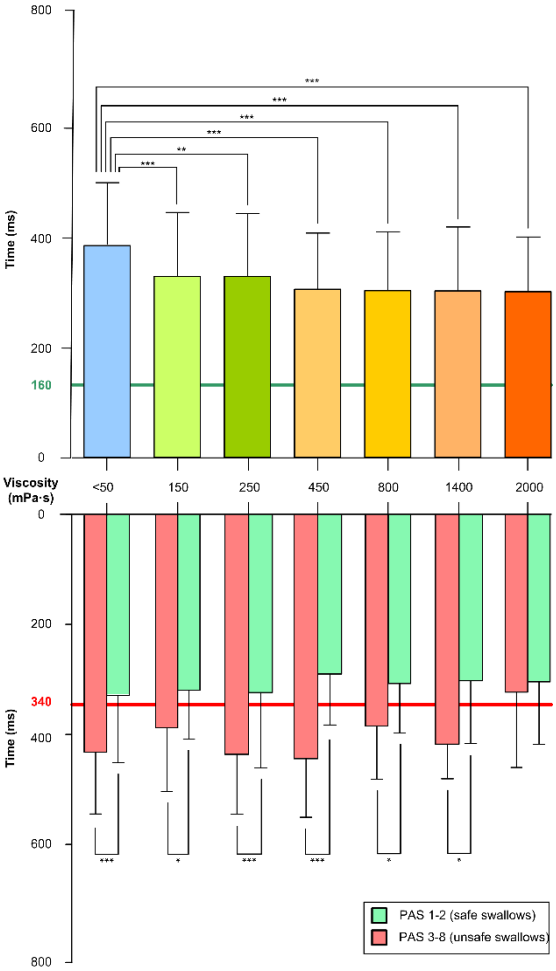

Figure 23. Time to LVC at each viscosity level. The upper panel shows mean time to LVC at each viscosity. The lower panel shows time to LVC plotted against safe/unsafe swallow at each viscosity level. Time to LVC was delayed in patients with unsafe swallowing at all viscosity levels except for 2000 mPa·s. Time to LVC<160ms (green line): safe swallowing as established in a study with healthy volunteers (4).Time to LVC≥340ms (red line): cut-off time to detect the presence of unsafe swallowing in post-stroke patients according to previous studies (12). *p<0.05; **p<0.01; ***p<0.001.

Kinematics of swallow

PSOD patients included in the study presented a mean bolus velocity at liquid of 0.3138±0.1265 (m/s). Increasing bolus viscosity, ≥450 mPa·s caused a significant reduction in bolus velocity for 450mPa·s (0.2835±0.0948; p<0.05), 800mPa·s (0.2613±0.0784; p<0.001), 1400mPa·s (0.2564±0.0803; p<0.001) and 2000mPa·s (0.2729±0.1010; p<0.01) vs. thin liquid (figure 24). Mean bolus propulsion force was 0.041±0.035 mN at thin liquid. A significant decrease was found at the thickened viscosities (all p<0.001 vs. thin liquid): 150 mPa·s (0.033±0.025), 250 mPa·s (0.035±0.032), 450 mPa·s (0.030±0.019), 800 mPa·s (0.026±0.014), 1400 mPa·s (0.025±0.015) and 2000 mPa·s (0.028±0.022).

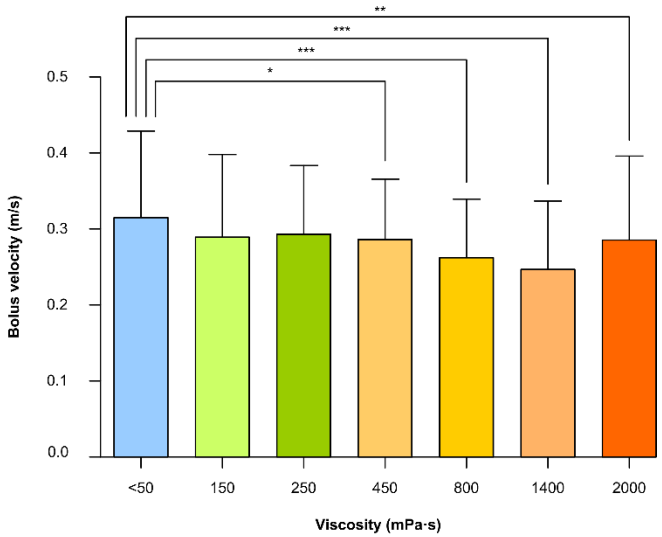

Figure 24. Mean bolus velocity from GPJO to UESO at each viscosity level. Bolus velocity was reduced above 450 mPa·s. **p<0.05; **p<0.01; ***p<0.001 vs. thin liquid.*

Palatability

Comfortability while swallowing scored highest at thin liquid (66.3%) and it decreased significantly to 46.3% and 31.3% during swallowing the main viscosities 800 and 2000 mPa·s, respectively. Categories of comfortability were differently distributed at all viscosities compared to thin liquid (all p<0.001 vs. thin liquid), except for 150 and 250 mPa·s.

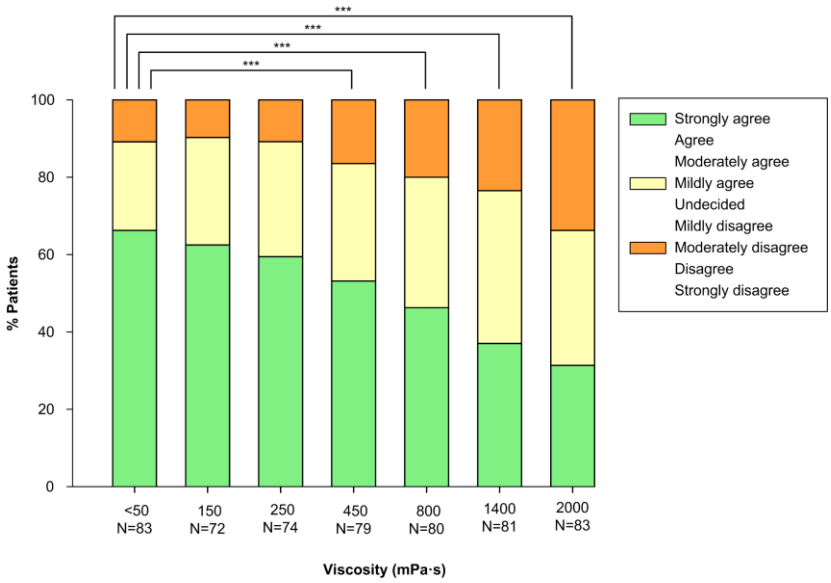

Figure 25. Palatability of the thickening product at each viscosity level assessed.

Adverse events

A total of 16 AE occurred in 11 patients out of the 116 in the AST population and were considered unrelated or unlikely to be related to the study product. The most frequent AE were mild

gastrointestinal disorders (14 AE in 10 patients): diarrhea, nausea, abdominal distension and pain, dyspepsia and stomatitis. No serious AE were reported during or following the study.

5.1.2 Sub-study 1.2

Study population

128 patients with OD were recruited for the study and divided in four groups according to OD etiology (Figure 26): a) 36 older patients, b) 31 patients following treatments for head and neck cancer (HNC), c) 31 post-stroke patients, and d) 30 Parkinson's disease patients. We found many demographic differences among the study population groups (age, sex, functionality, BMI, force and health status self-perception) (Table 10). As a summary, older and stroke patients were the oldest and weakest (handgrip) groups, while HNC was the youngest and with the best functional capacity. In the older group, unlike the others, the majority of patients were women. Regarding nutritional status, MNA-sf evaluation showed high and similar percentages of malnourished or at risk of malnutrition patients (from 63.34% to 40%) in all groups. Patients with HNC had the lowest BMI. Finally, health status self-perception was quite low except for the HNC group (Table 1).

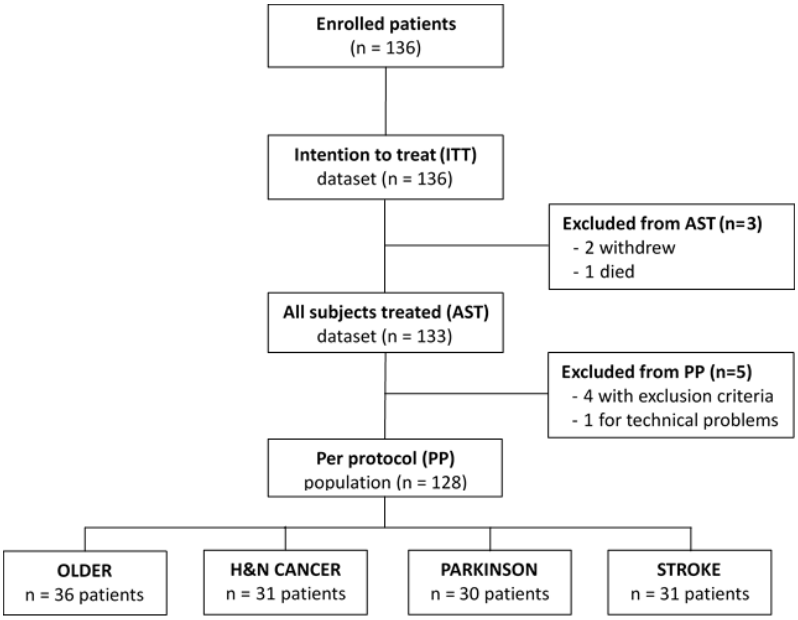

Figure 26. Study flow chart in the Sub-study 1.2.

Table 10. Demographic characteristics of the study population (continuous variables expressed as mean±SEM).

	OLDER	HNC	PARKINSON	STROKE	p-value
N	36	31	30	31	-
Age	82.96±1.24	68. 29±1.39****	72.34±1.92****	79.42±1.36#### ŦŦŦ	<0.0001
Sex (female) (%)	66.67 (24)	32.26 (10)**	20.00 (6)***	35.48 (11)*	0.0008
Barthel	78.33±4.25	96.50±1.68*	77.33±4.48#	74.83±5.31#Ŧ	0.0007
Optimum (100) (%)	38.89 (14)	67.34 (21)*	23.33 (7)###	43.33 (13)	0.005
Sub-optimum (<100) (%)	61.11 (22)	32.26 (10)	76.67 (23)	56.67 (17)	
MNA-sf	10.97±0.38	11.50±0.35	11.7±0.47#	10.6±0.51##	0.326
Well-nourished [12-14] (%)	47.22 (17)	48.57 (17)	60.00 (18)	36.67 (11)	
At risk [8-11] (%)	44.44 (16)	37.14 (13)	33.33 (10)	56.67 (17)	0.610
Malnourished [0-7] (%)	8.33 (3)	2.86 (1)	6.67 (2)	6.67 (2)	
BMI (kg/m2)	27.594±0.88	23.97±0.69*	27.50±0.85	27.78±0.74	0.002
Handgrip Force (kg)	16.33±1.42	22.78±1.97	25.38±1.78**	17.77±1.48Ŧ	0.0009
Health status self-perception (0-100)	63.57±3.32	70.83±3.85	56.25±3.74#	58.67±5.29	0.055

*MNA-SF indicates mini nutritional assessment short form; BMC, body mass index; HNC, head and neck cancer. * p-value <0.05, ** <0.01 vs. Older; # p-value <0.05, ## <0.01, ###<0.001, ####<0.0001 vs. HNC; Ŧ p-value <0.05, ŦŦŦ<0.001 vs. Parkinson.*

Therapeutic effect

VFS evaluation showed an overall study population with a high prevalence VFS signs of impaired safety (70.31%) and efficacy (98.44%) of swallow and residue and a severe PAS (4.44±0.20). However, there was a different pattern of swallowing impairment depending on OD phenotype. Regarding impaired safety of swallow, we found a high prevalence of penetrations (46.67% - 74.19%) and aspirations (13.33% - 41.94%) in all groups and high mean PAS score (3.80 - 5.36) at thin liquid (<50 mPa·s). Patients with HNC had the most severe impairment of safety with a mean PAS score of 5.36±0.41, 41.94% of them presenting aspirations and up to 25.81% of them, silent aspirations (Table 11). Older patients presented the highest prevalence of oral residue (91.67%) and those with stroke the lowest (61.29%, p<0.01). HNC had the highest prevalence of pharyngeal residue (96.80%) while older patients had the lowest (66.67%, p<0.01) (Table 11).

Table 11. Videofluroscopic signs of impaired efficacy and safety of swallow in the study groups (continuous variables expressed as mean ± SEM) for any viscosity level.

	ALL	OLDER	HNC	PARKINSON	STROKE	p-value
N	128	36	31	30	31	
Impaired Safety (%)						
Penetrations	70.31 (90)	63.89 (23)	83.87 (26)	56.67 (17)$^{\#}$	77.42 (24)	0.076
Aspirations	61.72 (79)	58.3 (21)	74.19 (23)	46.67 (14)$^{\#}$	67.74 (21)	0.135
Silent Aspirations	28.91 (37)	25.00 (9)	41.94 (13)	13.33 (4)$^{\#}$	35.48 (11)	0.071
(PAS = 8)	14.84 (19)	11.11 (4)	25.81 (8)	13.33 (4)	9.67 (3)	0.256

Impaired Efficacy (%)	98.44 (126)	100.00 (36)	100.00 (31)	100.00 (30)	96.67 (29)	nc
Oral Residue	82.81 (106)	91.67 (33)	80.65 (31)	76.67 (23)$^{\#\#}$	61.29 (19)$^{**\ \#\#\#}$	0.0002
Pharyngeal Residue	80.47 (103)	66.67 (24)	96.80 (30)**	86.67 (26)$^{\#\#\#\#}$	74.19 (23)$^{\#\ ₸}$	<0.0001
Higher PAS score	4.44±0.20	4.08±0.39	5.36±0.41	3.80±0.40$^{\#}$	4.55±0.41	0.038

PAS: penetration aspiration scale.

We found a strong shear viscosity-dependent therapeutic effect on safety of swallow, with a maximal significant therapeutic effect at 1000 mPa·s (80.56%, 96.67%, and 74.19% of safe swallows) for older, Parkinson and stroke patients respectively. This therapeutic effect was greatly reduced in HNC patients, at 58.06%. (Figure 27 and 28). Further increase of viscosity up to 2,000 mPa·s did not cause a significant increase in safety in any study group. We also found that the therapeutic effect of FCT depended on the phenotype of patient assessed: therapeutic effect was significantly reduced in HNC vs. all the other groups together ($p<0.05$ <50 mPa·s; $p<0.01$ 250 mPa·s; $p<0.01$ 1000 mPa·s; and $p<0.001$ 2000 mPa·s) (Figure 27 and 28). However, it was similar between the three other groups, achieving 82.47% of patients with safe swallow at 1000 mPa·s, and 95.88% of patients with safe swallow at 2000 mPa·s. We also found an important reduction in the severity of OD measured with the PAS with significant changes when comparing <50 mPa·s vs. 1000 mPa·s and 2000 mPa·s in all groups of patients and 250 mPa·s vs. 2000 mPa·s in older, Parkinson and stroke groups, but not between 1000 and 2000 mPa·s, even when grouped together (Figure 28). The therapeutic range of FCT was defined between 250 and 1000 mPa·s. On the other hand, 250 mPa·s was selected as the minimum level of viscosity presenting a significant therapeutic effect (compared to thin liquid); and 1000 mPa·s as the maximal significant therapeutic effect compared to 2000 mPa·s.

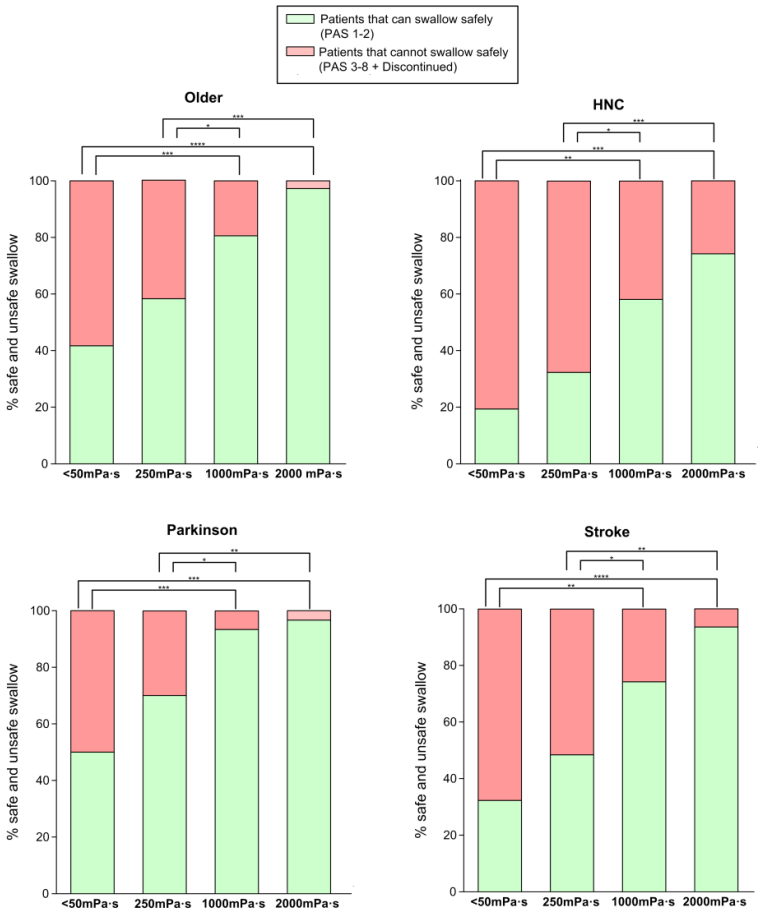

Figure 27. Percentage of patients who could swallow safely vs. those who could not swallow safely at each viscosity level. **p-value <0.05, **<0.01, ***<0.001, ****<0.001. HNC: head and neck cancer.*

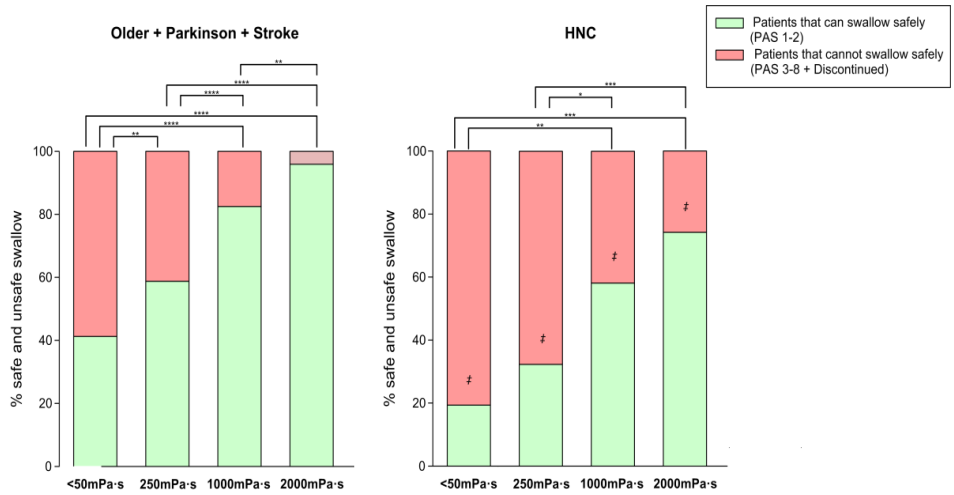

Figure 28. Percentage of patients who could swallow safely vs. those that cannot swallow safely comparing 3 groups together against HNC. Left: older, Parkinson and stroke patients; right: patients with head and neck cancer. **p-value <0.05, **<0.01, ***<0.001, ****<0.001. ‡p-value <0.05 Older+Parkinson+Stroke. HNC: Head and neck cancer.*

FCT presented a strong therapeutic effect depending on the shear viscosity. The minimum viscosity tested (250 mPa·s) showed a significant therapeutic effect vs thin liquid for all the

phenotypes studied and it increased until the maximum viscosity assessed (2000 mPa·s) achieving >90% of safe swallows in older, Parkinson and stroke groups and 74.19% in HNC patients (Figure 29). However, no significant differences were seen between 1000 mPa·s and 2000 mPa·s for each group compared individually (80.56%, 96.67%, 74.19% and 58.06 of patients with safe swallow for each group at 1000 mPa·s, respectively). Accordingly, the threshold viscosity of safety was 250 mPa·s and maximal viscosity, 1000 mPa·s.

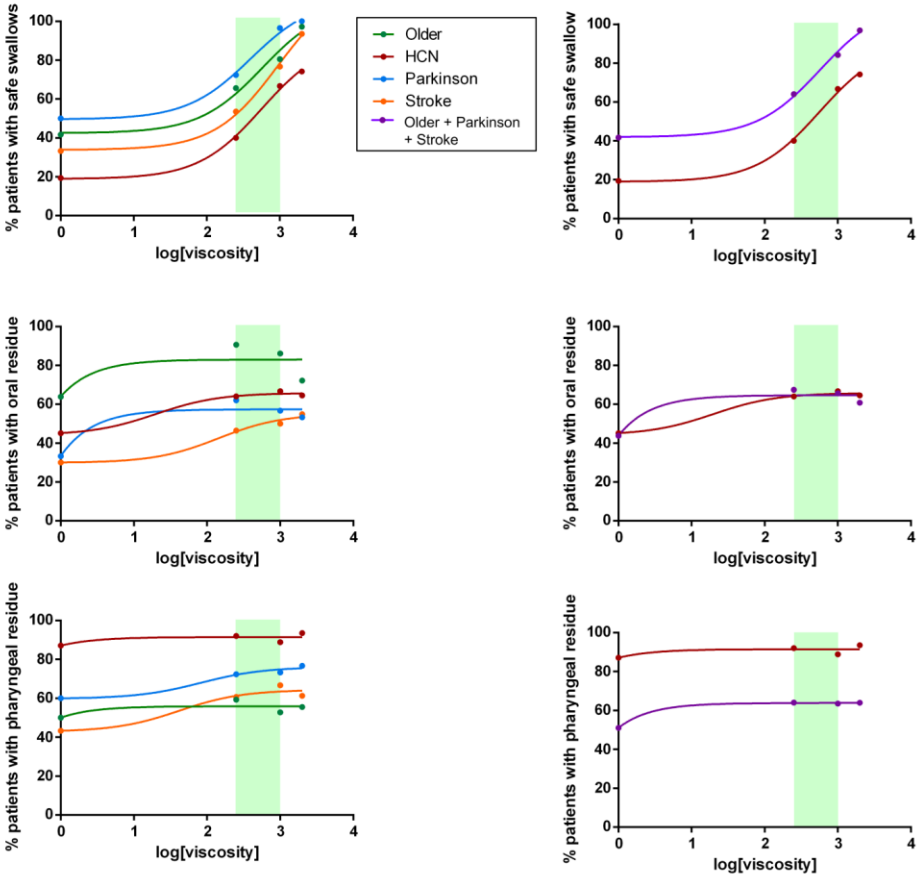

Figure 29. Viscosity-dependent effect of FCT on safety and efficacy of swallow (oral and pharyngeal residue). *Left: all groups of patients; right: older, Parkinson and stroke patients merged vs patients with head and neck cancer. The therapeutic range has been marked with a green rectangle.*

Regarding efficacy of swallow, a significant increment was observed in the prevalence of oral residue when compared the study viscosities with thin liquid in all phenotypes. Older patients that had the highest prevalence of oral residue and also presented significant differences in oral residue when comparing 2000 mPa·s vs. 250 and 1000 mPa·s (Table 12 and Figure 30). On the other hand, there were no differences regarding pharyngeal residue when we compared the tested viscosities in any of the studied phenotypes. The highest prevalence of pharyngeal residue was in HNC patients vs. the rest of the groups together: $p<0.001$ <50 mPa·s; $p<0.01$ 250 mPa·s; $p<0.05$ 1000 mPa·s; and $p=0.068$ 2000 mPa·s in all the tested viscosities (Figure 31).

Table 12. Comparison between viscosities of oral residue among the study groups.

OLDER	<50mPa·s (n=36)	250mPa·s (n=32)	1000mPa·s (n=36)	2000mPa·s (n=36)
Oral residue	63.89% (23)	90.63% (29)	86.11% (31)	72.22% (26)
<50mPa·s		0.008	0.013	0.752
250mPa·s			1.000	0.023
1000mPa·s				0.041
HNC	**<50mPa·s (n=31)**	**250mPa·s (n=25)**	**1000mPa·s (n=27)**	**2000mPa·s (n=31)**
Oral residue	45.16% (14)	64.00% (16)	66.67% (18)	64.52% (20)
<50mPa·s		0.041	0.046	0.114
250mPa·s			0.683	0.617
1000mPa·s				0.683
PARKINSON	**<50mPa·s (n=30)**	**250mPa·s (n=29)**	**1000mPa·s (n=30)**	**2000mPa·s (n=30)**
Oral residue	33.33% (10)	62.07% (18)	56.67% (17)	53.33% (16)
<50mPa·s		0.027	0.070	0.077
250mPa·s			1.000	0.683
1000mPa·s				1.000
STROKE	**<50mPa·s (n=30)**	**250mPa·s (n=28)**	**1000mPa·s (n=30)**	**2000mPa·s (n=31)**
Oral residue	30.00% (9)	46.46% (13)	50.00% (15)	54.84% (17)
<50mPa·s		0.131	0.073	0.023
250mPa·s			0.683	0.683
1000mPa·s				0.617

HNC: head and neck cancer

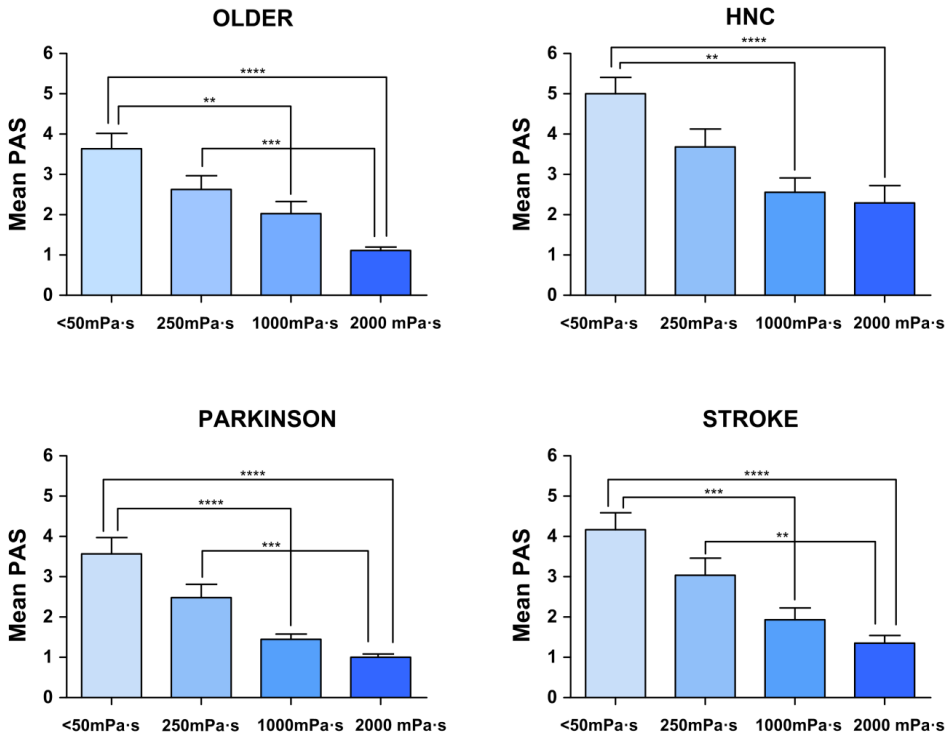

Figure 30. Mean penetration-aspiration scale (PAS) score in each viscosity of each of the study groups.
p-value <0.05, **<0.01, *<0.001, ****<0.001. HNC: head and neck cancer.*

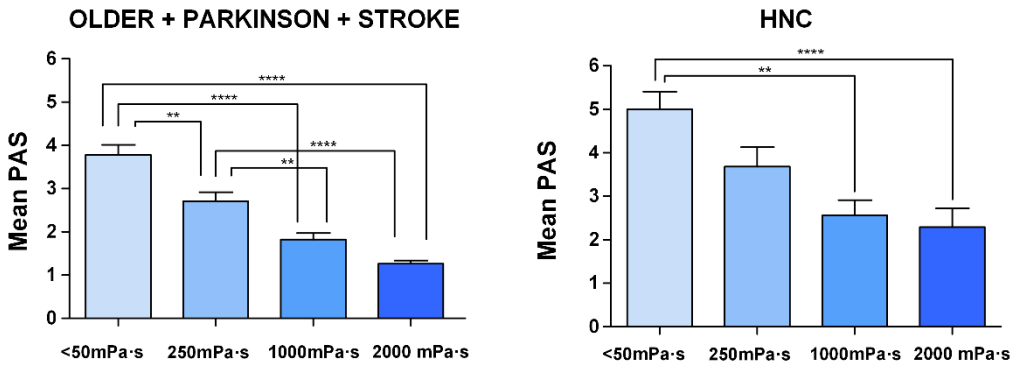

Figure 31. Mean penetration-aspiration scale (PAS) score in each viscosity comparing 3 groups together vs HNC. Left: older, Parkinson and stroke patients; right: patients with head and neck cancer. **p-value <0.05, **<0.01, ***<0.001, ****<0.001. HNC: Head and neck cancer.*

Oropharyngeal swallow response

OSR at 5 mL liquid bolus was severely impaired in all studied groups when compared with our earlier studies on healthy volunteers (HV) [26]. Time to LVC was severely delayed ranging from 360 to 428 ms among the different groups (HV <160 ms) and time to UESO was also delayed and ranged from 240 to 281.33 ms (HV 200 ms). Regarding bolus kinematics, bolus propulsion force was weak (11.90 to 18.57 mN vs. HV 22 mN) leading to decreased bolus velocity from 0.24 to 0.33 m/s [11] (Table 3). Although patients with HNC presented the most delayed LVC (428.00±35.11), there were no significant differences between groups. This impaired OSR, especially the severe delay in protection of the airway (LVC time), puts our patients at great risk of aspiration and also residue through weak propulsion.

When we assessed the effect of viscosity on the OSR within groups, we found a reduction in the time to LVC in two groups of patients, older ($p<0.05$, 2,000 mPa·s vs. <50mPa·s) and Parkinson ($p<0.05$, 1,000 mPa·s and 2,000 mPa·s vs. <50 mPa·s). The other OSR parameters affecting oropharyngeal reconfiguration or bolus propulsion forces were not affected by increasing bolus viscosity with FCT (Table 13).

Table 13. Comparison between viscosities of oropharyngeal swallow response among the study groups (continuous variables expressed as mean±SEM).

		<50 mPa·s (5 mL)	250 mPa·s (5 mL)	1000 mPa·s (5 mL)	2000 mPa·s (5 mL)	p-value
ALL	LVC (ms)	387.00±13.32	359.00±11.68	315.80±9.59$^{***\ \#}$	316.20±10.92$^{****\ \#\#}$	<0.0001
	UESO (ms)	259.50±9.79	257.20±9.40	260.00±8.61	290.10±15.34	0.066
	KE (mJ)	0.96±0.08	0.90±0.06	0.83±0.06	0.85±0.10	0.112
	Force (mN)	14.87±1.01	14.43±1.08	13.19±0.97	13.31±1.42	0.228
	$\bar{x}$ bolus vel. (m/s)	0.26±0.01	0.27±0.01	0.27±0.01	0.24±0.01	0.230
OLD	LVC (ms)	360.00±20.85	336.25±14.90	298.89±14.78	284.57±12.70*	0.006
	UESO (ms)	260.00±18.69	238.75±13.85	232.22±8.72	262.86±14.21	0.304
	KE (mJ)	0.90±0.11	0.90±0.11	0.92±0.10	0.80±0.15	0.408
	Force (mN)	14.64±1.63	15.24±1.65	14.73±1.23	12.48±1.83	0.228

ER	$\bar{x}$ bolus vel. (m/s)	0.26±0.02	0.27±0.01	0.27±0.01	0.25±0.02	0.541
HNC	LVC (ms)	428.00±35.11	370.00±30.48	338.46±26.37	360.00±32.72	0.222
	UESO (ms)	240.00±21.07	257.60±19.47	280.00±19.80	282.80±25.26	0.153
	KE (mJ)	1.28±0.25	0.82±0.12	0.62±0.07	0.81±0.16	0.160
	Force (mN)	18.57±3.08	12.13±1.57	9.50±1.11	12.28±2.22	0.136
	$\bar{x}$ bolus vel. (m/s)	0.33±0.03	0.28±0.02	0.25±0.01	0.27±0.02	0.157
PARKINSON	LVC (ms)	373.33±21.81	342.07±26.68	293.33±17.82*	289.33±12.08*	0.009
	UESO (ms)	257.33±20.18	249.66±24.20	237.33±16.49	241.33±15.16	0.862
	KE (mJ)	0.89±0.11	1.08±0.18	0.99±0.16	1.23±0.32	0.963
	Force (mN)	14.18±1.61	17.80±3.30	16.50±3.16	19.33±4.78	0.946
	$\bar{x}$ bolus vel. (m/s)	0.27±0.02	0.29±0.02	0.29±0.02	0.30±0.03	0.920
STROKE	LVC (ms)	392.00±27.55	390.67±21.92	338.06±18.35	335.48±23.14	0.148
	UESO (ms)	281.33±18.14	284.00±16.98	296.77±21.37	326.45±23.18	0.421
	KE (mJ)	0.74±0.07	0.77±0.10	0.73±0.10	0.58±0.07	0.317
	Force (mN)	11.90±1.14	12.17±1.44	11.34±1.47	9.26±1.13	0.325
	$\bar{x}$ bolus vel. (m/s)	0.24±0.01	0.24±0.02	0.24±0.02	0.21±0.01	0.3367
OLDER	LVC (ms)	374.20±13.47	356.40±12.59	309.70±9.88$^{***\ \#}$	302.5±9.78$^{***\ \#\#}$	<0.0001
	UESO (ms)	265.80±11.00	256.40±10.87	254.40±9.51	276.70±10.82	0.170
	KE (mJ)	0.85±0.06	0.92±0.08	0.88±0.07	0.87±0.12	0.220
	Force (mN)	13.66±0.87	15.07±1.31	14.22±1.19	13.63±1.72	0.147
	$\bar{x}$ bolus vel. (m/s)	0.26±0.01	0.27±0.01	0.27±0.01	0.26±0.01	0.267

*$\bar{x}$: mean; HNC: head and neck cancer; PK: Parkinson; STR: stroke; LVC: Laryngeal vestibule closure; UESO: upper esophageal sphincter opening; KE: kinetic energy; * p-value <0.05, *** <0.001, ****<0.0001 vs. <50mPa·s; # <0.05, ## <0.01 vs. 250mPa·s*

Rheological characterisation

FCT presented strong resistance to salivary α-amylase effect. Viscosity was not affected by oral incubation at any of the viscosity levels tested compared with control samples without saliva for any phenotype assessed (Table 5). Surprisingly, we only found a slight but significant increase of viscosity in the 1000 mPa·s thickened fluid in patients with OD and stroke (Table 14; Figure 32). FCT thickened fluids presented a non-Newtonian viscous behaviour (shear-thinning type) when submitted to shear. We found a similar percentage of viscosity reduction for all the samples between shear rates from 50 s^{-1} to 300 s^{-1}, that was unaffected by salivary α-amylase in any group. Linear regression curves performed between a shear rate from 0 to 1000 s^{-1} are presented for each level of viscosity tested (Table 14).

Table 14. Viscosity percentage change between comparisons of control vs. samples after oral incubation (amylase effect) and shear thinning effect represented with the linear regression from 0 to 1000s^{-1} shear rate. Positive values indicate increase and negative values decrease of viscosity.

	% change (viscosity at 50 s^{-1} before and after oral incubation)	p-value	Shear-thinning (linear regression from 0 to 1000 s^{-1})	Correlation coefficient 'r'
ALL	+7.85	0.637	fx=-0.91x + 4.18	0.99
	+7.16	0.135	fx= -0.93x + 4.71	0.99
	-2.96	0.560	fx= -0.91x + 4.94	0.99
OLDER	+30.47	0.062	fx= -0.83x + 4.067	0.99
	-4.01	0.471	fx= -0.83x + 4.39	0.99
	-2.29	0.659	fx= -0.81x + 4.69	0.99
HNC	+9.30	0.459	fx= -0.90x + 4.15	0.99
	+4.17	0.531	fx= -0.91x + 4.64	0.99
	-1.86	0.706	fx= -0.91x + 4.91	0.99
PARK.	+9.47	0.447	fx= -0.92x + 4.2	0.99
	+11.64	0.061	fx= -0.93x + 4.68	0.99
	-5.22	0.424	fx= -0.88x + 4.86	0.99
STR.	-10.17	0.327	fx= -0.90x + 4.09	0.99
	+14.11	**0.025**	fx= -0.92x + 4.69	0.99
	-2.62	0.683	fx= -0.91x + 4.93	0.99

HNC: Head and neck cancer; PARK: Parkinson; STR: stroke.

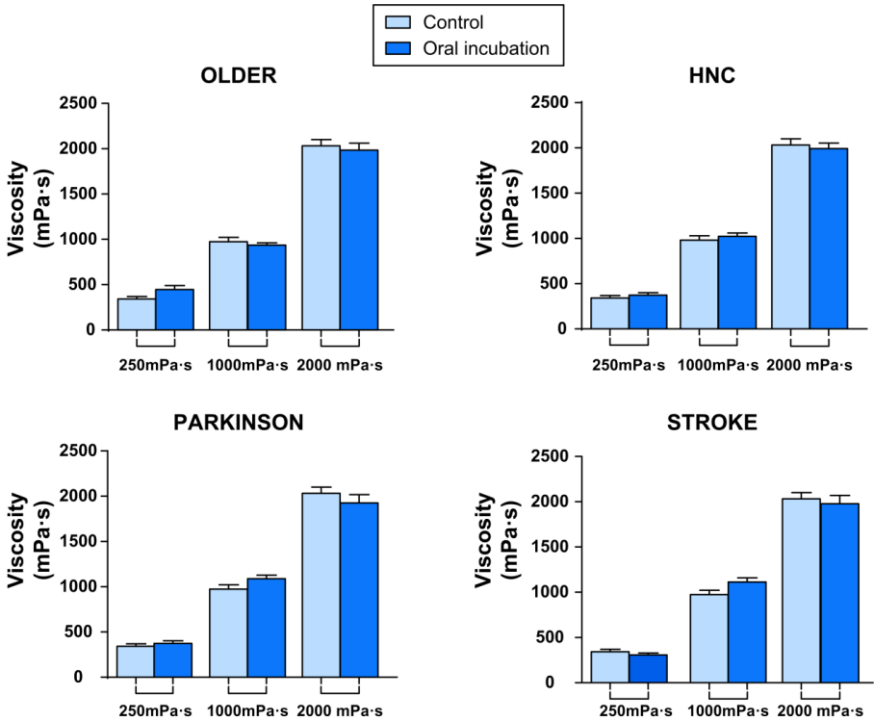

Figure 32. Mean viscosity (mPa·s) prior and after oral incubation for each phenotype of patient at each viscosity level assessed.

Palatability

Palatability was not significantly affected by increasing bolus viscosity. Palatability with the study product measured on a 10-points scale was similar in all the viscosities tested (6.73±0.48 with liquid; 6.22±0.48 with 250 mPa·s; 5.63±0.43 with 1000 mPa·s; and 5.48±0.42 with 2000 mPa·s). In addition, we did not find any difference in this item between volumes and viscosities in any of the study groups nor between groups.

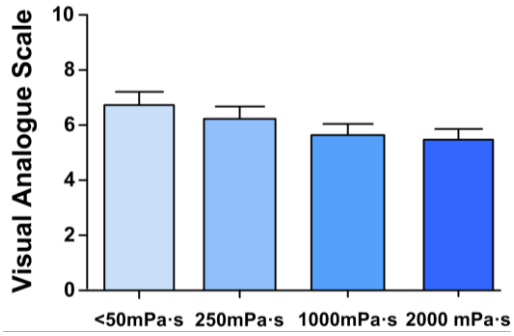

Figure 33. Palatability of the thickening product assessed at each viscosity level studied.

Adverse events

No adverse events or serious adverse events related or not related to the study product were reported during the study period.

5.1.3 Sub-study 1.3

Study population

305 patients were screened and entered the VFS study. Of these, 91 were excluded for presenting OD structural causes (29.83%), and 105 patients were excluded because they presented a safe swallow with PAS 1 and 2 (34.42%). Another 9 (2.9%) of recruited patients were not able to perform the VFS procedure and were excluded due to a protocol deviation or interruption such as nausea or inability to complete the series of boluses or to stay seated while performing the VFS. 13 patients (4.3%) were withdrawn due to technical problems during the study.

Mean age of the population included in the study was 83±6.93 years, and 53.33% were male. Regarding OD causes, 45% were only associated with ageing, 36.67% presented a previous stroke, and 18.33% presented neurodegenerative diseases such as Parkinson's or Alzheimer's disease.

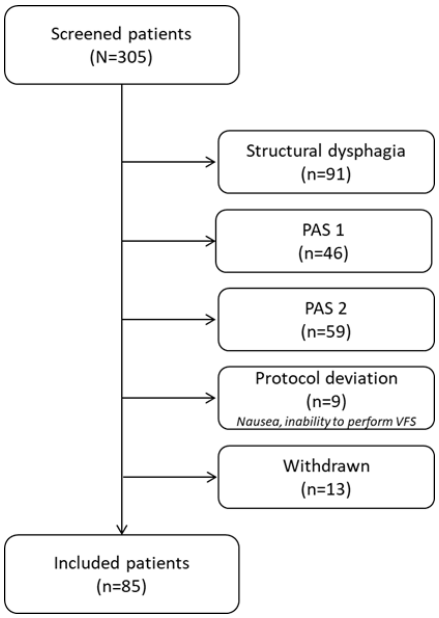

Figure 34. Consort flow chart of the patient recruitment and inclusion in the Sub-study 1.3.

Therapeutic effect

Safety of swallow. Up to 83.75% older patients with OD presented unsafe swallows with thin liquid, 45.00% (PAS 3-5) being patients with penetrations and 38.75% with aspirations (PAS 6-8). Fluid thickening with TQ caused a strong viscosity-dependent effect on the prevalence of patients with safe swallow, ranging from 62.90% at 100 mPa·s to 95.24% at 1600mPa·s. Significant differences appeared for all the thickened levels vs thin liquid ($p<0.0001$) shown in Figure 35. Prevalence of patients with safe swallow at each viscosity level are presented in Table 15 and in Figure 36.

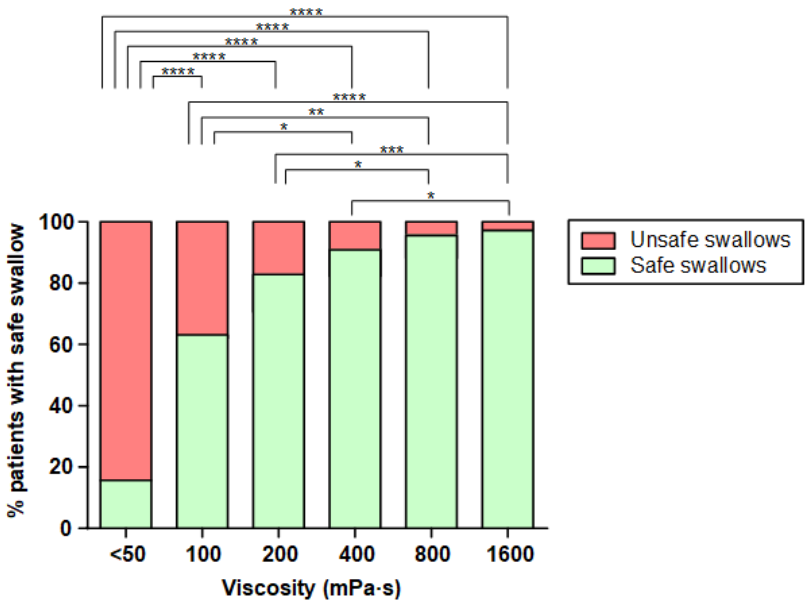

Figure 35. Prevalence of patients with safe swallow at each viscosity level assessed. Significant differences are also presented for all viscosities. **p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.*

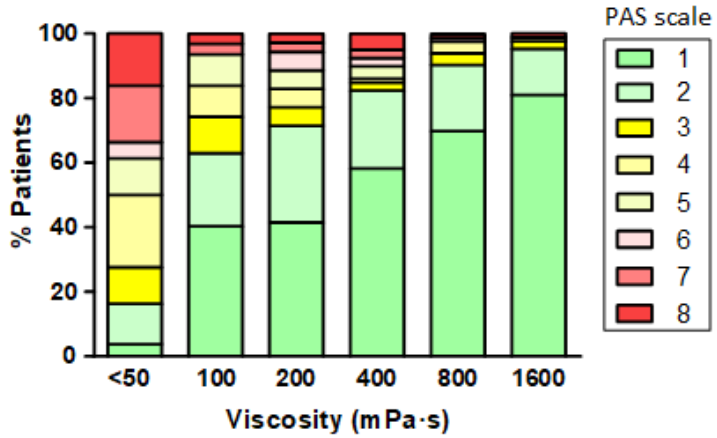

Figure 36. Penetration Aspiration Scale obtained for each viscosity level assessed in the study.

Increasing shear viscosity also greatly reduced the prevalence of patients with penetrations or aspirations. Penetrations were reduced in a viscosity-dependent manner ranging between 30.65 - 3.57% for 100 and 1600 mPa·s viscosity level, respectively (Figure 37). Aspirations were also reduced from 11.43% at 200 mPa·s to 1.19% at 1600 mPa·s. Prevalence of patients with penetrations and aspirations and the mean PAS score for each viscosity level are presented for all viscosity levels in Table 15.

Therapeutic range was determined between 100 and 800 mPa·s. The threshold effect on safety of swallow was observed at 100 mPa·s and maximal therapeutic effect was observed at 800 mPa·s with a protection of 90.36% and no significant differences with the highest viscosity level assessed (1600 mPa·s).

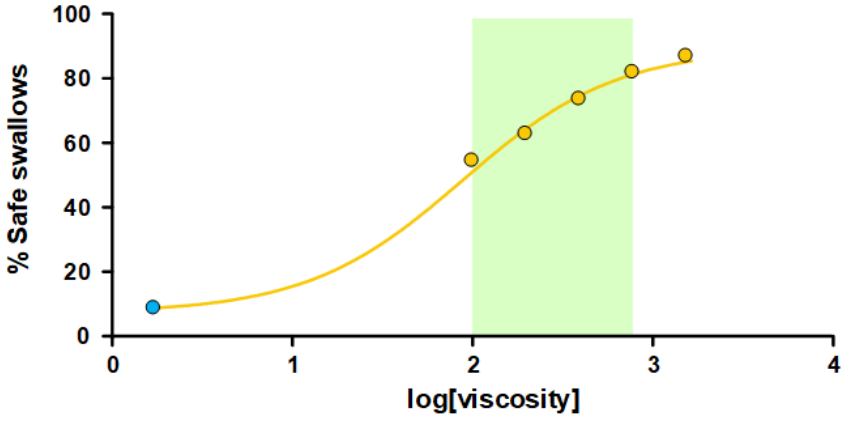

Figure 37. Dose-response curve. Effect of increasing viscosity on the prevalence of patients with safe swallow. The green frame depicts the therapeutic range of TQ in older patients with OD.

Efficacy of swallow. No viscosity-dependent effect was observed for oral nor pharyngeal residue. Oral residue was significantly increased for 200 (71.43%; p=0.003), 400 (68.35%;

p=0.006) and 1600 mPa·s (69.05%; p=0.004) when compared to thin liquid. In contrast, pharyngeal residue was maintained for all viscosity levels ranging between 11.25% to 20.24%). Coating residue according to Robbins Scale was the most prevalent in both oral and pharyngeal areas. Prevalence of coating and pooling residue are shown in Table 15 and Figure 38.

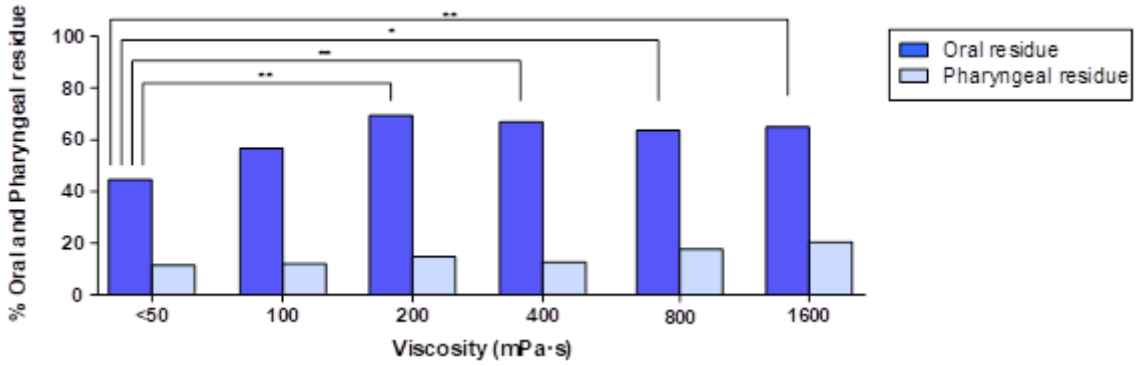

Figure 38. Prevalence of oral and pharyngeal residue at each viscosity level assessed. Significant differences are also presented for all viscosities.

Table 15. Prevalence of safe swallows, penetrations, aspirations, and oral and pharyngeal residues at each viscosity level assessed. Safe swallows = PAS 1,2; Penetrations = PAS 3-5, Aspiration PAS 6-8.

Prevalence (%)	**Viscosity level (mPa·s)**					
	<50	**100**	**200**	**400**	**800**	**1600**
Safe swallows	16.25	62.90	71.43	82.28	90.36	95.24
Penetrations	45.00	30.65	17.14	7.60	7.23	3.57
Aspirations	38.75	6.45	11.43	10.13	2.41	1.19
Mean PAS±SD	4.91±2.16	2.55±1.87	2.47±1.92	2.11±1.97	1.53±1.14	1.31±0.92
Oral residue	46.25	58.06	71.43	68.35	65.06	69.05
Coating	41.25	50.00	64.29	59.49	49.40	52.38
Pooling	5.00	8.07	7.14	8.86	16.66	16.67
Pharyngeal residue	11.25	11.29	14.29	12.66	16.87	20.24
Coating	8.75	8.065	11.43	8.86	13.25	14.29
Pooling	2.50	3.23	2.86	3.80	3.61	5.95

Oropharyngeal swallow response

Airway protection mechanisms. Time to LVC was decreased by the increment of viscosity between 360±90.18 and 300±84.16 ms (Figure 39). Significant differences appeared for the highest viscosity levels vs <50 mPa·s: 400, 800 and 1600 ($p<0.01$).

Bolus transit. Time to UESO was moderately increased by fluid thickening showing significant differences for <50 mPa·s vs 800 and 1600 mPa·s ($p<0.0001$). Mean UESO is represented in Figure 7 with the statistically significant differences obtained for all viscosity levels. LVC and UESO time values are presented in Table 3.

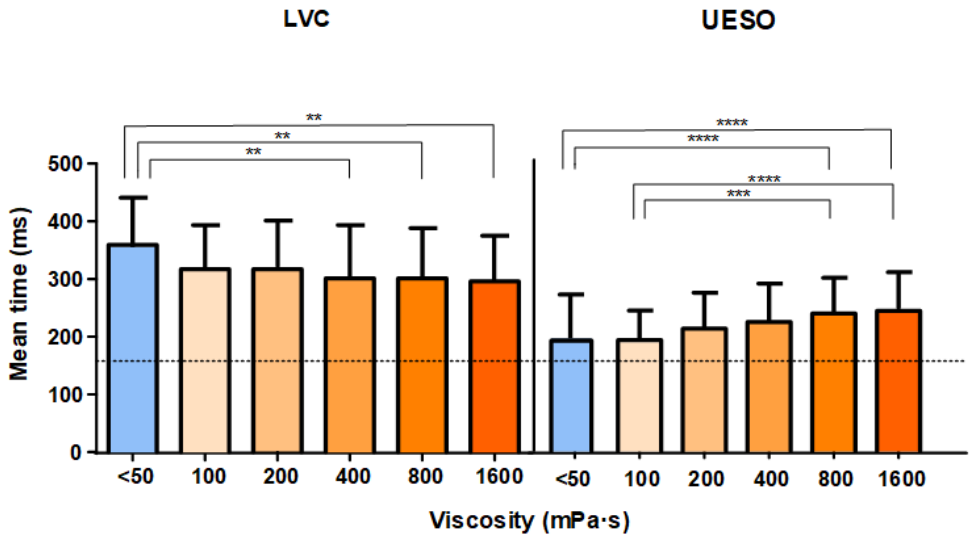

Figure 39. Effect of bolus velocity on oropharyngeal swallow response. Mean laryngeal vestibule closure (LVC) and upper esophageal sphincter opening (UESO) time for each viscosity level assessed. *Dotted line represents the reference value in healthy volunteers. **p<0.01; ***p<0.001; ****p<0.0001.*

Kinematics of swallowing. Mean bolus velocity was reduced by increasing bolus viscosity ranging between 0.33±0.18 to 0.22±0.08 m/s. Significant differences appeared for <50, 100 and 200 mPa·s vs 800 and 1600 mPa·s (Figure 40). Bolus kinetic energy went from 2.44±2.38 to 1.22±1.24 mJ. Values for kinematics of swallowing are presented in Table 16.

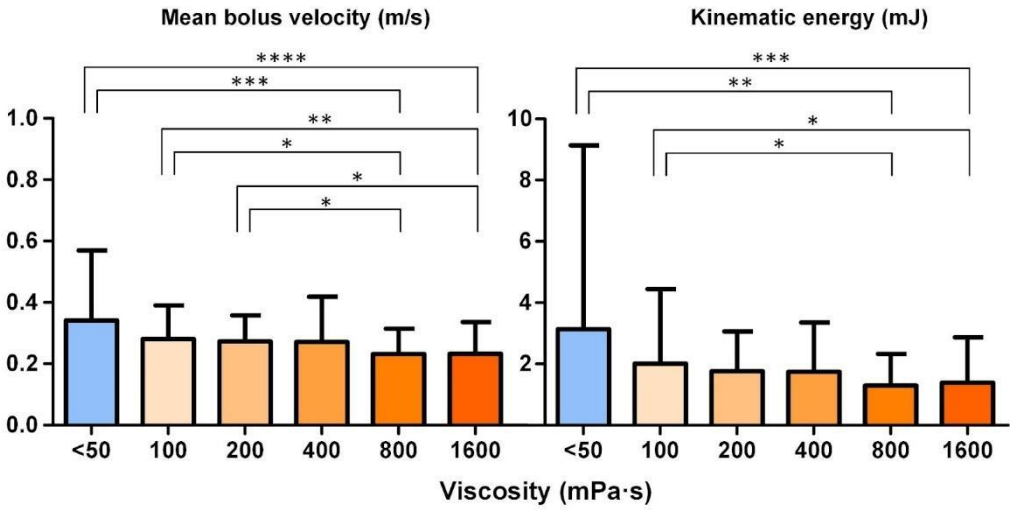

Figure 40. Mean bolus velocity and kinetic energy for each viscosity level assessed. **p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.*

Table 16. Laryngeal vestibule closure and upper ssophageal sphincter opening time, mean and final velocity and kinetic energy at each viscosity level assessed.

OSR and Kinematics	Viscosity level (mPa·s)					
	<50	**100**	**200**	**400**	**800**	**1600**
LVC (ms)	360±90.18	323±76.67	324±86.40	305±93.85	307±89.16	300±84.16

UESO (ms)	186±78.20	188±50.35	208±61.54	214±66.85	231±59.60	236±65.60
Mean vel. (m/s)	0.34±0.23	0.28±0.11	0.27±0.09	0.27±0.15	0.23±0.08	0.23±0.10
KE (mJ)	3.14±6.00	2.01±2.43	1.76±1.30	1.75±1.60	1.29±1.04	1.39±1.48

OSR: oropharyngeal swallow response; LVC: laryngeal vestibule closure; UESO: upper esophageal sphincter opening; KE: kinetic energy; mean Vel.: mean velocity

Adverse events

Analysis of the adverse events were obtained for 76% of the participants. A total of 22.95% (n=14) of patients reported adverse events: diarrhoea (85.71%), abdominal pain (7.14%) and nausea (7.14%). All of them were classified as unlikely to be related to the study product as gastrointestinal disorders are reported in the technical sheet of X-ray contrast. No serious adverse events were reported during or following the study.

Hedonic scale

Palatability decreased in a viscosity dependent manner. However, significant differences only appeared for the highest viscosity levels assessed (800 and 1600 mPa·s) vs thin liquid (<50 mPa·s) and vs 100 mPa.s. Mean punctuation for each viscosity level assessed: 3.93±1.34, 3.96±1.08, 3.25±1.24, 3.00±1.25, 2.57±1.36, 2.4±1.38 for thin liquid to 1600 mPa·s, respectively (figure 41).

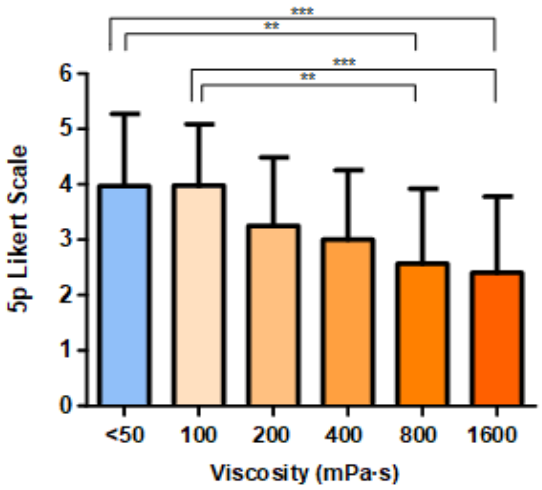

Figure 41. Mean±SD punctuation on palatability given by the participants according to the 5-point facial Likert Scale at each viscosity level assessed. **p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.*

Rheological characterisation

Measurements of shear viscosity at $50s^{-1}$ for both viscosity levels assessed were 189.65±2.39 and 768.90±19.76 for 200 and 800 target viscosity levels, respectively. After oral incubation in study participants we observed a non-significant decrease of viscosity at 50 s^{-1} for 200 mPa·s of 17.18% (157.07±49.75 mPa·s) and an increment of 2.01% for 800 mPa·s (784.38±99.84 mPa·s). In contrast, shear thinning produced a mean decrease of viscosity of 77.25%±1.02 at 300 s^{-1}. Both swallowing factors (oral incubation and pharyngeal shear thinning) caused a global shear-

viscosity decrease of 78.96%. Figure 42 shows the viscosity flow curve of shear viscosity for 200 and 800 mPa·s viscosity level pre and post oral incubation in patients with OD included in the study.

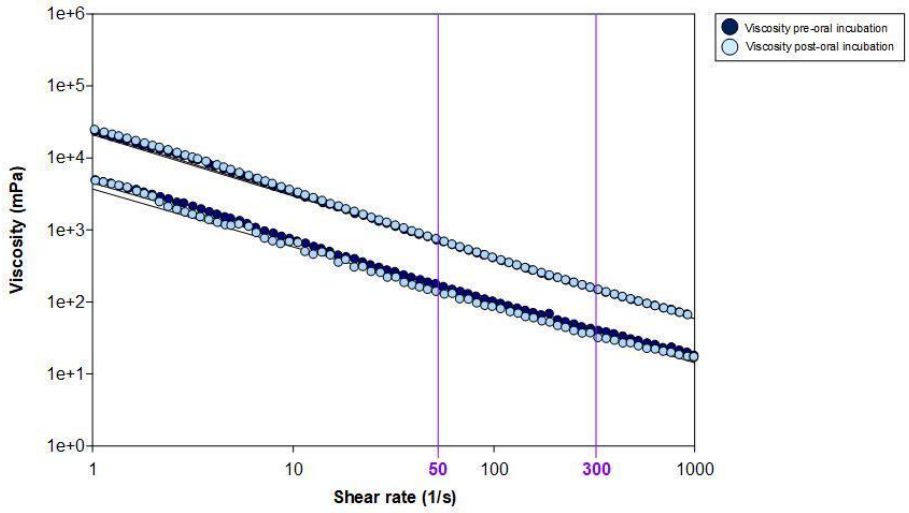

Figure 42. Viscosity flow curve of 200 and 800 mPa·s viscosity level pre and post-oral incubation in a shear rate range from 1 to 1000 s^{-1}.

5.2 Study 2 - Analysis of the risks by using qualitative descriptor for viscosity levels in TP's labelling

Description of the labels of thickening products

TPs composition included on the label is summarized in Table 17. All the labels presented a qualitative description of the TA in each product but not a quantitative description; 60% of TPs were composed of modified starch (including maltodextrin), 20% by a combination of modified starch and gums and the remaining 20% were composed by gums (guar or xanthan) and another ingredient (maltodextrin, modified cellulose or potassium chloride). None of the TPs was exclusively composed of gums.

Table 17. Thickening products and the batches used for the study. Description of the thickening agents described on the label of each TP included in this study. TPs have been divided into three categories according to their composition: MS-based TPs, mixtures of MS and gums, and gum-based TPs.

Code	Thickening Product	Batch	Qualitative composition	Product category
A	Fresubin Clear Thickener	29LL2307	Maltodextrin Xanthan gum Modified starch Modified cellulose	Mixture
B	Thick & Easy	V041020	Modified starch Maltodextrin	Modified Starch based
C	Bi1	BU P-01	Modified starch	Modified Starch based
D	Nutilis Powder	01374316111623	Maltodextrin Modified starch	Mixture

			Tara gum Xanthan gum Guar gum	
E	Wallax	185103	Modified starch	Modified Starch based
F	Nutavant	M-06	Modified starch	Modified Starch based
G	Espesante NM	180170101	Modified starch	Modified Starch based
H	Nutilis Clear	100906164	Maltodextrin Guar gum Xanthan gum	Gum based
I	Resource Thicken Up Clear	7321428200	Maltodextrin Xanthan gum Potassium chloride	Gum based
J	Resource Thicken Up	8177665300	Modified starch	Modified Starch based

Information provided on the label by manufacturers is summarized in Table 18. All manufacturers included the information and instructions required by the EU Regulations, except product G which did not include the instructions for preparation, as well as their category as FMSP and the statement that they be used under medical supervision. In contrast, the number of parameters described on the label that potentially affect the therapeutic effect was low: only one product (D) expressed the viscosity range in SI Units (mPa·s), only three TPs (C, G and I) described the dose (g/mL) to achieve the recommended thickness and four of them declared a resistance to amylase (A, D, H and I). None of the products described the potential changes in viscosity caused by oral amylase or shear rate (Table 18).

Dosage and descriptors for each viscosity level recommended by manufacturers are described in Table 19. All TPs use 3 thickness levels and 70% of them use the NDD descriptors. Up to 60% TPs described the dose only by scoops and only 40% included SI units (grams). Product G used only grams to describe the dose. 70% of the products described the dosage to prepare 100ml and 20% to prepare 200ml of thickened fluid. Product B described doses to prepare both volumes. Product D described a range of numbers of scoops needed to achieve each thickness level recommended. For the recommended thickness 2 for product C, the grams described in the label did not match the number of scoops recommended. Grams needed in 100ml water (g/100 mL) to achieve each recommended thickness level differed between products: 1.75–6 g; 4.8–8 g; and 7.2–10.5 g for the recommended thickness level 1, 2 and 3, respectively.

Table 20 presents the abbreviated preparation method for each TP divided into 4 stages: 1) first product to be added; 2) second product to be added; 3) stirring method and 4) resting time. Up to 90% of the products describe how to prepare them. Preparation procedure among TPs presented heterogeneous and even divergent information: 40% needed to first add the thickener; 90% defined the stirring method but only 2 of them defined the time and; resting time after preparing the thickened fluid was only provided by 40% of the TPs.

Table 18. Information affecting the therapeutic effect of thickening products.

Information included affecting the therapeutic effect of thickening products											
Parameters	**Concept / Units**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Quantitative thickening agent composition	**Composition with the % or mass of functional ingredients**	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
Dose	**g/mL**	NO	NO	YES	NO	NO	NO	YES	NO	YES	NO
Viscosity in SI Units	**Viscosity at $50s^{-1}$ (mPa·s)**	NO	NO	NO	YES	NO	NO	NO	NO	NO	NO
Amylase resistance	**Viscosity at $50s^{-1}$ after oral incubation**	NO *Amylase resistant*	NO	NO	NO *Amylase resistant*	NO	NO	NO	NO *Amylase resistant*	NO *Amylase resistant*	NO
Effect of shear thinning	**Viscosity at $300s^{-1}$ (mPa·s)**	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
Time for consuming	**h**	*Use promptly*	NO	YES	YES	*Stable over time*	NO	NO	YES	*Stable over time*	*Stable over time*
For preparation with water or other fluids	**Water or other fluids**	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
Different preparation between water and other fluids	**Explanation of different methods to achieve same viscosity**	NO	NO	NO	YES	NO	NO	NO	NO	YES	NO

Table 19. Label information. Dosage of each recommended thickness level for each thickening product, name of the descriptors used by each manufacturer and recommended volume of liquid to prepare the thickened fluid. Spanish descriptors (included as footnotes) have been translated into English.

Thickening Product	Recommended thickness 1			Recommended thickness 2			Recommended thickness 3			Fluid
	Scoops	Grams	Descriptor	Scoops	Grams	Descriptor	Scoops	Grams	Descriptor	Volume (ml)
A	1	1.75	Nectar	3	5.25	Honey	6	10.5	Purée	100
B	1	*4.5*	Nectar	1.5	*6.75*	Honey	2	*9*	Pudding	100
	2	*9*	Nectar	3	*13.5*	Honey	4	*18*	Pudding	200
C	1	4.5	Nectar	1.5	*6.75* 6.5	Honey	2	*9*	Pudding	100
D	2-3	*8-12*	Syrup*	3-4	*12-16*	Cream**	4-5	*16-20*	Semisolid[¥]	200
E	1	*4.5*	Nectar	1.5	*6.75*	Honey	2	*9*	Pudding	100
F	1	*4.1*	Nectar	1.5	*6.15*	Honey	2	*8.2*	Crème caramel[¥¥]	100
G	-	4.5	Nectar	-	6.6	Honey	-	*9*	Pudding	100
H	1	*3*	Nectar	2	*6*	Honey	3	*9*	Pudding	100
I	2	2.4	Nectar	4	4.8	Honey**	6	7.2	Pudding	200
J	1	*4.5*	Nectar	1.5	*6.75*	Honey**	2	*9*	Pudding	100

**Syrup: jarabe; [**]Cream: crema; [¥]Semisolid: semisólida; [¥¥]Crème caramel: flan; **Miel. Italics numbers have been calculated according to the recommended scoops (grams 1 scoop*number scoops).*

Table 20. Preparation method stated in the labelling of each thickening product divided in stages (1, first product to be added; 2, second product to be added; 3, stirring method and 4, resting time).

Thickening product	Preparation method	Thickening product	Preparation method
A	1. Thickener 2. Liquid 3. Stir 20s with spoon in different directions 4. 1 minute	**F**	1. Liquid 2. Thickener 3. Shake vigorously 4. -
B	1. Liquid 2. Thickener 3. Stir correctly till its dissolution 4. -	**G**	1. - 2. - 3. - 4. -
C	1. Liquid 2. Thickener 3. Stir 15 seconds 4. 30 seconds	**H**	1. Thickener 2. Liquid 3. Stirring continuously 3. -
D	1. Thickener 2. Liquid 3. Mix with a mixer, rods or fork 4. Rest some minutes	**I**	1. Thickener 2. Liquid 3. Stir till its complete dissolution 4. -
E	1. Liquid 2. Thickener 3. Stirring vigorously until completely dissolved 4. 15 seconds	**J**	1. Liquid 2. Thickener 3. Removing until achieving an homogenous texture 4. -

Measurements of rheological properties of the thickening products and the thickness levels recommended by the manufacturers

Viscosity classifications and viscosity values are represented in Table 21. For Thickness 1, viscosity measurements at 50 s^{-1} ranged from 74.93±4.49 to 350.26±46.19 mPa·s (78.61% variability); for Thickness 2, values went from 255.59±6.29 to 1272.67±198.03 mPa·s (79.92% variability) and for Thickness 3, viscosities ranged from 377.11±14.09 to 6205.77±1237.91 mPa·s (93.92% variability).

According to the NDD classification, all the viscosities assessed for Thickness 1 were described by manufacturers as “Nectar”. Viscosities for Thickness 2 matched with “Honey” except two and viscosities for recommended Thickness 3 were described as “Pudding” except two. Following the application of the IDDSI syringe flow test, up to 3 IDDSI descriptors (Slightly, Mildly and Moderately Thick) fell into Thickness 1; 2 IDDSI descriptors (Moderately and Extremely Thick) into Thickness 2; and another 2 (Moderately and Extremely Thick) into Thickness 3. “Slightly thick” covered a viscosity range from 74.93 to 143.73 mPa·s; “mildly thick” from 83.47 to 350.26 mPa·s; “moderately thick” from 167.12 to 1272.67 mPa·s and “extremely thick” from 723.83 to

6205.77 mPa·s. For viscosities with an IDDSI level 4, the fork drip test was performed and products D and H decreased to IDDSI level 3. For the JDD2013, 3 descriptors (Mildly, Moderately and Extremely thick) matched viscosities obtained in Thickness 1; 3 descriptors in Thickness 2 (Moderately, Extremely and Over extremely thick), and 2 in Thickness 3 (Extremely and Over extremely thick). Table 5 also shows that the names of the descriptors used by IDDSI and JDD2013 for the same viscosity value rarely correlated.

The descriptor "Moderately thick", used by IDDSI and JDD2013, includes products with objective viscosity measurements from 170 to 1300 mPa·s, and the descriptor "Extremely thick" includes products with objective viscosity from 320 to 6200 mPa·s. Table 5 Viscosity measurements at 50 s^{-1} and viscosity levels assessed by the IDDSI syringe flow test. Descriptors used by the NDD, IDDSI and JDD2013 for the doses recommended by each manufacturer. Milliliters remaining in the syringe after applying IDDSI flow test are also presented (IDDSI level: remaining milliliters).

Viscosity achieved for each TP with the doses recommended by manufacturers for each recommended thickness level (expressed in grams of TP/100ml mineral water) is presented in Figure 1.

Thickening capacity is presented in figure 43 and table 22. Viscosity values of solutions prepared with 4.5 g of each thickener in 100ml mineral water varied up to 95.6% between each other. Viscosity for MS-based TP ranged from 56.66 to 111.99mPa·s (49.41% variability). For Mx, viscosity varied from 106.28 to 1159.33 mPa·s (90.83%) and for gum-based composition, viscosity ranged from 240.27 to 806 mPa·s (70.19% variability). Shear thinning behaviour also differed according to the main TA: 48.16% - 64.11%, 53.16 - 76.03% and 77.90% - 80.05% for MS-based, Mx and gum-based TPs, respectively.

Table 21. Viscosity measurements at 50 s^{-1} and viscosity levels assessed by the IDDSI syringe flow test. Descriptors used by the NDD, IDDSI and JDD2013 for the doses recommended by each manufacturer. Millilitres remaining in the syringe after applying IDDSI flow test are also presented (IDDSI level: remaining millilitres).

Thickening Product	Recommended thickness 1				Recommended thickness 2				Recommended thickness 3			
	Mean 50 ± SD	NDD	IDDSI	JDD2013	Mean 50 ± SD	NDD	IDDSI	JDD2013	Mean 50 ± SD	NDD	IDSSI	JDD2013
A	322.51 ± 13.44	Nectar	Mildly thick (2; 7.5ml)	Extremely thick	1230.90 ± 29.55	Honey	Extremely thick (4; 10ml)	Over extremely thick	2727.13 ± 72.32	Pudding	Extremely thick (4; 10ml)	Over extremely thick
B^1	82.09 ± 12.58	Nectar	Slightly thick (1; 3.25ml)	Mildly thick	610.46 ± 41.57	Honey	Moderately thick (3; 9.5ml)	Over extremely thick	3080.17 ± 320.35	Pudding	Extremely thick (4; 10ml)	Over extremely thick
B^2	123.22 ± 16.91	Nectar	Mildly thick (2; 4.75ml)	Mildly thick	643.77 ± 81.37	Honey	Moderately thick (3; 9.5ml)	Over extremely thick	2828.87 ± 138.22	Pudding	Extremely thick (4; 10ml)	Over extremely thick
C	143.73 ± 19.41	Nectar	Slightly thick (1; 3ml)	Mildly thick	1018.053 ± 225.46	Honey	Moderately thick (3; 9.75ml)	Over extremely thick	6205.77 ± 1237.91	Pudding	Extremely thick (4;10ml)	Over extremely thick
D_1	74.93 ± 4.49	Nectar	Slightly thick (1; 1.25ml)	Mildly thick	350.26 ± 46.19	Nectar	Mildly thick (2; 6.75ml)	Extremely thick	1272.67 ± 198.03	Honey	Moderately thick (3; 9.75ml)	Over extremely thick
D_2	350.26 ± 46.19	Nectar	Mildly thick (2; 6.75ml)	Extremely thick	1272.67 ± 198.03	Honey	Moderately thick (3; 9.75ml)	Over extremely thick	2724.17 ± 216.51	Pudding	Extremely thick* (4; 10ml)	Over extremely thick
E	78.864 ± 7.52	Nectar	Slightly thick (1; 3.5ml)	Mildly thick	567.77 ± 113.20	Honey	Moderately thick (3; 9.75ml)	Over extremely thick	2450.77 ± 82.02	Pudding	Extremely thick (4; 10ml)	Over extremely thick
F	83.67 ± 12.59	Nectar	Slightly thick (1; 3ml)	Mildly thick	453.88 ± 49.32	Honey	Moderately thick (3; 9.5ml)	Extremely thick	2306.36 ± 521.24	Pudding	Extremely thick (4; 10ml)	Over extremely thick
G	85.40 ± 9.01	Nectar	Slightly thick (1; 3.25ml)	Mildly thick	430.29 ± 31.78	Honey	Moderately thick (3; 9ml)	Extremely thick	2098 ± 212.79	Pudding	Extremely thick (4; 10ml)	Over extremely thick

H	167.12 ± 34.48	Nectar	Moderately thick (3; 9.5)	Moderately thick	490.016 ± 39.63	Honey	Moderately thick (3; 9.75ml)	Extremely thick	723.83 ± 19.98	Honey	Extremely thick* (4;10ml)	Over extremely thick
I	83.47 ± 6.81	Nectar	Mildly thick (2; 5)	Mildly thick	255.59 ± 6.29	Nectar	Moderately thick (3; 8.75ml)	Moderately thick	377.11 ± 14.09	Honey	Moderately thick (3; 9.5ml)	Extremely thick
J	98.61 ± 2.90	Nectar	Slightly thick (1; 3.75)	Mildly thick	748.58 ± 27.19	Honey	Moderately thick (3; 9.75ml)	Over extremely thick	3564.1 ± 457.41	Pudding	Extremely thick (4; 10ml)	Over extremely thick

*[1]for 100ml recommendation; [2]for 200ml recommendation; 1 2 first and second level recommended for same thickness; *Applying the fork drip test, IDDSI level 3 was obtained.*

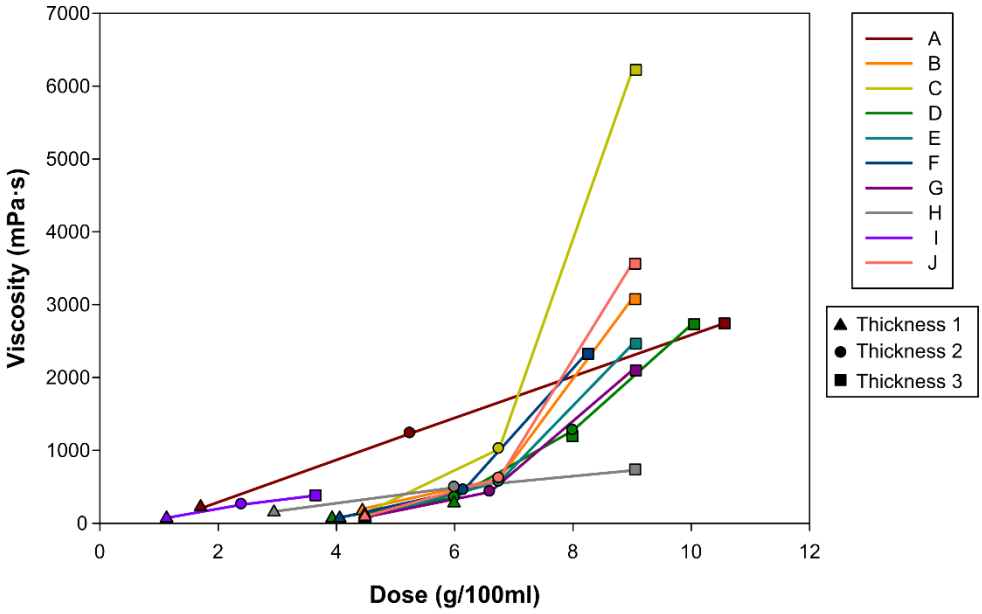

Figure 43. Viscosity in mPa·s for each level of thickness recommended by the manufacturers.

Table 22. Viscosity achieved at 50 s^{-1} and at 300 s^{-1} with 4.5 g of each thickening product in 100 ml mineral water.

Thickening Product	Viscosity		
	Mean 50s^{-1} ± SD	Mean 300s^{-1} ± SD	Shear thinning %
A	1159.33 ± 99.77	277.93 ± 9.21	76.03
B	69.05 ± 6.21	32.97 ± 1.81	52.25
C	103.20 ± 6.21	52.3 ± 13.43	49.32
D	106.28 ± 19.94	49.78 v 4.76	53.16
E	83.84 ± 12.20	30.09 ± 7.15	64.11
F	101.27 ± 3.66	39.08 ± 2.07	61.41
G	56.66 ± 5.27	29.37 ± 17.02	48.16
H	806 ± 9.11	160.8 ± 2.42	80.05
I	240.27 ± 22.055	53.10 ± 4.63	77.90
J	111.99 ± 18.05	51.02 ± 7.77	54.44

Salivary amylase after oral incubation produced a reduction in viscosity that ranged from 96.78 to 99.26% of their initial viscosity for MS-based TP at recommended Thickness 1. In contrast, for gum-based TPs (H and I), viscosity was much less affected (decreasing up to 16%) or even increased (34.31%). Viscosity of TPs containing mixtures varied enormously following oral incubation depending on their TA composition: viscosity decreased up to 26.66% for product A and 82.91% for product D. Recommended Thickness 2 and 3 presented similar behaviours for each TP. Table 23 shows viscosity values at 50 s^{-1} after oral incubation of TPs in HV for each recommended viscosity.

TP were grouped in three main rheologic profiles. Figure 44, shows the three different patterns determined by the viscosity before and after oral incubation at recommended Thickness 3. TPs with Pattern 1 (products B, C, D, E, F, G and J) presented a decrease in viscosity caused by salivary amylase of between 60 and 100%; Pattern 2 (product A), between 20 and 60% and Pattern 3 (products H and I) with a maximum viscosity decrease of 20%. MS-based TPs are represented by Pattern 1 while gum-based TPs by Pattern 3. Pattern 2 represents a mixture (A) but the other mixture (product D) followed Pattern 1 but was declared as *amylase resistant* on its label. Product I presented an increase of viscosity after oral incubation.

Table 23. Viscosities of the thickening products assessed at 50 s^{-1} after 30 s oral incubation in healthy volunteers.

Thickening Product	Recommended thickness 1		Recommended thickness 2		Recommended thickness 3	
	After oral incubation ($50s^{-1}$ ± SD)	% reduction by amylase	After oral incubation ($50s^{-1}$ ± SD)	% reduction by amylase	After oral incubation ($50s^{-1}$ ± SD)	% reduction by amylase
A	236.54 ± 18.90	26.66	834.10 ± 231.31	32.24	1721.68 ± 122.94	36.87
B*	0.61 ± 0.97	99.26	10.20 ± 16.12	98.33	49.08 ± 75.70	98.41
C	1.67 ± 1.19	98.84	5.07 ± 2.03	99.50	4.06 ± 4.87	99.93
D_1	26.11 ± 2.86	65.15	59.91 ± 10.51	82.90	131.08 ± 34.86	89.70
D_2	59.91 ± 10.51	82.91	131.08 ± 34.86	89.70	218.08 ± 100.4	92.00
E	1.44 ± 0.92	98.17	4.00 ± 4.92	99.30	1.10 ± 1.32	99.96
F	2.3 ± 2.56	97.25	3.04 ± 3.65	99.33	5.99 ± 5.45	99.74
G	2.75 ± 1.62	96.78	1.41 ± 1.09	99.67	5.23 ± 5.56	99.75
H	140.38 ± 6.72	16.00	397.06 ± 6.18	18.97	697.33 ± 79.62	3.66
I	112.11 ± 5.74	-34.31 (increase)	308.54 ± 5.96	-20.72 (increase)	446.59 ± 20.95	-18.42 (increase)
J	1.71 ± 1.53	98.27	8.22 ± 10.57	98.66	1.88 ± 1.59	99.95

**Salivary amylase test performed for the 100ml doses*

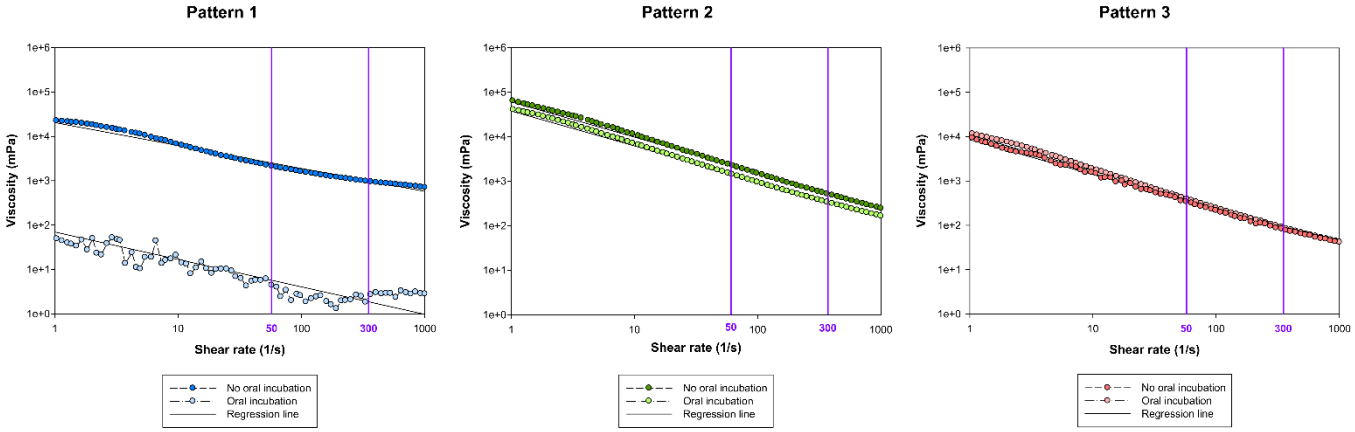

Figure 44. Representative examples of thickening products (TPs) for each pattern of rheological behaviour by adding the effect of oral amylase and shear thinning. Three TPs (Product F -Pattern 1-, Product A -Pattern 2- and Product I -Pattern 3-) have been represented at the recommended Thickness 3. Viscosity was assessed before and after oral incubation in a shear rate range from 1 to 1000 s^{-1}.

Shear rate effect showed that all TPs presented an index flow lower than 1, indicating the shear thinning behaviour (pseudoplasticity) of those fluids. Shear thinning observed from the initial viscosity at 50s^{-1} to 300s^{-1} in recommended Thickness 1 ranged between 40.55% and 58.53% for MS-based TPs, between 50.19 and 73.86% for mixtures and from 71.64 to 73.35% for gum-based TPs. Table 24 presents viscosities at 300 s^{-1}, index flow (n) and consistency (k), and the decrease from the initial viscosity at 50 s^{-1} caused by the increment of shear rate for recommended Thicknesses 1, 2 and 3.

Table 24. Viscosities of the thickening products assessed at 300 s^{-1}, flow Index, consistency index and their shear thinning at each manufacturer's recommended thickness

Thickening Product	Recommended thickness 1				Recommended thickness 2				Recommended thickness 3			
	Flow Index (n)	**Consistency index (K; Pa·s^n)**	**300 s^{-1} (mPa·s)**	**Shear thinning %**	**Flow Index (n)**	**Consistency index (K; Pa·s^n)**	**300 s^{-1} (mPa·s)**	**Shear thinning %**	**Flow Index (n)**	**Consistency index (K; Pa·s^n)**	**300 s^{-1} (mPa·s)**	**Shear thinning %**
A	0.20	3.71	84.29	73.86	0.21	4.45	312.25	74.63	0.21	4.78	669.81	75.44
B[1]	0.64	2.50	40.00	51.27	0.46	3.78	277.99	54.46	0.39	4.84	2029.58	34.11
B[2]	0.69	2.52	63.10	48.79	0.40	3.86	325.67	49.41	0.51	4.46	1213.90	57.09
C	0.53	2.95	59.61	58.53	0.43	4.04	417.68	58.97	0.35	4.93	2118.44	65.12
D_1	0.65	2.49	37.32	50.19	0.58	3.17	131.80	62.37	0.49	3.83	371.76	70.79
D_2	0.58	3.17	131.80	62.37	0.49	3.83	371.76	70.79	0.40	4.38	804.16	70.48
E	0.54	2.66	33.51	57.51	0.43	3.80	247.19	56.46	0.38	4.56	1090.01	55.52
F	0.67	2.41	43.30	40.55	0.50	3.58	213.24	52.91	0.51	4.34	965.45	58.14
G	0.54	2.74	39.99	53.17	0.47	3.62	197.37	54.14	0.42	4.47	1057.17	49.61
H	0.22	3.59	44.54	73.35	0.18	4.08	115.31	76.47	0.20	4.21	175.79	75.71
I	0.22	3.30	23.67	71.64	0.17	3.86	64.16	74.90	0.21	3.93	96.34	74.45
J	0.69	2.46	49.12	50.19	0.40	3.98	309.60	58.64	0.51	4.33	1295.01	63.67

When combining oral incubation and shear thinning at "pharyngeal" shear rates, final viscosities varied enormously between TP. Final viscosity achieved for the recommended Thickness 1 in the pharyngeal phase after oral incubation ranged between 1.13 and 2.29 mPa·s for MS-based TPs, 14.25 and 60.89 mPa·s for mixtures and 31.33 and 37.74 mPa·s for gum-based TP. Total viscosity decrease, caused by the effect of salivary amylase and shear rate effect at the pharyngeal shear rate, varied according to the main composition: 96.86 - 98.73% for MS-based TP, 81.22 - 90.74% for mixtures and 62.47-77.42% for gum-based TP. Final viscosity achieved after oral incubation and the pharyngeal shear rate and their decrease is also presented for recommended Thicknesses 2 and 3 in Table 25.

Table 25. Viscosities of the thickening products assessed at 300 s^{-1} after oral incubation and their total decrease.

Thickening Product	Recommended thickness 1		Recommended thickness 2		Recommended thickness 3	
	300s^{-1} after oral incubation	**% reduction by amylase and shear rate**	**300s^{-1} after oral incubation**	**% reduction by amylase and shear rate**	**300s^{-1} after oral incubation**	**% reduction by amylase and shear rate**
A	60.89	81.12	202.7	83.53	433.1	84.12
B	1.13	98.62	5.93	99.03	17.00	99.45
C	1.84	98.72	2.10	99.79	3.00	99.95
D_1	14.25	80.98	32.44	90.74	57.96	95.45
D_2	32.44	90.74	57.96	95.45	92.33	96.61
E	1.49	98.11	2.5	99.56	1.25	99.95
F	2.29	96.86	2.09	99.54	2.29	99.88
G	1.79	97.90	1.76	99.59	3.61	99.83
H	37.74	77.42	84.80	82.69	147.27	79.65
I	31.33	62.47	75.73	70.37	108.87	71.13
J	1.25	98.73	4.52	99.40	1.81	99.95

5.3 Study 3 - Design and validation of a rheological protocol to standardize shear viscosity measurements

Study population

Mean age of the volunteers was 32±3.76 years and 25% were females.

Design and validation of a common protocol to standardize rheological measurements

Harmonization of the preparation protocol (Table 26). a) Stirring conditions: an homogeneus protocol was selected to be performed in the 4 different labs; TP was added to the solvent in 5s, the speed of stirring was determined at 4rps and the stirring time was assessed at 30 s; b) Stirrer: metallic spatula was selected for presenting the lowest viscosity variability ranging between 1.2 and 5.8% for all viscosity levels; in contrast, both plastic spoons obtained a higher variability ranging from 2.4 to 18.3% and 3.9 to 14.6% for the 160 mm and 100 mm length spoon, respectively ; c) Container: variability for each viscosity level was similar for the glass beaker (1.8-6.3%) and the clear plastic cup (1.9-5.7%). The white plastic cup presented the highest viscosity variation (5.2-12.0%). A clear plastic cup was selected for this study; d) Standing time before measurement: Thickened viscosities varied widely when assessed immediately after preparation when prepared in a glass beaker (2.9-12.8%) and in a clear plastic cup (3.1-12.9%) and variability was reduced after 10 minutes. Leaving the preparations standing for 10 and 30 minutes after mixing reduced the varia-tion to maximum of 6.6%. All the data is presented in Table 1.

Laboratory variability (Table 27). Mean intralaboratory coefficient of variation on the measurements at all target Shear viscosity levels (100-1600 mPa·s) was very low: 0.9% (Lab1); 3.9% (Lab2); 2.7% (Lab3); 2.9% (Lab4), and similar for all viscosity levels. Mean interlaboratory variability was higher: 81-105 (22.9%); 190-205 (7.3%); 381-403 (5.5%); 768-818 (6.1%) and 1552-1632mPa·s (4.9%) for 100, 200, 400, 800 and 1600 mPa·s target Shear viscosity, respectively but they did not exceed 10% differences except for the lowest viscosity level tested, and this was caused by measurements only in Lab 3 at 100 mPa·s No significant differences on viscosity values were obtained for the Shear viscosity levels assessed except for 100 mPa·s ($p=0.038$). Table 2 presents the mean viscosity value for each level determined for the various facilities. Finally, for the rheological characterisation, (Lab 3) the dose to achieve 100 mPa·s level was increased to 1.45g in order to reduce the variation between the analysed viscosity values and the target viscosity which was of 19% for 1.25 g/100 ml and reduced to 7.70% when using 1.45 g.

Table 26. Stirring conditions tested by the reference lab to harmonize the rheological protocol between laboratories: stirrer, container and standing time.

Stirrer						
Target viscosity at 50 s^{-1} (mPa·s)	**Metallic spatula 180 mm**		**Plastic spoon 160 mm**		**Plastic spoon 100 mm**	
	Viscosity at 50 s^{-1} (mPa·s)	**Variability (%)**	**Viscosity at 50 s^{-1} (mPa·s)**	**Variability (%)**	**Viscosity at 50 s^{-1} (mPa·s)**	**Variability (%)**
100	97.6±3.3	1.2-5.8	107.0±10.8	2.4-18.3	109.1±9.0	3.9-14.6
200	203.8±6.0		212.0±5.4		202.3±9.1	
400	400.4±4.2		402.2±8.6		388.2±8.0	
800	802.8±7.1		767.8±31.1		811.6±21.6	
1600	1602.4±10.1		1572.4±19.6		1595.5±31.7	
Container						
Target viscosity at 50 s^{-1} (mPa·s)	**Glass beaker**		**White plastic cup**		**Clear plastic cup**	
	Viscosity at 50 s^{-1} (mPa·s)	**Variability (%)**	**Viscosity at 50 s^{-1} (mPa·s)**	**Variability (%)**	**Viscosity at 50 s^{-1} (mPa·s)**	**Variability (%)**
100	98.1±3.5	1.8-6.3	107.6±4.7	5.2-12.0	101.9±3.3	1.9-5.7
400	398.4±3.90		401.0±25.6		407.4±5.0	
1600	1597.0±18.1		1555.3±42.1		1573.1±16.0	
Standing time – Glass beaker						
Target viscosity at 50 s^{-1} (mPa·s)	**0 min**		**10 min**		**30 min**	
	Viscosity at 50 s^{-1} (mPa·s)	**Variability (%)**	**Viscosity at 50 s^{-1} (mPa·s)**	**Variability (%)**	**Viscosity at 50 s^{-1} (mPa·s)**	**Variability (%)**
100	100.9±6.2	2.9-12.8	100.0±3.0	1.4-5.6	98.4±3.0	1.3-5.4
400	394.3±29.6		403.7±6.8		398.5±4.1	
1600	1620.3±24.7		1588.3±13.0		1595.1±12.0	
Standing time – Clear plastic cup						
Target viscosity at 50 s^{-1} (mPa·s)	**0 min**		**10 min**		**30 min**	
	Viscosity at 50 s^{-1} (mPa·s)	**Variability (%)**	**Viscosity at 50 s^{-1} (mPa·s)**	**Variability (%)**	**Viscosity at 50 s^{-1} (mPa·s)**	**Variability (%)**
100	102.2±6.4	3.1-12.9	100.8±3.2	1.3-6.0	99.4±3.4	1.4-6.6
400	415.1±29.6		401.4±7.8		407.4±5.0	
1600	1670.1±27.4		1644.3±11.1		1605.4±12.5	

Table 27. Mean values and the coefficient of variation from the triplicates of each viscosity level performed by the four labs. Variations within measurements in the same facility are shown as well as variations between facilities for each viscosity level; **p<0.05*

Dosage TP (g/100 ml)	Viscosity (mPa·s) at 50 s^{-1}								Mean interlaboratory variability (%)	p-value
	Lab1		Lab2		Lab3		Lab4			
	Mean (mPa·s)	±CV (%)	Mean (mPa·s)	±CV (%)	Mean (mPa·s)	±CV (%)	Mean (mPa·s)	±CV (%)		
1.25	101	±2.6	105	±4.9	81	±5.2	101	±4.3	22.9	*
2	205	±0.09	199	±4.5	190	±1.3	197	±2.3	7.3	0.08
3.2	403	±0.85	396	±4.1	386	±0.97	381	±3.2	5.5	0.13
5.8	805	±0.38	818	±3.0	768	±2.6	797	±3.4	6.1	0.11
10.5	1602	±0.35	1601	±2.8	1552	±3.5	1632	±1.3	4.9	0.16

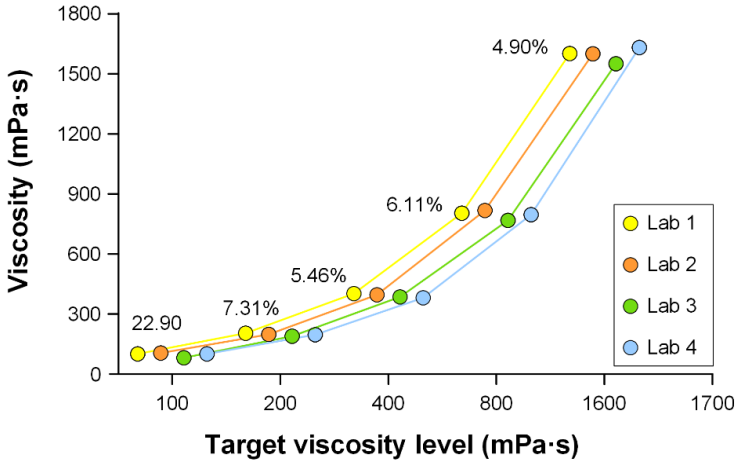

Figure 44. Inter-laboratory variability for each shear viscosity level tested (100, 200, 400, 800 and 1600mPa·s).

Rheological characterization

In healthy volunteers, amylase effect produced a viscosity decrease ranging from -0.4 (increase) to 16%. Mean shear viscosity values are presented in Table 28 for each dose of the TP assessed. Similar viscosity effect was observed after oral incubation in older patients: 16 – 18% (200 mPa·s) and 0 – 2% (800 mPa·s). Mean results of HV and patients are presented in Table 29 and Figure 45.

Shear rate effect for this specific TP, TQ, produced an index flow ranging from 0.16-0.29 which confirms the pseudoplastic behaviour of the fluid (n<1). Shear thinning at 300 s^{-1} caused a significant reduction in apparent viscosity ranging between 77 and 78% (p<0.05 vs 50 s^{-1}) which is in line with xanthan gum-based TP previously determined [144] (Table 28).

Table 28. Index flow, consistency and shear thinning effect from 50 to 300 s^{-1} for daily condition (DC) doses.

DC doses			
Target viscosity (mPa·s) at 50 s^{-1}	**Index flow (n)**	**Consistency (K)**	**Shear thinning (%)**
100	0.29	3.1	77.1
200	0.19	3.7	76.5
400	0.16	4.0	78.0
800	0.16	4.3	77.8
1600	0.17	4.6	77.2

DC: Daily condition

The combined effect of salivary amylase (oral phase) and shear rate (pharyngeal flow) produced a decrease in viscosity ranging from 75-79% in HV (Table 29, Figure 45). The combination of both parameters did not present a summatory effect but a slight increase in the impact on Shear viscosity values.

Table 29. Shear viscosity decrease caused by both swallowing factors (salivary amylase in the oral phase and shear thinning in the pharyngeal phase) in healthy subjects; *****p<0.0001 vs pre-oral incubation shear viscosity values.*

Healthy Volunteers (n=8)			
Target viscosity (mPa·s) at 50 s^{-1}	**Viscosity (mPa·s) at 50 s^{-1} Mean±SD**	**Amylase effect (%)**	**p-value**
100	93.1±6.5	5.9	0.99
200	160.0±7.3	15.6	0.75
400	355.5±23.7	7.1	0.81
800	771.8±42.0	-0.37	>0.99
1600	1449.0±72.8	6.7	***
Target viscosity (mPa·s) at 50 s^{-1}	**Viscosity at 300 s^{-1} Post-oral incubation (mean±SD)**	**Shear rate + amylase effect (%)**	**p-value**
100	25.2±1.7	74.5	****
200	39.1±1.8	79.4	****
400	78.8±4.1	79.4	****
800	168.1±9.0	78.1	****
1600	329.3±20.0	78.8	****

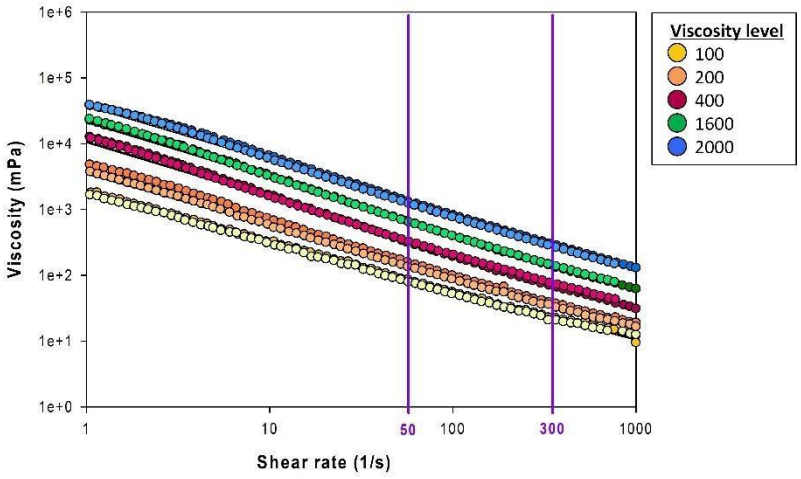

Figure 45. Viscosity curves from a shear rate range 1-1000 s^{-1} for daily condition doses before and after oral incubation in healthy volunteers. Dark colors correspond to viscosity levels pre-oral incubation. Soft colors correspond to viscosity post-oral incubation. Purple lines mark the two main shear rate landmarks (50 and 300 s^{-1}) during deglutition in patients with OD.

Time effect produced a variability of 16% was observed at 200mPa·s level for the Shear viscosity values assessed at the 5 different times (Table 30). At 800mPa·s, variability in Shear viscosity values was reduced to a maximum of 10%. No significant differences appeared between Shear viscosity values assessed for multiple comparisons nor between all values. After 120 min, Shear viscosity experienced a decrease ranging from 9 to 17% at 200mPa.s and from 3 to 10% at 800mPa.s (Table 30, Figure 46).

Increasing temperature by 5 ºC caused a viscosity variation ranging from 1.9 to 5.1% at 200 mPa·s at 50s^{-1} ($p>0.05$) and between 0.74 and 6.9% ($p>0.05$) at 800 mPa·s at 50s^{-1}. The maximal temperature assessed (40ºC) caused a decrease of viscosity between 6-7% for both Shear viscosity levels assessed. Viscosity values assessed for each temperature level and for both viscosity levels are presented in Table 6. Shear viscosity values presented no significant differences by increasing temperature for the whole temperature frame nor by multiple comparisons (Table 31, Figure 46).

Table 30. Viscosity values (mean±SD) by standing time for 200 and 800 mPa·s; *p>0.05 (ns).*

Standing time (min)	200 mPa·s (Target viscosity at 50 s^{-1})			800 mPa·s (Target viscosity at 50 s^{-1})		
	Viscosity (mPa·s) at 50 s^{-1} (mean±SD)	Differences 0-120 min (%)	p-value	Viscosity (mPa·s) at 50 s^{-1} (mean±SD)	Differences 0-120 min (%)	p-value
0	183.3±11.0	16.6	0.46	848.2±38.4	10.2	0.054
30	177.0±9.0			827.1±42.7		
60	169.5±8.3			784.9±32.7		
90	167.5±15.3			809.4±38.4		
120	152.9±19.1			762.1±39.4		
	200 mPa·s (Target viscosity at 50 s^{-1})			800 mPa·s (Target viscosity at 50 s^{-1})		

Standing time (min)	Viscosity (mPa·s) at 300 s^{-1} (mean±SD)	Differences 0-120 min (%)	p-value	Viscosity (mPa·s) at 300 s^{-1} (mean±SD)	Differences 0-120 min (%)	p-value
0	42.1±2.0			166.0±35.3		
30	41.1±1.5			157.7±5.9		
60	38.5±0.3	14.9	0.051	159.2±3.1	3.9	0.40
90	39.2±2.2			159.4±2.3		
120	35.8±3.9			159.6±2.9		

Table 31. Viscosity values (mean±SD) by increasing temperature from 20ºC to 40ºC for 200 and 800 mPa·s levels for daily conditions doses; *p>0.05 (ns).*

Temperature (ºC)	200 mPa·s (Target viscosity at 50 s^{-1})			800 mPa·s (Target viscosity at 50 s^{-1})		
	Viscosity (mPa·s) at 50 s^{-1} (mean±SD)	Differences 0-40ºC (%)	p-value	Viscosity (mPa·s) at 50 s^{-1} (mean±SD)	Differences 0-40ºC (%)	p-value
20	190.2±6.5			848.2±38.4		
25	180.5±5.8			827.1±42.7		
30	185.3±15.3	6.3	0.17	784.9±32.7	5.9	0.09
35	181.6±5.6			809.4±38.4		
40	178.1±8.9			762.1±39.4		
	200 mPa·s (Target viscosity at 50 s^{-1})			**800 mPa·s (Target viscosity at 50 s^{-1})**		
Temperature (ºC)	**Viscosity (mPa·s) at 300 s^{-1} (mean±SD)**	**Differences 0-40ºC (%)**	**p-value**	**Viscosity (mPa·s) at 300 s^{-1} (mean±SD)**	**Differences 0-40ºC (%)**	**p-value**
20	42.4±3.1			166.03±5.34		
25	39.7±0.9			157.70±5.90		
30	42.8±2.2	7.0	0.20	159.23±3.10	5.8	0.11
35	42.0±1.9			159.43±2.27		
40	39.4±0.3			159.57±2.87		

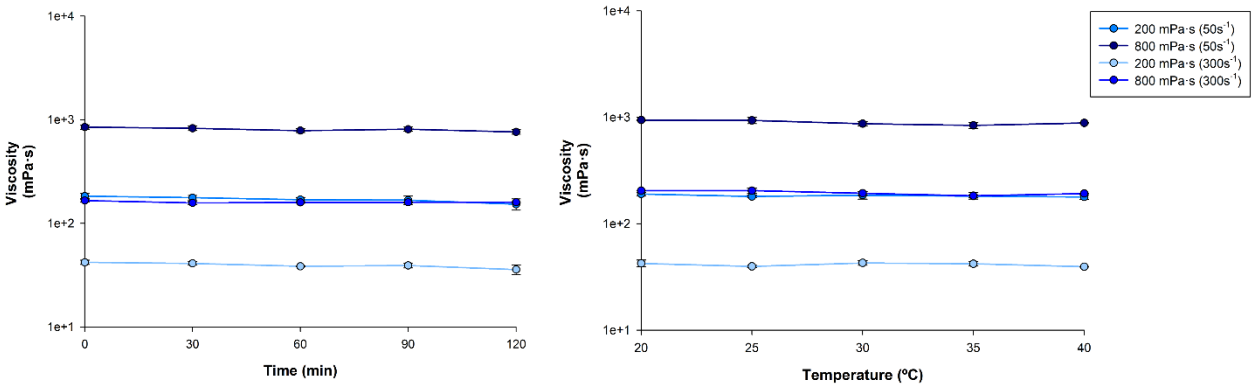

Figure 46. Viscosity values (mean±SD at 50 and 300 s^{-1}) by increasing time from 0 to 120 minutes (left) and temperature from 20 to 40ºC (right) for 200 and 800 mPa·s levels for daily condition doses.

Effect of the X-ray contrast Omnipaque on the rheological properties

Dose adaptation. Solutions with X-ray contrast in water (1:1 vol/vol) were adapted to the volume normally used for VFS at Lab 3 (50ml) to achieve the target Shear viscosity. Doses were calculated to achieve the same viscosity levels obtained by the DC doses previously described. Table 32 shows the dose of TQ necessary to obtain each target viscosity level when using the X-ray contrast as a solvent. Doses needed to achieve each Shear viscosity level were slightly lower than for DC doses (Table 32) varying between: 20.0, 0, 9.4, 15.5 and 18.1% for 100, 200, 400, 800 and 1600 mPa·s, respectively.

Table 32. Doses used to mix the videofluoroscopy doses

VFS Doses (g/ml)			
Target viscosity (mPa·s) at 50 s^{-1}	**Tsururinko Quickly (g)**	**Final volume (ml)**	**Dissolvent (ml)**
100	0.58	50	1:1 (water : Omnipaque)
200	1	50	1:1 (water : Omnipaque)
400	1.45	50	1:1 (water : Omnipaque)
800	2.45	50	1:1 (water : Omnipaque)
1600	4.3	50	1:1 (water : Omnipaque)

VFS: videofluoroscopy

VFS doses presented similar results at the shear rate of 50 s^{-1} between Lab1 and Lab3. Variability was calculated for each viscosity: 17.5%, 2.5%, 0.7%, 2.3%, and 4.6% for 100, 200, 400, 800 and 1600mPa·s, respectively. Maximal difference appeared for the lowest viscosity level (100 mPa·s) according to previous results presented above. Similar variations appeared for viscosities at 300 s^{-1} (Table 33). VFS doses were calculated in order to obtain no significant differences in Shear viscosity according to DC viscosity doses.

Table 33. Viscosity assessment between Lab1 (reference lab) and Lab3 (Health care centre for the CT) for VFS doses.

Target viscosity at 50 s^{-1} (mPa·s)	**Dosage (g/50 ml)**	**Average viscosity at 50 s^{-1} (mPa·s), n=3**		**Variations within facilities (%, n=3)**		**Variations between facilities (%)**	**Lab1-targeted viscosity (%)**	**Lab3-targeted viscosity (%)**
		Lab1	**Lab3**	**Lab1**	**Lab3**			
100	0.58	114	94	1.0	7.2	17.5	14	6
200	1	239	233	0.54	6.7	2.5	19.5	16.5
400	1.45	446	443	0.68	12.0	0.7	11.5	10.8
800	2.45	833	852	1.0	8.4	2.3	4.1	6.5
1600	4.3	1598	1672	0.6	3.7	4.6	0.13	4.5
Target viscosity at 50 s^{-1} (mPa·s)	**Dosage (g/50 ml)**	**Average viscosity at 300 s^{-1} (mPa·s, n=3)**		**Variations within facilities (%, n=3)**		**Variations between facilities (%)**		
		Lab1	**Lab3**	**Lab1**	**Lab3**			
100	0.58	32	28	0.77	1.9	12.5		

200	1	58	57	0.52	4.23	1.7
400	1.45	99	100	0.5	13.4	1.0
800	2.45	178	187	0.82	9.2	5.1
1600	4.3	349	384	0.63	5.6	10.0

Amylase effect after oral incubation caused a decrease of viscosity in healthy volunteers ranging from 11 to 20% for VFS doses. A slight increase was seen after oral incubation for the lowest viscosity level (100 mPa·s). Table 34 and figure 47 presents the results for VFS doses after oral incubation. No significant differences appeared when comparing Shear viscosity values post-oral incubation between DC doses and VFS doses.

Shear rate effect produced an index flow ranged between 0.16 and 0.32. The shear thinning effect caused a viscosity decrease from 50 s^{-1} to 300 s^{-1} of 70-78% for VFS (table 34 and figure 47). Significant differences appeared between Shear viscosity at 50 s^{-1} and 300 s^{-1} for all the levels tested ($p<0.0001$). Similar results have been obtained for VFS and DC doses.

Table 34. Index flow, consistency and shear thinning effect from 50 to 300 s^{-1} for VFS doses.

VFS doses			
Target viscosity at 50 s^{-1} (mPa·s)	**Index flow (n)**	**Consistency (K)**	**Shear thinning (%)**
100	0.32	3.1	70.2
200	0.23	3.7	75.5
400	0.16	4.1	77.4
800	0.16	4.4	78.1
1600	0.18	4.6	77.0

VFS: videofluoroscopy

The combined effect of salivary amylase (oral phase) and shear rate (pharyngeal flow) produced a viscosity decrease ranging from 69 to 83% (table 35). No significant differences appeared when comparing Shear viscosity values post-oral incubation for DC doses and VFS doses.

Table 35. Shear viscosity decrease caused by both swallowing factors (salivary amylase in the oral phase and shear thinning in the pharyngeal phase) in healthy subjects and in patients with oropharyngeal dysphagia; *****p<0.0001 vs pre-oral incubation shear viscosity values.*

Healthy Volunteers (n=8)			
Target viscosity (mPa·s) at 50 s^{-1}	**Viscosity (mPa·s) at 50 s^{-1} Mean±SD**	**Amylase effect (%)**	**p-value**
100	99.0±11.6	-4.47 (increment)	>0.99
200	187.0±21.4	19.7	0.98
400	355.5±23.7	19.8	0.75
800	731.5±33.1	14.1	**
1600	1476.3±115.9	11.7	****

Target viscosity (mPa·s) at 50 s^{-1}	Viscosity at 300 s^{-1} Post-oral incubation (mean±SD)	Shear rate + amylase effect (%)	p-value
100	28.9±3.1	69.5	****
200	47.5±5.0	79.6	****
400	78.8±4.1	83.1	****
800	161.9±6.3	82.2	****
1600	331.6±29.0	80.5	****

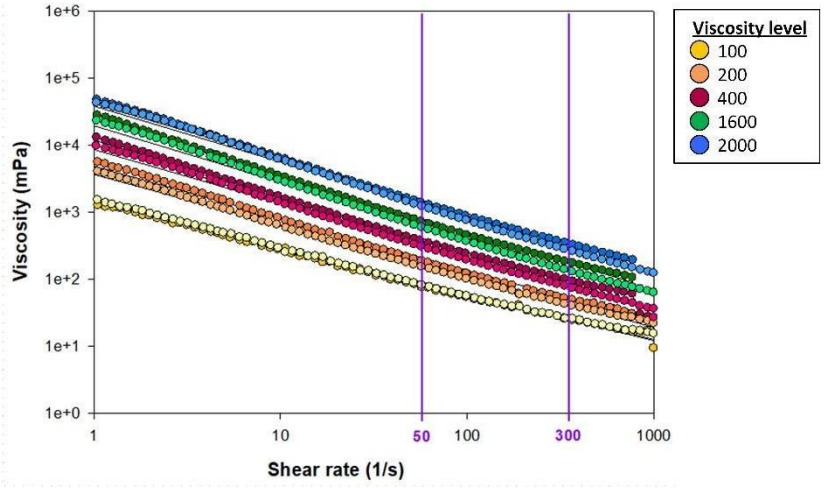

Figure 47. Viscosity curves from a shear rate range 1-1000 s^{-1} for VFS doses before and after oral incubation in healthy volunteers. Purple lines mark the two main shear rate landmarks during deglutition in patients with oropharyngeal dysphagia.

5.4 Study 4 - The rheological properties of thickening products that have a therapeutic effect on safety of swallow in patients with post-stroke oropharyngeal dysphagia

Shear viscosity

Viscosity for solutions prepared with thin liquid at 50 s^{-1} were: 2.20±0.06 and 1.66±0.10 mPa·s for solutions with Gastrografin and Omnipaque, respectively. TP A presented a viscosity at 50 s^{-1} of 116±6.21 mPa·s for nectar viscosity and incremented until 3375.06±302.03 mPa·s for spoon thick viscosity levels, respectively. In contrast, viscosity levels for XG TPs (B and C) ranged between 229.25±8.28 mPa·s and 2264.73 mPa·s. TP D (Mx) presented a viscosity of 281.90±28.41 mPa·s for the lowest viscosity level and 3256.80±148.33 mPa·s for the highest. Results on shear viscosity are presented in Table 36. Shear thinning effect ranged between 50-62% for MS product (TP A) and between 69-76% for products with XG (TP B, C and D).

Table 36. Rheological characterisation of shear viscosity. Viscosity at 50 s^{-1} and 300s^{-1} are presented for each thickening product.

Thickening Product	Viscosity descriptors	Viscosity at 50 s^{-1} (mPa·s)	Viscosity at 300 s^{-1} (mPa·s)	Shear thinning effect (%)
A	Nectar	116.33±6.21	57.95±13.60	50.18
	Spoon thick	3375.06±302.03	1268.41±57.17	62.42
B	Nectar	294.33±18.00	92.00±1.93	68.74

	Extreme spoon thick	1259.38±4.01	313.92±5.63	75.07
C	250	229.25±8.28	55.66±1.54	75.72
	800	848.35±59.90	204.11±12.98	75.94
	2000	2264.73±80.83	582.17±20.25	74.29
D	250	281.90±28.41	79.35±7.73	71.85
	1000	1449.87±56.00	346.60±16.26	76.09
	2000	3256.80±148.33	801.21±28.23	75.40

Flow index confirmed the Non-Newtonian pseudoplasticity behaviour of all the TP selected for the study (Table 37, Figure 49). Highest values were obtained for product A (0.66-0.38). Flow index for products B, C and D ranged between 0.18 and 0.29. Consistency index ranged between 2.60 and 4.90 $Pa{\cdot}s^n$ according to the shear viscosity achieved (Table 2).

Table 37. Flow index and consistency index of each thickening product viscosity level assessed.

Thickening product	**Viscosity at $50s^{-1}$**		
	Viscosity descriptors	**Flow index (n)**	**Consistency index (K; $Pa{\cdot}s^n$)**
A	Nectar	0.66	2.60
	Spoon thick	0.38	4.70
B	Nectar	0.29	3.72
	Extreme spoon thick	0.22	4.42
C	250	0.20	3.70
	800	0.22	4.26
	2000	0.24	4.64
D	250	0.27	3.70
	1000	0.18	4.58
	2000	0.20	4.90

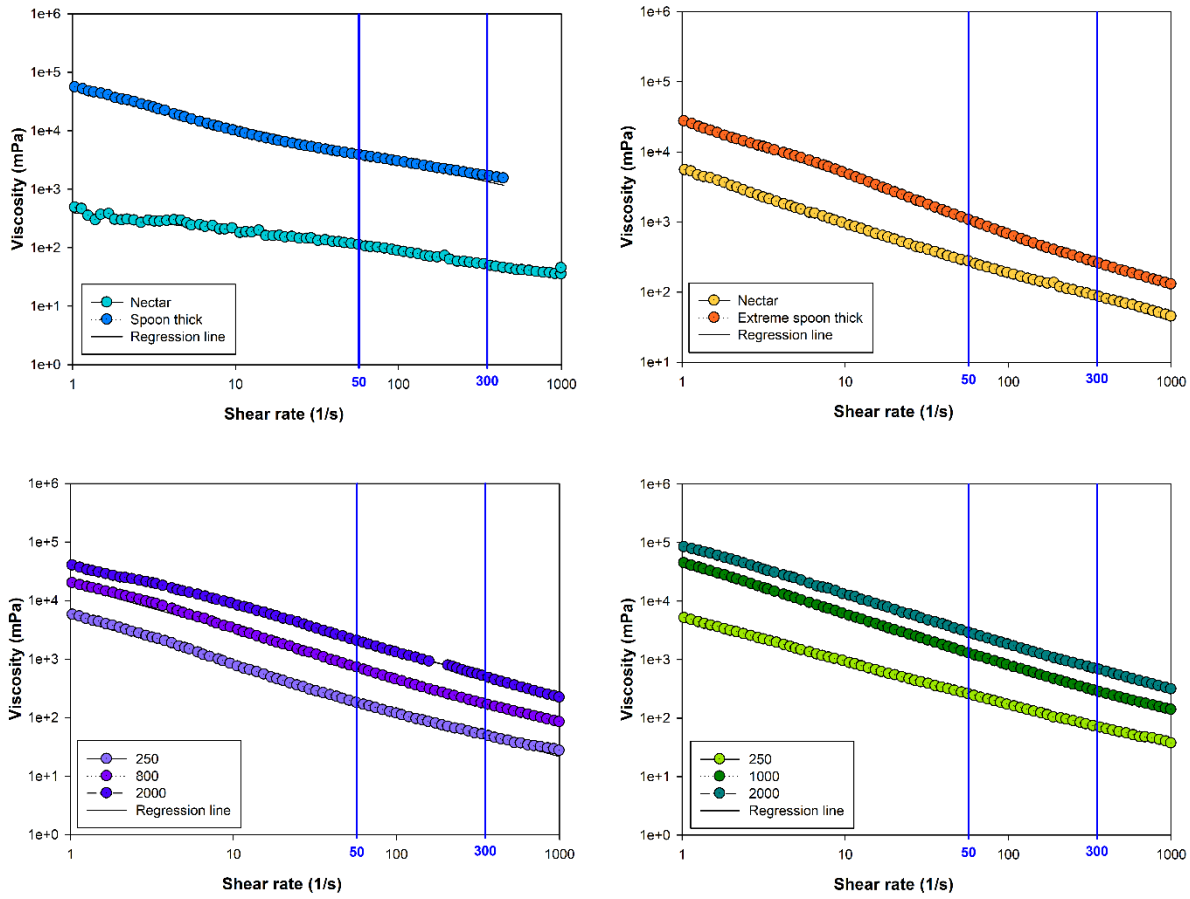

Figure 49. Viscosity flow curves for each thickening product level. *Blue: thickening product A; Orange: thickening product B; Purple; thickening product C; Green: thickening product D.*

Extensional deformation

For product A, time to BUD (mean±SD) was extremely low ranging between 0.04 and 0.02 ms for nectar and spoon thick viscosity, respectively. In contrast, for products B, C and D, time until diameter 0 was increased by the increment of shear viscosity. Time to BUD is presented in Table 38 for each thickening product dose and the graphical representation is shown in Figure 50.

Table 38. Time to break up diameter for each thickening product.

Thickening Product		Time to Break Up Diameter (ms; mean±SD)
A	Nectar	40.34±41.65
	Spoon thick	24.00±5.45
B	Nectar	250.57±63.16
	Extreme spoon thick	3040.99±2392.68
C	250	237.41±103.93
	800	756.56±328.54
	2000	6506.00±4563.31 (+ not broken)

D	250	68.36±13.01
	1000	599.50±156.75
	2000	21743.67±2990.92

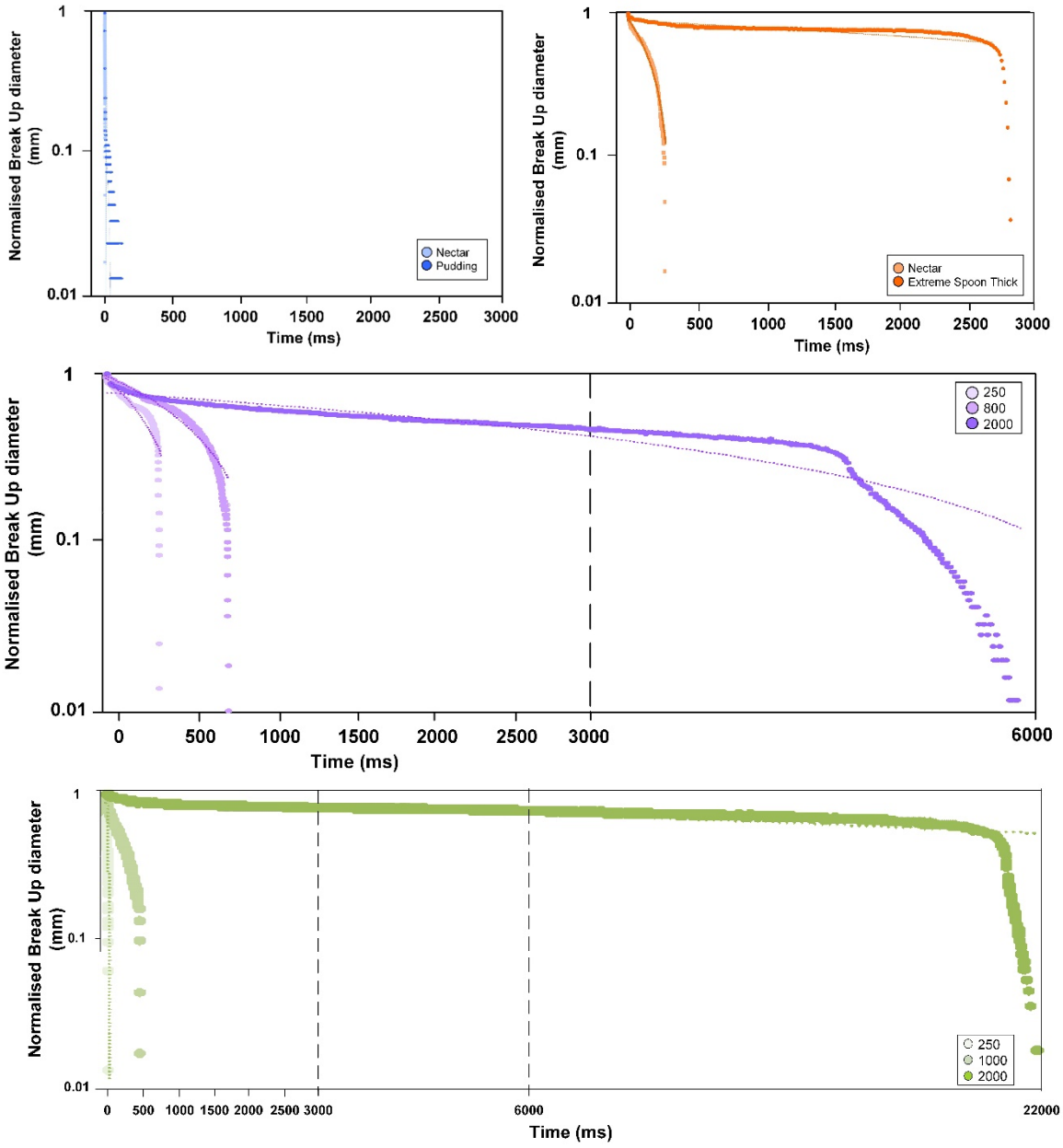

Figure 50. Sample examples of the normalized break up diameter vs time at each thickening products' viscosity level assessed in the study.

Time required to BUD was extremely low for all shear viscosity values assessed except for the highest viscosity levels for TP B, C and D. Lowest time to BUD was observed for TP A (MS) which presented the highest shear viscosity value (3400 mPa·s). For each TP, the increment of viscosity produced an increment in the time to BUD but no consistent relationship was observed between shear viscosity and extensional deformation (figure 51).

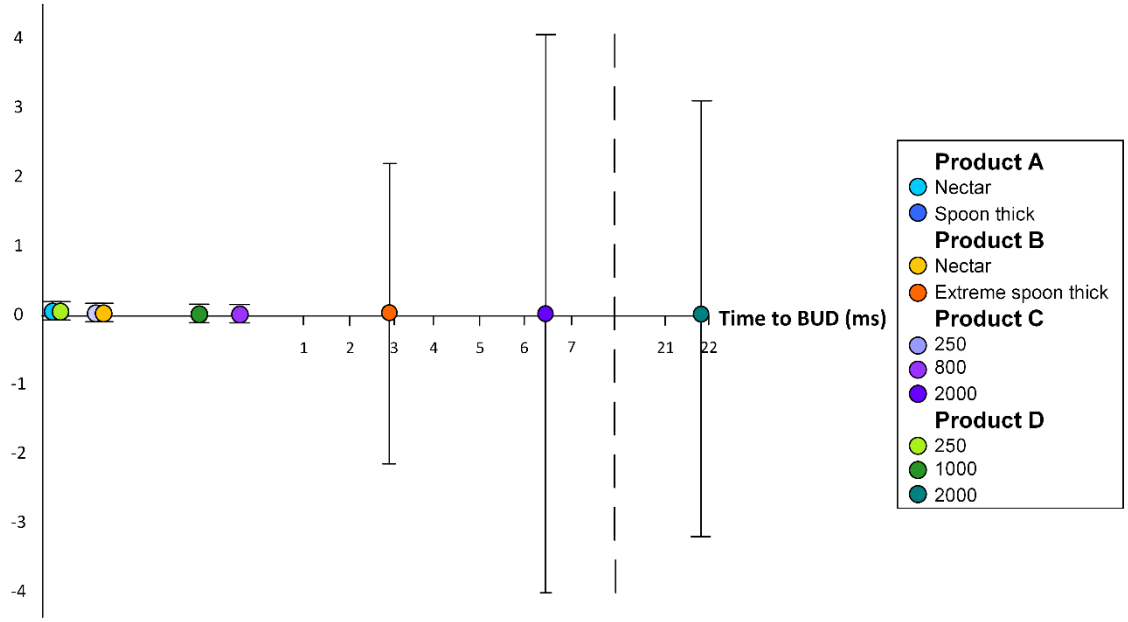

Figure 51. Time to break up diameter (BUD; mean±SEM) for all thickening product viscosity levels.

Textural characterization

Maximal force was increased by increasing the viscosity level for all the TP assessed: 0.45-1.01 N (A), 0.19-0.52 N (B), 0.26-1.03 (C), 0.27-0.80 (D). Adhesiveness was also increased according to the viscosity level: 0.56-1.95 (A), 0-0.53 (B), 0.05-1.04 (C), 0.003-1.12 (D). In contrast, cohesiveness increased for TP A with the increment of shear viscosity but decreased for TP B, C and D. Table 39 and figure 52 shows the textural results of all the TP at each viscosity level assessed.

Table 39. Maximum force, cohesiveness and adhesiveness for each viscosity level assessed for all the thickening products selected.

TP	Viscosity level	Maximum force (N)	Adhesiveness (N·s)	Cohesiveness
A	**Nectar**	0.45±0.10	0.56±0.03	0.80±0.02
	Pudding	1.01±0.03	1.95±0.17	0.93±0.02
B	**Nectar**	0.19±0.002	0.00	1.09±0.05
	Extreme spoon thick	0.52±0.01	0.53±0.01	0.74±0.03
C	**250**	0.26±0.008	0.05±0.005	0.82±0.021
	800	0.50±0.02	0.42±0.001	0.74±0.01
	2000	1.03±0.02	1.04±0.05	0.71±0.04
D	**250**	0.27±0.004	0.003±0.005	0.95±0.06
	1000	0.47±0.03	0.47±0.04	0.81±0.02
	2000	0.80±0.02	1.12±0.04	0.78±0.009

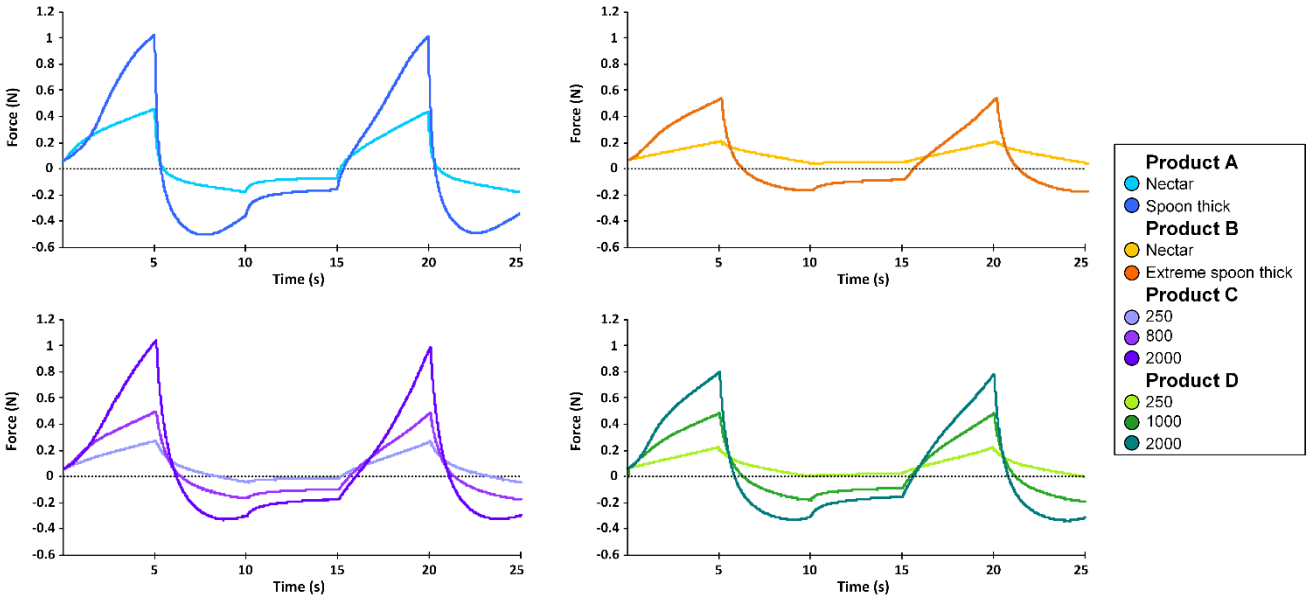

Figure 52. Texture profile analysis graphs for each thickening product at each viscosity level. *Maximal force is represented by the first peak force and adhesiveness by the area under the curve. Cohesiveness is determined by Area 2 divided by Area 1.*

Therapeutic effect

Demographics and stroke characteristics of the population selected for each CT are detailed in Table 40. CT for TP A and B recruited patients in the acute post-stroke phase. In contrast, product C was tested in patients at post-stroke chronic phase. The study testing product D recruited patients in both chronic and acute stroke phase.

Table 40. Population obtained from each CT

Thickening Product		A	B	C	D	Total
Number participants		46	76	114	31	267
Gender (% men)		65	57	54.4	89	66.40
Age (mean±SD)		75.63 ± 8.40	74.83 ± 10.86	76.70 ± 8.9	79.42 ± 1.36	76.65±2.00
PAS (mean±SD)		-	-	3.70 ± 2.30	3.23 ± 2.34	
Time from stroke (months)		7.45±8.97	27.32±72.04	431.7±1031.9	-	16.39±10.08
Barthel index		83.26±22.64	72.92±26.32	-	74.83±5.31	77.34±5.15
Type of stroke (%)	*Ischemic*	42.35	34.20	78.1	-	51.55
	Hemorrhagic	5.88	43.42	11.4	-	20.23
	Unknown	5.88	22.37	10.5	-	12.92

Safety of swallow for thin liquid ranged between 36 and 53%. Prevalence of safe swallows for thickened viscosities went from 62 to 97%. For low viscosities (Nectar and 250) the highest percentage was seen for product B achieving 79% of safe swallows at its first thickened level. For higher viscosities, including spoon thick (A), extreme spoon thick (B), 800, 1000 and 2000 levels, safe swallows ranged between 90 – 97%. Prevalence of safe swallows for the different doses of the TPs are presented in Table 41.

Table 41. Percentage of patients with safe swallows for each viscosity level and thickener.

Thickening Product	Viscosity descriptors	Safe swallows (% patients)	Penetrations (% patients)	Aspirations (% patients)
A	Thin liquid	37.21	58.14	4.65
	Nectar	62.22	37.78	0.00
	Spoon thick	93.33	6.67	0.00
B	Thin liquid	35.82	56.72	7.46
	Nectar	79.22	15.58	5.19
	Extreme spoon thick	90.91	7.79	1.30
C	Thin liquid	41.20	41.20	17.50
	250	78.10	9.60	12.30
	800	92.10	2.60	5.3
	2000	91.20	6.10	2.70
D	Thin liquid	53.33	30.00	16.67
	250	75.00	14.29	10.71
	1000	90.00	10.00	0.00
	2000	96.77	3.23	0.00

All TP showed a strong shear viscosity-dependent effect on prevalence of patients with safe swallow. A significant dose-response correlation was obtained between shear viscosity and safety of swallow ($p<0.0001$; Figure 53). In contrast, extensional deformation (time to BUD) did not present a dose-response effect on safety of swallow ($p=0.123$): widely divergent extensional deformation values (756.56±328.54 vs 21743.67±2990.92 ms) provided similar safety levels (92% and 96%, respectively). Regarding textural properties, MF significantly correlated with safety of swallow ($p<0.0001$) but adhesiveness ($p=0.06$) or cohesiveness ($p=0.11$) did not.

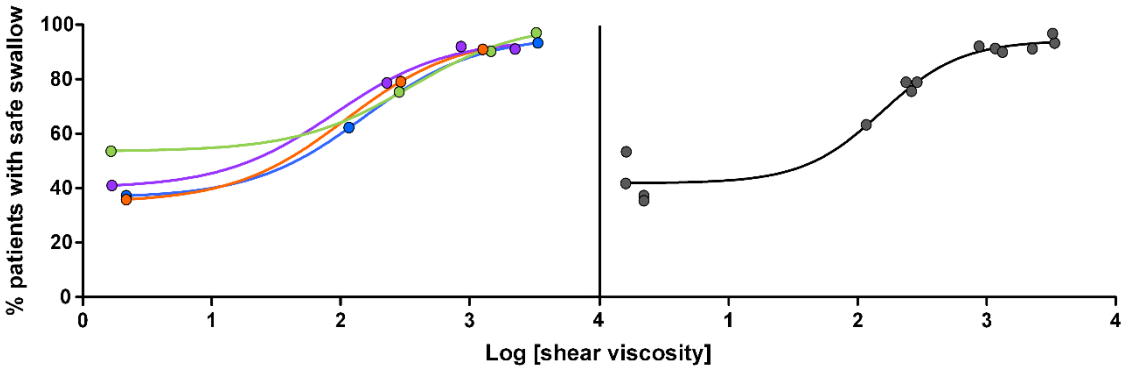

Figure 53. Log viscosity-response effect for safety of swallow at the different viscosity levels assessed for each thickening product. On the right, curves for each thickening product; on the left, dose-response curve for all viscosities assessed.

Shear viscosity was strongly, linearly and significantly correlated to MF ($r^2=0.85$; $p<0.0001$) and weakly inversed correlated to cohesiveness ($r^2=0.29$; $p=0.044$). Linear regressions of both parameters are presented in Figure 54.

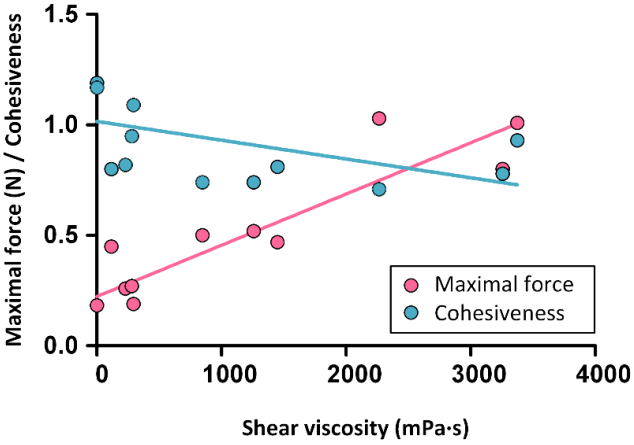

Figure 5. Linear regression for maximal force (N) and cohesiveness vs shear viscosity.

6. DISCUSSION

The present doctoral thesis includes several studies published or in the process of publication with the aim to better explore the therapeutic effect of TP on safety and efficacy of swallow in the main OD phenotypes, to determine the rheological properties affecting it and select the optimal viscosity values to provide safe swallow to most patients with OD. This project set out to address different issues related to the therapeutic effect of the main types of TP used in the treatment of dysphagia (Figure 54).

To assess the therapeutic effect of TP, three CT (Study 1) were performed on the main OD phenotypes: older, post-stroke, neurodegenerative and head and cancer patients by using XG TP [140] or mixtures [141]. Firstly, there is a need to characterize the pathophysiology and swallowing response with TP in patients with OD by studying the swallowing response when different viscosity levels are given. Accurate techniques such as VFS can specifically determine OD causes and severity (including safety and efficacy) OSR and kinematics of swallowing [26]. These studies confirm that the main biomechanical alteration associated with impaired safety of swallow are a delay in time to LVC and slow bolus velocity caused by reduced bolus propulsion from the tongue. Once patients' swallowing is analyzed, in order to assess the therapeutic range and optimal viscosities for each TP, a viscosity dose-response curve was performed. This pharmacological approach applied to shear viscosity levels (mPa·s) in TP can determine the viscosities for threshold and maximal therapeutic effect on safety of swallow as well as the number of viscosity levels required to cover the therapeutic range.

Study 1 determined the therapeutic effect of several viscosity levels on the main phenotypes of patients with OD. Results from 327 patients with OD (including all phenotypes) showed the high prevalence of patients with unsafe swallows at thin liquid viscosity (<50mPa·s). This prevalence highlights the enhanced risk patients face when swallowing unthickened liquids and also those TP highly affected by salivary amylase such as MS [144]. As observed in this thesis, MS-based TP lose between 80 and 100% of their oral shear viscosity acquiring a similar viscosity to thin liquids. In contrast, the increment in shear viscosity presented a viscosity-dependent effect for safety of swallow independently of the phenotype of patient or the TP. In addition, this study determined that **only two viscosity levels were needed to cover more than 90% of the population with dysphagia,** ranging between 200 and 1000 mPa·s for all the TP assessed. Regarding the efficacy of swallow, it is important to mark the increment of oral residue for all the TP evaluated with the increment of viscosity but no viscosity-dependent effect was observed. This effect can be related to the decrease in tongue strength which is usually reduced in older patients with OD. In contrast, pharyngeal residue was maintained by the increment of shear viscosity and no significant differences were observed between thin liquid and thickened levels. When analyzing OSR, a new and unexpected finding was observed with the XG-based TP (not for the Mx): a significant reduction in time to LVC over thin liquid and of the bolus velocity for viscosity levels above 450 mPa·s. These reductions in time suggest an additional mode of action to the compensatory effect obtained by the increment of viscosity with TP which needs to further be explored in future studies.

As shear viscosity was found to be an important parameter linked to safe swallows, Study 2 was designed to analyze the different shear viscosity levels recommended by manufacturers for TP

already commercialized. In addition, to simulate oral and pharyngeal conditions, salivary amylase and shear rate effect were applied to determine the effect of swallowing on viscosity [129]. The analysis of several TP commercialised in Spain showed that manufacturers recommend the use of 3 viscosity levels randomly selected with no CT to prove their therapeutic effect [144]. In addition, the levels were described in a qualitative manner. When determining shear viscosity in SI units, we observed that very different viscosity values were described with the same qualitative descriptor and that different qualitative descriptors could refer to the same viscosity value. Another relevant parameter to take into account is the wide variability between the preparation protocol recommended by each manufacturer. All this scenario endangers the safety of our patients, therefore**, the use of qualitative descriptors should be avoided and shear viscosity must be described in a scientific manner** (Pa·s / cP) on the label of all TP, as stated by the legal system of measurements in the European Union [145]. Main conclusion arising from this study is that an international and scientific manner must be established to describe the properties of products used for dysphagia. This information can enhance patients' safety by providing the specific values of viscosity when swallowing and improve clinical practice and clinical research by following an international and homogeneous description.

In order to standardize this scenario exposed above, Study 3 was performed. To further explore the effect of the mode of preparation on shear viscosity, different equipment was studied. We determined that the stirring element, the container or the time can alter the shear viscosity achieved in a significant manner. Due to these results, a protocol to assess the rheological behaviour of TP in a scientific and objective manner was designed and validated. This protocol showed **that is possible to obtain shear viscosity measurements (mPa·s) worldwide when applying the same protocol with a variability below 10%.** This proposal includes a simulation of oral, pharyngeal and other external factors such as stability to temperature, time, etc. by applying an *in vitro* and *ex vivo* protocol and avoiding the use of invasive techniques. We have developed a rheological protocol which can be applied around the world and simulates TP behavior during swallowing by patients with OD in an accurate and reproducible manner. We believe that, step by step, we can move from the qualitative approach to the quantitative and SI system which will improve not only the safety of our patients but also the quality of the care provided by healthcare professionals.

Finally, the main conclusion from this thesis emerges from the combination of the therapeutic effect and the rheological characterization of TP. Study 4 presents the analysis of the therapeutic effect on safety of swallow of 4 TP (MS, Mx and XG) already determined by previous CT [112], [140], [141]. In parallel, an accurate rheological characterisation was also performed including shear viscosity, extensional deformation, maximal force, adhesiveness and cohesiveness. This study demonstrated that shear viscosity was strongly, directly and significantly associated in a dose-response manner with the safety of swallow. However, the most relevant finding arising from this Study was the confirmation that viscosities over 800 mPa·s did not provide any significant improvement in safety of swallow for any of the XG TP assessed. Therefore, there is no scientific evidence to commercialize or prescribe viscosities above this specific level. This outcome must lead to a change in the usual clinical practice applied in patients with OD to increase the effectiveness of the treatment and their quality of life. This study confirmed that **shear viscosity is the main parameter causing the safety of swallow of TP both being linked in**

a strong dose-dependent effect. MF was also related to safe swallow due to the significant and strong correlation with shear viscosity. No other dose-related correlation with safe swallow was observed for the rest of the rheological parameters.

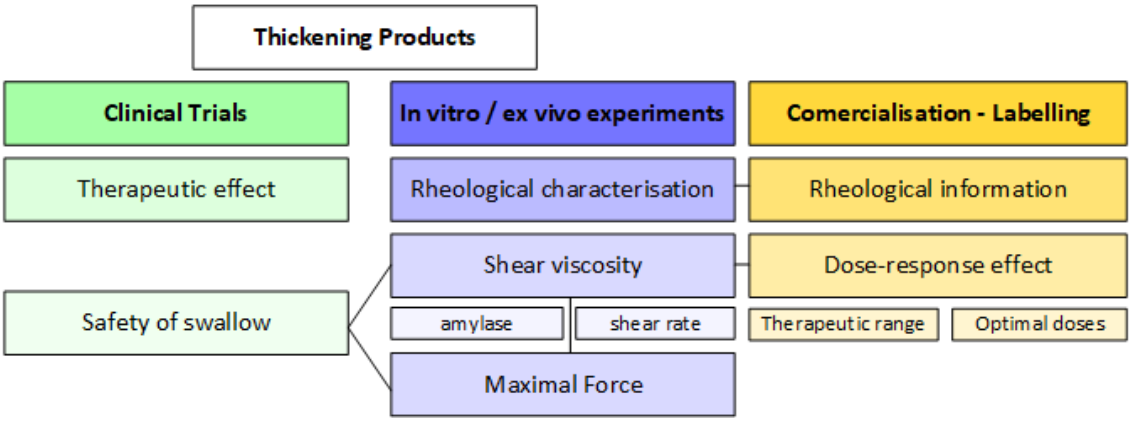

Figure 54. Summary of studies to determine thickening products' therapeutic effect and their rheological characteristics. Information obtained from the combination of both fields that needs to be reported is also presented.

7. CONCLUSIONS

C1. TP improve the safety of swallow by the increment of shear viscosity in dose-response effect in patients with dysphagia caused by aging, stroke, neurodegenerative diseases and head and neck cancer. The increment of shear viscosity by the use of TP produced a significant increase inr oral residue. In contrast, pharyngeal residue was not affected by the increase of viscosity levels for any of the TP assessed. A therapeutic range can be established between 100 and 1000 mPa·s for all OD phenotypes. Increasing viscosity above 800 mPa·s for XG and 1000 mPa·s for Mx TP did not cause any further significant improvement in therapeutic effect suggesting that higher viscosities are useless.

C2. The use of qualitative viscosity classifications leads to several risks and contradictions which endangers the safety of patients with dysphagia. An objective, international and scientific labelling must be established to describe the rheological characteristics with an impact on the therapeutic effect of TP according to the three patterns described. Pattern 1 is highly affected by salivary amylase which endangers the therapeutic effect offered by the TP (MS and some Mx) and, given this situation, should be removed from the market. Optimal viscosity levels for each TP should be determined by clinical trials and stated in the labelling in SI units.

C3. A rheological protocol to measure shear viscosity simulating TP behavior during swallowing by patients with OD can be applied worldwide in an accurate and reproducible manner with a variability between different institutions below 10%. The application of a standardised protocol will support a labeling system based on SI units to help to improve the management of patients with OD and being prescribed with TP.

C4. Shear viscosity is the main property causing the therapeutic effect of TP. Shear viscosity has a strong dose-dependent effect on safety of swallow. Maximal force was also related to safety of swallow due to the strong correlation obtained with shear viscosity. Extensional deformation, adhesiveness and cohesiveness was not significantly related to the effect of TP on safety of swallow.

8. FUTURE PERSPECTIVES

This doctoral thesis increased the evidence on the need to establish a scientific and common methodology to evaluate TP and to determine the optimal doses to treat patients with swallowing disorders according to their abilities and needs. Results from these studies demonstrate that OD population can be covered (>90%) with only two viscosity levels of XG TP (250 and 800 mPa·s) and that there is no need to increment viscosity above 800 mPa·s as higher viscosities cause no significant improvement on the therapeutic effect and decrease palatability of the treatment and thus, the patients' quality of life.

Viscosity can be quantitatively assessed in SI units (mPa-s) and tested in OD patients in order to obtain evidence of their clinical effect. It is also important to comment on the main property causing the therapeutic effect of TP: shear viscosity, which presents a strong dose-response effect on safety of swallow. Shear viscosity can be altered during the oral and pharyngeal phases of swallowing, therefore, its behavior when submitted to the swallowing factors (salivary amylase and shear rate) needs to be characterized prior to its recommendation when commercializing TP. However, other properties such as MF, which is strongly related to shear viscosity, should also be further explored to assess their potential role in TP's therapeutic effect.

In summary, main future perspectives arising from these studies are:

a) Commercialisation of TP's viscosity levels recommended by each manufacturer must be adjusted according to the optimal viscosity levels previously determined and tested in CT and this should be controlled by authorities. XG above 800 mPa·s and Mx above 1000 and 1000 do not provide any additional therapeutic effect on safety of swallow.

b) TP must guarantee optimal properties according to the swallowing factors affecting shear viscosity: resistance to salivary amylase and minimal affection to shear rate to obtain the maximal therapeutic effect while being swallowed. MS thickening agents should be avoided because of the strong effect of oral amylase on shear viscosity with this type of TP.

c) The labels on TPs must include all the information regarding its therapeutic effect and the effect on it of rheological swallowing factors. This information should be described in a precise, accurate and objective manner following the SI units for each parameter expressed.

According to these perspectives, main progress in this field would be the design and development of TP not affected by salivary amylase or shear rate (Newtonian behaviour) which would reduce the viscosity levels prescribed to minimal doses. This would increase the security and the adherence to the OD compensatory treatment.

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10. ANNEXES

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10.2 Publications

Some of the results obtained in this doctoral thesis have been published in indexed scientific journals:

10.2.1 Article 1

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ORIGINAL ARTICLE

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Effect of a gum-based thickener on the safety of swallowing in patients with poststroke oropharyngeal dysphagia

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Abstract

Background: Increasing viscosity with thickening agents is a valid therapeutic strategy for oropharyngeal dysphagia (OD). To assess the therapeutic effect of a xanthan gum-based thickener (Nutilis Clear®) at six viscosities compared with thin liquid in poststroke OD (PSOD) patients.

Methods: A total of 120 patients with PSOD were studied in this controlled, multiple-dose, fixed-order, and single-blind study using videofluoroscopy (VFSS). A series of boluses of 10 mL thin liquid and 2000, 1400, 800, 450, 250, and 150 mPa s viscosities were given in duplicate, interrupted in case of aspiration. We assessed the safety and efficacy of swallow and the kinematics of the swallow response.

Key Results: A total of 41.2% patients had safe swallow at thin liquid which significantly increased for all viscosities from 71.9% at 150 mPa s to 95.6% at 1400 mPa s ($P < .001$). PAS score (3.7 ± 2.3) at thin liquid was also reduced by increasing bolus viscosity ($P < .001$). The prevalence of patients with aspiration at thin liquid was 17.5% and decreased at all viscosities ($P < .01$), except at 150 mPa s. Increasing viscosity shortened time to laryngeal vestibule closure (LVC) at all viscosities ($P < .01$) and reduced bolus velocity at ≥450 mPa s ($P < .05$). The prevalence of patients with pharyngeal residue at each viscosity 37.7%-44.7% was similar to that at thin liquid (41.2%).

Conclusions and Inferences: The prevalence of unsafe swallow with thin liquids is very high in PSOD. Increasing shear bolus viscosity with this xanthan gum-based thickener significantly increased the safety of swallow in patients with PSOD in a viscosity-dependent manner without increasing the prevalence of pharyngeal residue.

KEYWORDS
aspiration, deglutition disorders, shear viscosity, stroke, swallow response, thickener, xanthan gum

Clinical trial registration: Netherlands Trial Register with code NTR5628.

1 | INTRODUCTION

Oropharyngeal dysphagia (OD) is a motility disorder characterized by difficulty forming or moving the alimentary bolus from the mouth to the esophagus and can include aspiration.[1] Poststroke OD (PSOD) is classified in the ICD under the code: 438.82 (ICD-9) and I69.391 (ICD-10).[2] OD is a prevalent complaint following stroke, with high incidence (45%) on hospital admission.[3] It is associated with poor short- and long-term prognosis and several complications, such as malnutrition, dehydration,[4] and aspiration pneumonia, increasing the risk of mortality [5-7] in comparison with poststroke patients without OD.[5,8-10] It is an independent risk factor for prolonged hospital stay and institutionalization after discharge, and for poorer functional capacity and increased mortality 3 months after stroke.[3] While some patients recover spontaneously, 50% assessed 6 months poststroke were found to have chronic OD.[11] The pathophysiology of PSOD is characterized by several motor impairments in the kinematics of the swallow response including delayed laryngeal vestibule closure (LVC) and decreased bolus propulsion forces [12]; also, patients affected by unilateral stroke showed a disrupted pattern of sensory cortical activation after pharyngeal stimulation as a distinctive marker of abnormal sensory integration of swallowing pathways in PSOD.[13]

Thickening agents increase the viscosity of fluids and thin liquids, enhancing the safety of swallow by avoiding aspirations and their associated complications,[14,15] as stated in a review by the European Society for Swallowing Disorders (ESSD).[16] Viscosity is a rheological property which measures the resistance of a fluid to flow, expressed in SI units as mPa s [15,17]—rheology is the study of the flow and deformation of fluids.[18,19] Several factors can affect the viscosity of thickened fluids: salivary α-amylase breaks down starch molecules during the oral phase of swallow,[15] and shear thinning decreases viscosity with increasing bolus velocity and shear rate [18,20] in the pharyngeal phase.

The ESSD review also recommended (a) the development of new thickening agents with less residue, more palatability and, thus, better compliance (gum-based thickeners have proven to be better than starch) [20]; and (b) clinical trials to establish the optimal viscosity level for each phenotype of dysphagic patients.[16] Few viscosity levels per product have been studied, and the optimal viscosity levels for patients suffering poststroke OD have not been determined yet.[16]

The aim of this study was to assess the effect of a gum-based thickener (Nutilis Clear®) on the safety and efficacy of swallowing in patients with poststroke OD by evaluating seven different shear viscosities (150-2000 mPa s) during swallowing with videofluoroscopy swallowing study (VFSS). There are no previous studies that have evaluated such a wide range of viscosities. The primary objective was to assess the percentage of patients that could swallow safely at each of the three main viscosities (2000, 800 or 250 mPa s) compared with thin liquid. Secondary and exploratory objectives were to assess the effect of all viscosities on penetrations, aspirations, the Penetration-Aspiration Scale (PAS) developed by Rosenbek,[21] the presence and severity of oral and pharyngeal residue, and the effects on the biomechanics of the swallow response.[13,15]

Key Points

- Oropharyngeal dysphagia (OD) occurs in 45% poststroke patients. Increasing bolus viscosity with thickeners reduces aspirations, but optimal viscosity levels need to be determined.
- We assessed 7 shear viscosity levels with a xanthan gum-based thickener in stroke patients with dysphagia and found a viscosity-dependent improvement in swallowing safety from 150 mPa s to 800 mPa s through reduced time to laryngeal vestibule closure and bolus velocity.
- This is the first study to show the full dynamics and mechanisms of gum-based thickeners in poststroke OD.

2 | PATIENTS AND METHODS

2.1 | Study population

This study included 120 PSOD outpatients who were consecutively recruited from March 2016 to December 2017 at the GI Physiology Lab of the Hospital de Mataró, Barcelona following hospital discharge. Assuming discordant proportions of 7.5% (safe swallow on thin liquid and unsafe swallow on main viscosities) and 30% (unsafe swallow on thin liquid and safe swallow on main viscosities), a sample size of 95 patients would be sufficient to have 90% power to detect statistical significant differences in safe swallowing between each of the three main viscosities and thin liquid, using a two-sided Mcnemar's test with an α of 0.5, assuming 20% of patients do not complete the measurements. Main inclusion criteria were patients older than 18 years, minimum of 28 days since diagnosis of stroke, clinical signs or symptoms of swallowing dysfunction in the volume-viscosity swallow test (V-VST) [22] or referral by physician for VFSS or current use of thickened products, no alteration in consciousness, and written informed consent. Main exclusion criteria were need of oxygen therapy, OD not related to stroke, history of other neurological disorders or head and neck cancer, xerostomia induced by drugs, severe cognitive disorder, incapability to perform VFSS, pregnancy or lactation, participation in another research study, and allergy to any ingredient tested. In addition, for the description of study population, we collected demographic parameters such as age, sex, weight, height, type of stroke, time after stroke, severity of dysphagia, nutritional status, comorbidities, medication, and stroke severity according to the National Institute of Health Stroke Scale (NIHSS).[23]

The Ethics Committee of the Hospital de Mataró (Spain) approved the study protocol, information given to patients about the study and the informed consent form with code 41/15. The study was conducted according to the principles of the "World Medical Association Declaration of Helsinki" (2013) and the International Conference

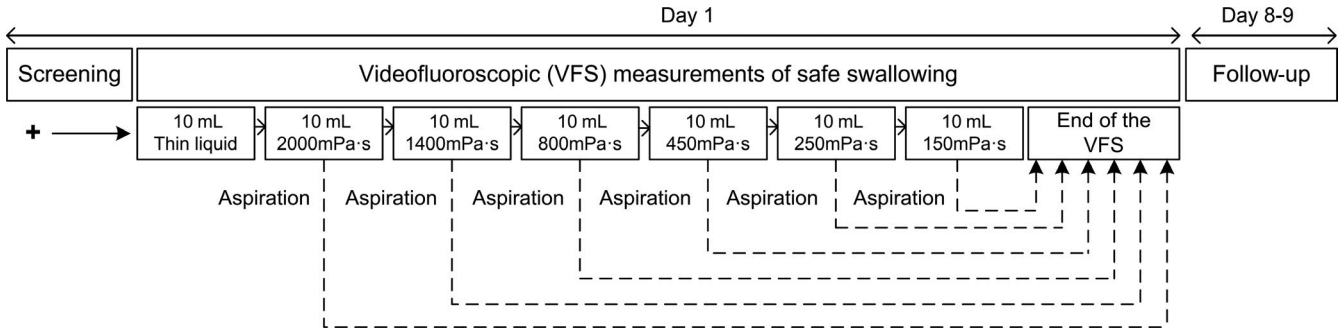

FIGURE 1 Study design

on Harmonization (ICH) guidelines for Good Clinical Practice (GCP, September 1997) as appropriate for nutritional products legislation of Spain where the study took place. This study has been registered in The Netherlands Trial register with code: NTR5628.

2.2 | Experimental design

This was a reference-controlled, multiple-dose, fixed-order, single-blind, and single-center study. The study procedure (Figure 1) was performed in one single visit. Firstly, the V-VST—a clinical assessment tool for dysphagia—was performed on each patient to assess clinical signs of OD [23-25] and those positive for OD were referred for VFSS. One week after the completion of the study, a follow-up call was performed to assess potential adverse events.

During the VFSS, 10mL boluses were given in duplicate to each patient, following the algorithm shown in Figure 1 (only one bolus is shown in the algorithm, but two were given if the patient swallowed safely). Briefly, the procedure started with thin liquid (when aspirations occurred, the second bolus of thin liquid was not administered to protect patients from a new aspiration) and continued with boluses from the highest viscosity to the lowest. If the patient aspirated any of the thickened boluses, the study was terminated to avoid any further aspiration as a safety measure.[16,26]

2.3 | Outcome parameters

The main outcome parameter was the percentage of patients with safe swallow (PAS score 1 and 2) [21] for the main viscosities (250, 800, and 2000 mPa s). Secondary outcome parameters were as follows: (a) safety of swallowing expressed by the mean PAS score,[21] and the percentage of patients with penetration (PAS score of 3,4,5), or aspiration (PAS score of 6,7,8); and (b) the efficacy of swallowing expressed by the presence and severity of oral and pharyngeal residue. Exploratory parameters included physiology of swallowing (time to LVC, total duration of swallowing response –LVO–, mean bolus velocity, and bolus propulsion force), distribution of PAS scores, subjective swallowing experience at all viscosities (150, 250, 450, 800, 1400, and 2000 mPa s) and safety and efficacy of swallowing at the 3 exploratory viscosities (150, 450, and 1400 mPa s). Due to the relevance of the information for patient safety, comparisons on the prevalence of patients with safe swallow and mean PAS scores were also performed between all the different viscosities assayed in this study.

2.4 | Methods

2.4.1 | Videofluoroscopy (VFSS)

VFSS is a dynamic radiological exploration that evaluates the swallowing process of boluses of various volumes and viscosities marked with a radiopaque iodine contrast.[12] Boluses were tested while the patient was seated in a lateral projection. Boluses were prepared with water, X-ray contrast solution (Omnipaque™, GE Healthcare), and the required amount of thickener (g) to achieve each viscosity level (mPa s). In our research group, volumes of 5, 10, and 20mL are routinely used in the clinical practice to test the swallowing ability of the patient in an effort test.[12,22] As not all the patients are capable of swallowing the maximum volume (20mL), 10mL was chosen as an optimal comfortable bolus for the patient to swallow in this study. The oral cavity, pharynx, larynx, and cervical esophagus were recorded on video during swallowing. VFSS recordings were obtained using a Super XT-20 Toshiba Intensifier (Toshiba Medical Systems Europe) and recorded at 25 frames/s using a Canon DM-XM2 E video camera (Canon Inc.). The VFSS recordings were analyzed and the measurements obtained using specialized software (Swallowing Observer; Image & Physiology SL) by an expert blinded clinician.[14] VFSS signs. Safety of swallow was assessed by the identification of the PAS score and the prevalence of safe swallows (PAS 1,2), penetrations (PAS 3,4,5), or aspirations (PAS 6,7,8) of each bolus.[21] We considered the following signs as indication of impaired efficacy: piecemeal deglutition, oral, pharyngeal wall, and vallecular or pyriform sinus residue. The prevalence of residue was described as the presence or absence of residue in the oral cavity or the pharynx including the pharyngeal wall, the vallecula, and pyriform sinus.[27] Timing of oropharyngeal swallow response (OSR) and bolus kinematics. Timing of swallow response was assessed for each bolus given to the patient during VFSS.[4,27] We measured the time to LVC (time from the glossopalatal junction (GPJ) opening to the LVC) and the total duration of the swallow response. In addition, mean bolus velocity of the bolus between the GPJ and the upper esophageal sphincter (UES), propulsion forces, and kinetic energy were calculated as published before by our group.[4]

2.4.2 | Bolus rheology

The viscosity levels used in the study were selected according to the descriptors of the National Dysphagia Diet Task Force: 1-50 mPa s

for thin liquid, 51-350 mPa s for nectar, 351-1750 mPa s for honey, and >1750 mPa s for pudding viscosities at 25°C and 50 seconds^{-1}.[28] For VFSS, we prepared a total of seven different viscosities in 10 mL bolus, consisting of liquid X-ray contrast as control vs six thickened X-ray contrasts thickened with Nutilis Clear®—consisting of maltodextrin, xanthan gum, and guar gum (Nutricia N.V., Zoetermeer, The Netherlands)—at each viscosity level. To achieve those viscosities, varying amounts of thickener were added to 50 mL solution composed of 1:1 mineral water and the iodine X-ray contrast: 150 and 250 mPa s viscosities were obtained by adding 0.56 g and 0.75 g, respectively; 450, 800, and 1400 mPa s viscosities were obtained by adding 1.27 g, 2.08 g, and 3.81 g, respectively; and 2000 mPa s was obtained with 5.01 g.

2.4.3 | Comfortability

During the VFSS, patients were asked whether they felt comfortable during the swallowing experience ("*I felt comfortable during swallowing this product*") using a 9-point Likert scale, at each viscosity level. Results are presented by using three categories: (a) strongly agree, agree, and moderately agree; (b) mildly agree, undecided, and mildly disagree; and (c) moderately disagree, disagree, and strongly disagree.

2.4.4 | Safety of the product

All adverse events (AEs) occurring during the study and one week after the procedure (follow-up telephone call) were recorded and assessed for relationship with the study product according to the guideline of categories described by the World Health Organization and the Uppsala Monitoring Centre (WHO–UMC).[29]

2.4.5 | Data analysis and statistical methods

Binary data were described as relative and absolute frequencies, and the viscosity levels were compared with thin liquid by applying the McNemar's test. For ordinal data, the comparisons were done by applying the Bhapkar's test; in case of zero counts, the McNemar's test on aggregated categories was used. Continuous data are presented as mean ± standard deviation (SD), and comparisons were done by applying a repeated measure mixed model including all six viscosities or a paired-sample Wilcoxon signed rank test in case assumptions were not met. The McNemar's test, Bhapkar's test, paired-sample Wilcoxon signed rank test, and repeated mixed model all take into account the within-subject design and paired data. The statistical analysis was performed with SAS® software for Windows, SAS Institute Inc. (SAS version 9.4_M1).

Safety of swallow of each patient at a particular viscosity level was expressed as the worst PAS score of the duplicates, and all the parameters of that replicate were analyzed according to the scheme in Figure S1. Data on safety of swallowing were handled as binary by dividing the patients in two categories: patients who can swallow safely (PAS 1-2) vs patients who cannot swallow safely (PAS 3-8) over the "per protocol" population. The efficacy of swallowing was also handled as binary data (presence or absence): if residue was observed at any of the three pharyngeal locations (pharyngeal wall, vallecular, and pyriform sinus), the residue was present (yes); if no residue was observed at any of the locations, the residue was absent (no); and if at least one was missing (not performed due to the safety rule) and the others were absent, the residue was handled as missing. Data of the duplicates for residue were handled according to the algorithm shown in Figure S1. For efficacy of swallow, an additional procedure for handling duplicates was used to explore the "worst case" scenario. This selection was independent of PAS score, and the replicate was selected based on the worst value for the presence of pharyngeal or oral residue.

Statistical tests were conducted two-sided with a significance level of 5%. All confidence intervals are presented two-sided with a confidence level of 95%. A resultant probability value of $P < .05$ was judged as statistically significant. For the primary outcome parameter, percentage of patients that swallow safely, the null-hypothesis of no effect on safe swallowing of 2000, 800, and 250 mPa s compared with liquid will be rejected if all three (two-sided) P values are <.05 with correct directional decisions. An additional explorative analysis was performed on safety of swallowing and the mean PAS scores to evaluate the therapeutic effects between viscosities. As a post hoc test, the bolus propulsion force was analyzed, and dose-response curves for the viscosity-dependent effect of the thickening agent on safety and efficacy were obtained by representing the prevalence of patients with safe swallowing and those with residue respectively at each level of viscosity using Graphpad Prism 6.

3 | RESULTS

3.1 | Sample demographics

Of the 120 patients enrolled, 4 were excluded from the all subjects treated (AST) population because they did not receive any of the thickened viscosities. Additionally, two patients were excluded from the per protocol population (PP) because they discontinued due to reasons other than aspiration which was regarded as a protocol deviation. The originally planned analysis was on the intention-to-treat population (ITT). However, because there were 4 patients in this population who did not receive any of the thickened product, it was decided to present the results for the PP population (n = 114) (Figure S2). The results of the ITT and PP populations were comparable. The majority of our population, 76% (N = 87) were in the subacute phase (28-180 days after stroke) and 24% (N = 27) were chronic (>180 days after stroke). Mean age of the participants was 76.7 ± 8.9 years, and 54.4% were men. The MNA-SF total score indicated that 54.4% of patients were malnourished or at risk of malnutrition when enrolled in the study. Stroke type was predominantly ischemic 78.1% (n = 89), and the prevalent severity of the stroke valued with the NIHSS was scored (mean ± SD) 7.5 ± 6.8 on admission and 5.3 ± 5.9 on discharge. More details of the epidemiological and clinical characteristics of the population are provided in Table S1.

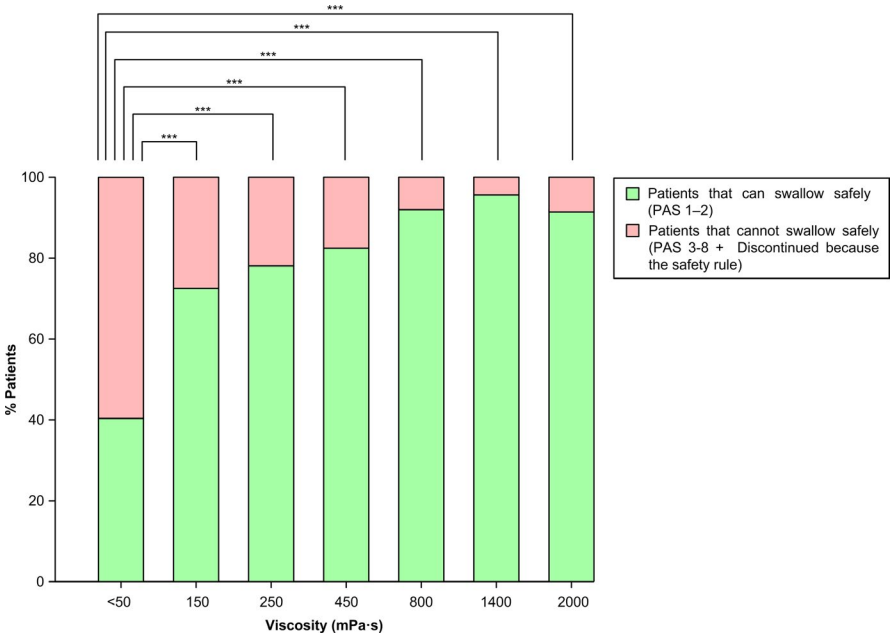

FIGURE 2 Percentage of PSOD patients with safe/unsafe swallow at each level of viscosity. "N" represents the number of patients who performed the bolus out of the PP population (114). The percentage of patients with unsafe swallow includes those with aspirations at the former viscosity who discontinued due to the safety rule. Percentage of patients who discontinued at each viscosity: thin liquid (0.0%), 150 mPa s (12.3%), 250 mPa s (8.8%), 450 mPa s (4.4%), 800 mPa s (1.8%), 1400 (1.8%), 2000 mPa s (0.9%). *P < .05; **P < .01; ***P < .001 vs thin liquid

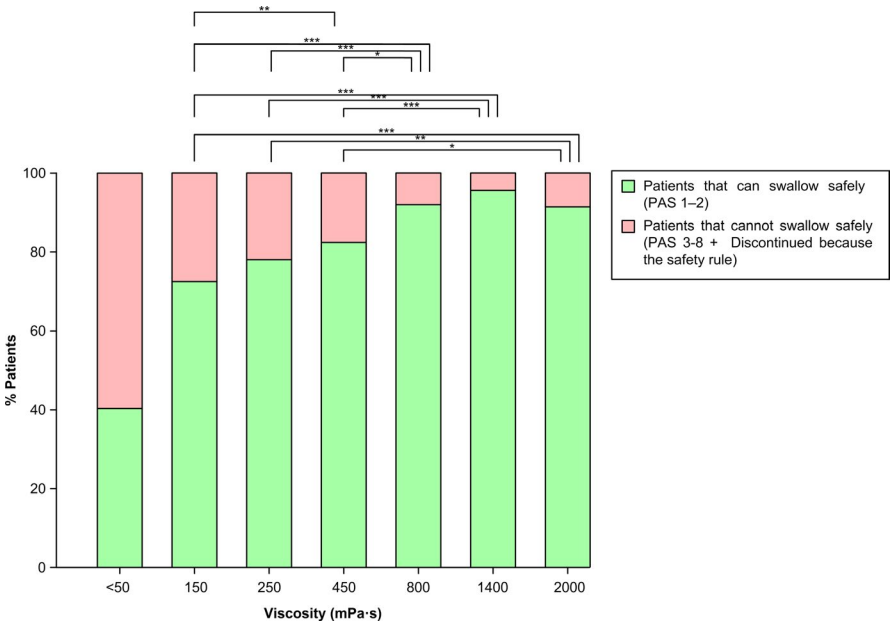

FIGURE 3 Percentage of PSOD patients with safe/unsafe swallow compared between levels of viscosity. Data of patients who discontinued due to the safety rule were imputed with the last observation carried forward. Values are presented for the PP population (114).*P < .05; **P < .01; ***P < .001

3.2 | Effect of range of viscosities on prevalence of VFSS signs of OD

3.2.1 | Safety of swallow

Primary parameter

Safe swallowing was observed in only 41.2% (n = 47) of the patients at thin liquid but the percentage significantly increased with the main viscosities (all P < .001 vs thin liquid) (Figure 2). Similarly, safety of swallowing significantly increased with the explorative viscosities compared with thin liquid (all P < .001 vs thin liquid) (Figure 2).

Mean PAS score at thin liquid was 3.7 ± 2.3, and it significantly decreased to 1.9 ± 1.4, 1.8 ± 1.6, 1.7 ± 1.6, 1.4 ± 1.2, 1.2 ± 0.6, and 1.4 ± 1.2 by increasing viscosity from 150 to 2000 mPa s (all P < .001 vs thin liquid). The distribution of safe swallowing, penetration, and aspiration was significantly different at all viscosities compared with thin liquid (all P < .001 vs thin liquid). The percentage of patients with penetration and aspiration decreased when viscosity increased (Figure S3). The prevalence of patients with penetrations at thin liquid was 41.2% and ranged between 2.6% and 13.2% for the thickened viscosities. The prevalence of patients with aspirations showed significant differences (P < .01) with thin liquid (17.5%) vs all viscosities (0.0%-4.4%) except for 150 mPa s (2.5%, P = .180).

Figure 3 shows the explorative analysis of the between viscosity comparisons. Among the different viscosity levels, there were

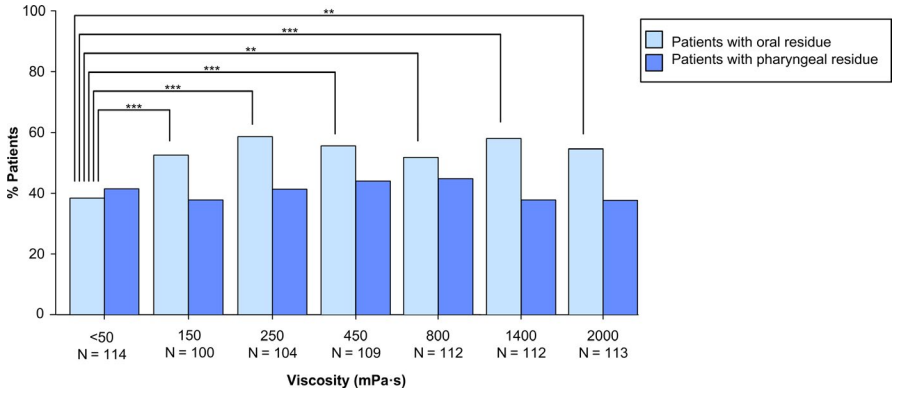

FIGURE 4 Percentage of patients with PSOD of the PP population (114) with oral and pharyngeal residue at each viscosity level. "N" represents the population who performed the bolus. *P < .05; **P < .01; ***P < .001 vs thin liquid

significant differences between the therapeutic effect of 250 mPa s (78.9%) vs 800 (92.1%), 1400 (95.6%), and 2000 mPa s (91.2%) (all P < .01 vs 250 mPa s), but not between 800 and 2000 mPa s or between 800 and 1400 (P > .05). The maximal therapeutic effect (ceiling effect) was observed at 800 mPa s (92.1% of patients with safe swallowing).

3.2.2 | Efficacy of swallow

At thin liquid, pharyngeal residue was present in 41.2% (n = 47) of patients and it did not increase at any of the tested viscosities (37.7%-44.7%, all P > .05 vs thin liquid) (Figure 4). Oral residue was present in 38.6% (n = 44) at thin liquid and significantly increased at all thickened viscosities (all P < .01 vs thin liquid) (Figure 4). Selecting the duplicate with the "worst case" scenario resulted in comparable results (not shown).

3.2.3 | Dose-response effect of range of viscosities on safety of swallowing and pharyngeal and oral residue

Figure 5 shows the viscosity-dependent therapeutic effect on safety of swallowing for the tested viscosities. 150, 250, and 450 mPa s offered a protection on safety of swallowing between 71.9% and 82.5% and 800, 1400, and 2000 mPa s a protection between 91.2% and 95.6%. Safety increased in a viscosity-dependent manner. Pharyngeal residue was not statistically different compared with thin liquid at any of the tested viscosities. Oral residue slightly, but significantly, increased at all viscosities.

3.3 | Effect of range of viscosities on oropharyngeal swallow response (OSR)

3.3.1 | Timing of OSR

Time to laryngeal vestibule closure (LVC)

Time to LVC at liquid viscosity was severely delayed (382.5 ± 139.1 ms) in patients with PSOD. Increasing bolus viscosity ≥150 mPa s shortened time to LVC for all viscosities (Figure 6): mean LVC for each viscosity was 327.3 ± 108.2 (150 mPa s), 330.1 ± 143.4

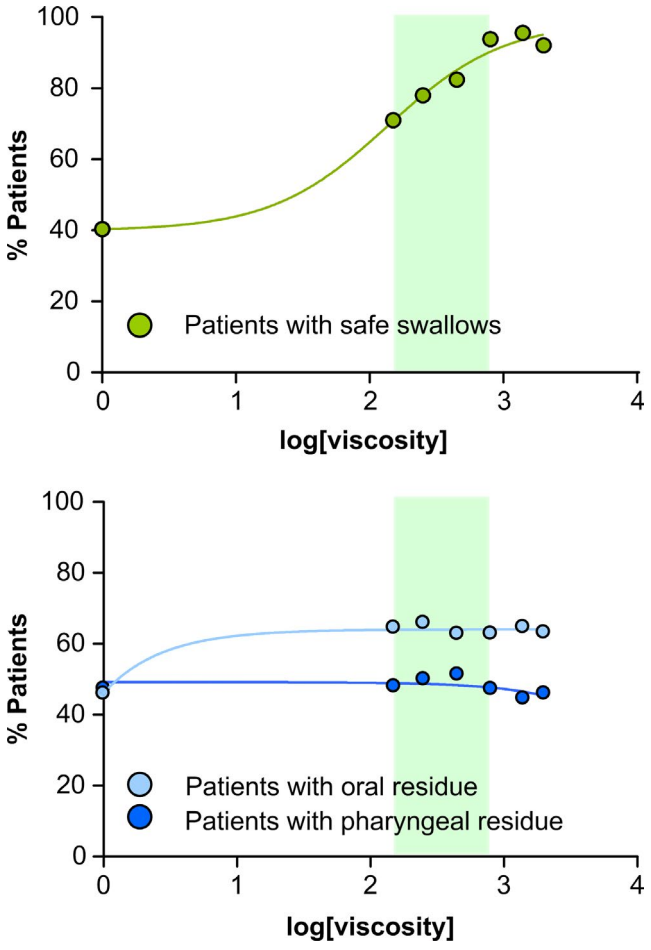

FIGURE 5 Dose-response curves for the therapeutic effect of the gum-based thickener on safety and efficacy of swallowing in patients with PSOD. The upper panel shows the curve of the viscosity-dependent response represented by the percentage of patients with safe swallows vs the log of the viscosity. The lower panel shows the curve representing the effects on the prevalence of oral and pharyngeal residue vs the log of the viscosity. The shadowed area represents the therapeutic range (150-800 mPa s) of the product

(250 mPa s), 304.8 ± 109.6 (450 mPa s), 303.3 ± 94.7 (800 mPa s), 300.5 ± 110 (1400 mPa s), and 300.4 ± 107.8 (2000 mPa s) ms (P < .01 vs liquid). Time to LVC was shorter in patients with safe

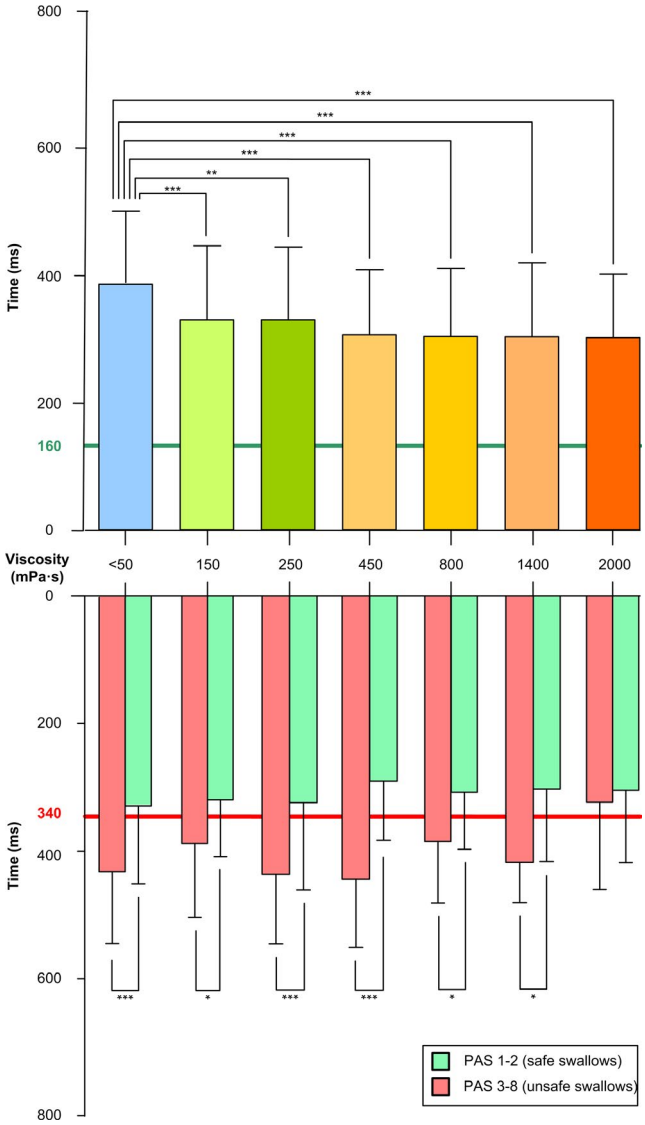

FIGURE 6 Time to LVC at each viscosity level. The upper panel shows mean time to LVC at each viscosity. The lower panel shows time to LVC plotted against safe/unsafe swallow at each viscosity level. Time to LVC was delayed in patients with unsafe swallowing at all viscosity levels except for 2000 mPa s. Time to LVC <160 ms (green line): safe swallowing as established in a study with healthy volunteers.[4] Time to LVC ≥340 ms (red line): cutoff time to detect the presence of unsafe swallowing in poststroke patients according to previous studies.[12] *P < .05; **P < .01; ***P < .001

(PAS 1-2) vs unsafe swallow (PAS 3-8): significant differences were detected in all viscosities except for 2000 mPa s (Figure 6).

At thin liquid, the total duration of the swallow response was 1020.9 ± 220.8 ms and significantly decreased to 947.1 ± 228.7, 998.8 ± 472.1, 944.1 ± 180.2, 943.1 ± 221.4, 953.5 ± 225.3, and 943.2 ± 234.8 ms at 150, 250, 450, 800, 1400, and 2000 mPa s, respectively (all P < .01 vs thin liquid).

3.3.2 | Bolus kinematics

Mean bolus velocity

Poststroke patients included in the study presented a mean bolus velocity at liquid of 0.3138 ± 0.1265 (m/s). Increasing bolus viscosity, ≥450 mPa s, caused a significant reduction in bolus velocity for 450 mPa s (0.2835 ± 0.0948; P < .05), 800 mPa s (0.2613 ± 0.0784; P < .001), 1400 mPa s (0.2564 ± 0.0803; P < .001), and 2000 mPa s (0.2729 ± 0.1010; P < .01) vs thin liquid (Figure 7).

Bolus propulsion forces

Mean bolus propulsion force was 0.041 ± 0.035 mN at thin liquid. A significant decrease was found at the thickened viscosities (all P < .001 vs thin liquid): 150 mPa s (0.033 ± 0.025), 250 mPa s (0.035 ± 0.032), 450 mPa s (0.030 ± 0.019), 800 mPa s (0.026 ± 0.014), 1400 mPa s (0.025 ± 0.015), and 2000 mPa s (0.028 ± 0.022).

3.4 | Comfortability

Comfortability while swallowing scored highest at thin liquid (66.3%), and it decreased significantly to 46.3% and 31.3% during swallowing the main viscosities 800 and 2000 mPa s, respectively (Figure 8). Categories of comfortability were differently distributed at all viscosities compared with thin liquid (all P < .001 vs thin liquid), except for 150 and 250 mPa s (Figure 8).

3.5 | Safety of the product. Adverse events (AEs)

A total of 16 adverse events occurred in 11 patients out of the 116 in the AST population and were considered unrelated or unlikely to be related to the study product. The most frequent AEs were mild gastrointestinal disorders (14 AEs in 10 patients): diarrhea, nausea, abdominal distension and pain, dyspepsia, and stomatitis. No serious AEs were reported during or following the study.

4 | DISCUSSION

The main result of this study is that increasing bolus viscosity with the xanthan gum-based thickener Nutilis Clear® significantly increased the safety of swallow in patients with PSOD in a viscosity-dependent manner. The study also shows that these patients presented a pattern of OD with highly prevalent and severe signs of impaired safety and efficacy of swallow, aspirations and oropharyngeal residue, a severely impaired swallow response, and a high prevalence of malnutrition or risk of malnutrition. Together these characteristics place these patients at high risk for severe nutritional and respiratory complications. Impaired safety of swallow in these patients with PSOD is associated with a severe delay in time to LVC. An unexpected, but very relevant, result of this study was that increasing viscosity with this gum-based thickening agent significantly improved airway protection mechanisms by reducing time to LVC. Increasing bolus viscosity also caused a slight, but significant, increase in oral residue and decreased tongue propulsion forces, and decreased bolus velocity at high viscosity levels without any significant effect on pharyngeal residue. Finally, the study shows that the gum-based thickener is safe and well tolerated in patients with PSOD as reflected by the low number of AEs.

The chronic PSOD population is a phenotype of patients with OD that is growing in Europe, due to the increasing incidence of stroke events (from 1.1 million per year in 2000 to an estimated 1.5 million per year in 2025,[30] the progressive increase in the prevalence of stroke survivors, and the high prevalence of OD among these patients (50%-81%),[5] even among those with mild strokes (45%).[3] We and others have found that mild stroke survivors are at high risk of malnutrition [3,31] and that aspiration pneumonia is the main cause of 1-year mortality among them.[6] The main result of our study is the viscosity-dependent effect on safety of swallow with this xanthan gum-based thickening agent in these patients with PSOD allowing safe deglutition in almost all these poststroke survivors with OD. The therapeutic range of this thickening agent in this phenotype of patients is 150-800 mPa s, as 150 mPa s was the lowest viscosity to have a significant effect on the safety of swallowing and 800, 1400, and 2000 mPa s showed a similar level of protection. Aspiration is the most severe impairment in swallowing safety. For this parameter, the minimal viscosity with a significant effect was 250 mPa s, which suggests a therapeutic range starting at 250 mPa s. However, current results on aspiration can be considered inconclusive to establish the lower level of the therapeutic range because the study was not powered for this parameter. Low sample size, which was partly driven by the safety rule, might have prevented us from finding significant effects on aspiration at the lowest tested viscosity, that is, 150 mPa s., which was proven effective with regard to safe swallowing. For the main viscosities tested, significant differences in the therapeutic effect on safety of swallow vs liquid were found, and increasing bolus viscosity above 800 mPa s did not cause any further significant increase in the safety of swallow in this phenotype of patients. As far as we know, this is the first study to assess the effect of seven different viscosities in patients with PSOD. Our results suggest that, using this specific thickening agent, healthcare providers can cover the therapeutic needs of this phenotype of dysphagic patients by using a viscosity between 150 and 800 mPa s.

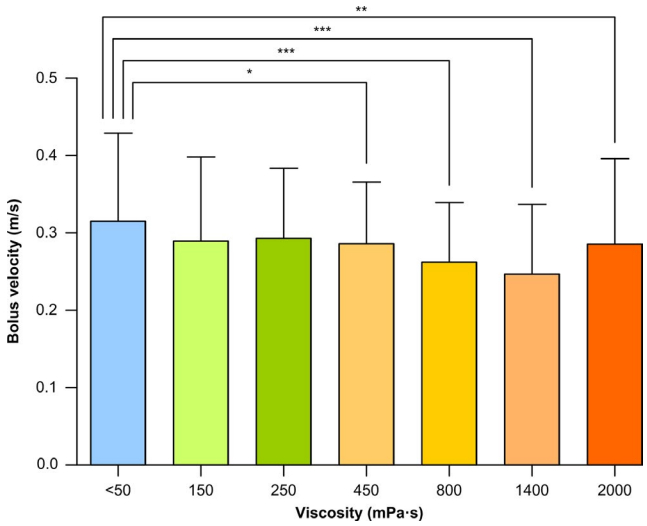

FIGURE 7 Mean bolus velocity from GPJO to UESO at each viscosity level. Bolus velocity was reduced above 450 mPa s. *P < .05; **P < .01; ***P < .001 vs thin liquid

A major question that arises from these results is how to prescribe the optimal viscosity level of this thickening agent to PSOD. Firstly, these products should be labeled appropriately to promote their safe use.[32] Secondly, accurate clinical methods should be used to diagnose OD and to prescribe which viscosity is the most appropriate for each patient with PSOD, as not all these patients can be assessed by instrumental exploration.[34] Multiple consistency methods for clinical diagnosis of poststroke OD—such as GUSS and the V-VST—have been recently recommended in PSOD in a guideline developed by the ESO and the ESSD and can be adapted to these viscosities.[33] For the V-VST—that uses only two levels of thickened viscosity—250 and 800 mPa s can be considered as the most appropriate; 800 mPa s as the viscosity providing the maximal significant therapeutic effect for this thickener; and 250 mPa s for patients with

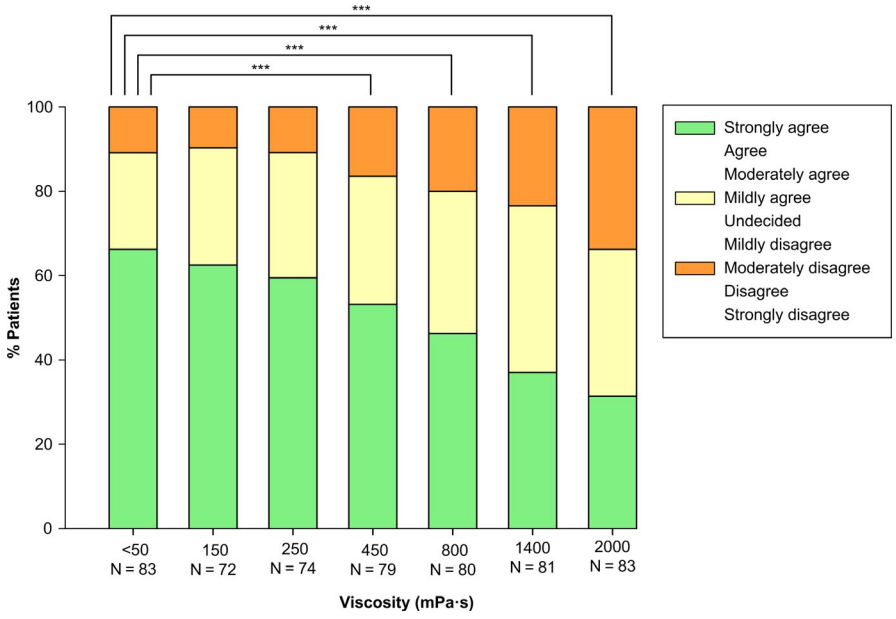

FIGURE 8 Comfortability while swallowing the product. The comfortability while swallowing the product at each viscosity level was evaluated by using a 9-point Likert scale to the following sentence: "I felt comfortable while swallowing this product." Likert scale score is divided into three categories for each viscosity. For the statistical analysis, these three catergories and the category of missing values were used. "N" represents the population who answered the question, the category of missing values is not shown in the figure. *P < .05; **P < .01; ***P < .001 vs thin liquid

less severe safety impairment as a safe and comfortable intermediate value providing a significant therapeutic effect vs thin liquid and vs 800 mPa s.[22,24]

Thickeners are widely used in poststroke OD as a compensatory therapeutic strategy to avoid aspiration. In a previous study on similar patients with PSOD, it was found that thickening liquids with either modified starch (MS) or xanthan gum-based (XG) thickeners had a strong therapeutic effect on safety of swallow.[20] The prevalence of safe swallow using MS and XG thickeners increased with bolus viscosity reaching up to 89%-92% of patients with PSOD at higher viscosity levels (4000 mPa s for MS and 1700 mPa s for XG), above those used in the present study. In this previous study, the MS thickener strongly increased pharyngeal residues, whereas the XG increased oral residue at 1700 mPa s but did not increase pharyngeal residue at any viscosity. Timing of airway protection mechanisms (LVC) and bolus velocity were not affected by either of the thickener agents.[20] This was one of the first studies showing an advantage for XG thickeners over MS in PSOD, due to its strong therapeutic effect on safety, low pharyngeal residue, and amylase resistance. The present study is a step forward as the therapeutic effect on safety of swallow is also very high (92.1% for 800 mPa s) and is achieved at lower viscosity levels, the absence of pharyngeal residue is similar, Nutilis Clear® is unaffected by amylase, and—a new finding—increasing viscosity with this thickener causes a significant reduction of time to LVC over thin liquid. Videofluoroscopic studies have shown that the time to LVC is a critical event in the occurrence of penetrations and aspirations, causing unsafe deglutition, and time to LVC ≥340 ms predicts unsafe swallow in chronic PSOD patients.[20] Such a delay in time to LVC in PSOD associated with impaired safety of swallow was also observed in this study, almost doubling the time to LVC of healthy people,[4] and was slightly above that previously described in comparable patients.[12] Reduced pharyngeal sensitivity and impaired conduction and cortical integration of pharyngeal sensory inputs at the stroke site is a key feature of chronic PSOD and has been closely associated with impaired safety of swallow and delayed time to LVC.[13] In fact, sensory feedback from the bolus is critical to tailor the motor component of the swallow response. Therefore, the reduction in time to LVC caused by the thickening agent suggests a mode of action beyond a simple "compensatory" effect.[12,13] Another relevant result of the study is that increasing viscosity—which is a measure of the fluid resistance to bolus flow—reduces bolus propulsion force and bolus velocity at viscosities greater than 450 mPa s. This effect might explain the slight, but significant, increase in oral residue as tongue strength is reduced in these patients.[12] This result agrees with a previous study from our group which concluded that impaired safety of swallow in chronic poststroke patients was caused by specific impairments in swallow response such as a delay in the airway protection mechanisms and weak tongue propulsion force.[12] Those results led to a claim that treatments for these patients should be targeted to improve these critical biomechanical events (delay in LVC and reduce tongue strength). We recently studied the natural history of swallow function during the 3-month period after stroke and found 26% of poststroke patients developed new signs/symptoms of ineffective swallow related to poor functional, nutritional, and health status and institutionalization.[35] Another study on stroke patients concluded that tongue weakness was also caused by reduce muscle mass of swallow muscles and poststroke sarcopenia.[36] Our present results of a reduced bolus propulsion force with the higher viscosities further suggest that stroke patients also need specific nutritional and rehabilitation procedures to increase bolus propulsion forces and tongue strength by fighting poststroke sarcopenia. Interestingly, pharyngeal residue, more related to pharyngeal clearance caused by pharyngeal constrictors, was unaffected by increasing viscosity.[35]

In the present study, increasing shear viscosity was obtained by adding increasing amounts (grams) of the gum-based thickener to a mixture of water and contrast agent. The obtained shear viscosity is the independent variable for this study. Besides shear viscosity, other rheological proprieties such as elasticity, adhesiveness, and cohesiveness and different extensional viscoelastic behaviors also may play a role in swallow physiology.[18] The assessment of the effect of extensional flows on viscosity of thickening agents is now under development, and the potential influence of these rheological properties on swallow safety and efficacy in patients with OD is still unknown.

Our study has some limitations. The first one arises from its experimental design as we included a pass/fail safety rule to protect patients from dangerous and unnecessary repeated aspirations. Due to our design, not all patients received all the viscosities, especially the lowest levels. This is a quite common situation in pharmacologic/physiologic studies, to minimize the possibility of serious AEs to patients, for example, during progressive effort tests.[27] A similar "safety rule" was used in all our previous studies with thickening agents as requested by the Ethical Committee.[16,25] Because it is clinically relevant information, in-between viscosity comparisons were performed by imputing the data of the missing values from the safety rule by carrying the last observation forward. As a consequence of the design of the study, care should be taken interpreting these results. However, this design and our interpretation is the safest from the patient's perspective. Another limitation is that the transversal design of the study does not provide information on longer term clinical outcome, for instance whether the observed improved safety of swallowing with the thickener agent results in fewer respiratory infections. Future longitudinal randomized clinical trials should be performed to confirm the translation of the strong therapeutic effect of the gum-based thickener on swallowing safety into clinical outcomes including incidence of nutritional and respiratory complications.[3] Nutritional support and oral care must also be included in these protocols.

In summary, this study shows that increasing bolus viscosity with Nutilis Clear® causes a strong viscosity-dependent effect on safety of swallow in PSOD without increasing pharyngeal residue. Our study suggests that the therapeutic effect of the thickener might be caused by specific effects on oropharyngeal physiology (mainly time to LVC and bolus velocity). To optimize this strong therapeutic effect, clinicians must provide early diagnosis of PSOD and the prescription of the required appropriate viscosity by multiconsistency clinical and/or instrumental methods. This might be appropriate to reduce nutritional

and respiratory complications and improve the prognosis of patients with PSOD. We believe these findings will have implications for current clinical practice. Our study clearly shows that the therapeutic effect of thickening agents depends on shear viscosity levels, with a therapeutic range of 150-800 mPa s for this xanthan gum-based thickener multiple consistency methods for clinical diagnosis, and management of poststroke OD can be adapted to this viscosity range for this specific phenotype of patients with OD. This information will improve clinical practice by providing the specific levels of viscosity to cover the therapeutic needs of this phenotype of dysphagic patients. Fluid thickening must be integrated into compensatory multimodal treatments, such as the minimal-massive intervention [37] or neurorehabilitation approaches, aiming to restore swallow function.[38]

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CONFLICT OF INTEREST

Declaration of personal interests: Clavé has served as consultant and received research funding from Danone Nutricia Research. Guida, Ripken, and Lansink are employees of Danone Nutricia Research.

AUTHOR CONTRIBUTION

Clavé is a submission's guarantor. Clavé, Rofes, Vilardell, and Lansink involved in study concept and design. Arreola, Rofes, Martín, Nascimento, Ortega, and Bolívar-Prados involved in selection of patients. Rofes, Vilardell, and Bolívar-Prados involved in acquisition of data. Clavé, Guida, Ripken, and Bolívar-Prados involved in statistical planning and analysis. Clavé, Guida, Ripken, Lansink, and Bolívar-Prados involved in analysis and interpretation of data. Clavé, Guida, and Bolívar-Prados involved in drafting of the manuscript. Clavé and Bolívar-Prados involved in revision of bibliography. All authors approved the final version of the manuscript, including the authorship list.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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10.2.1 Article 2

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Therapeutic Effect, Rheological Properties and α-Amylase Resistance of a New Mixed Starch and Xanthan Gum Thickener on Four Different Phenotypes of Patients with Oropharyngeal Dysphagia

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Abstract: Thickened fluids are a therapeutic strategy for oropharyngeal dysphagia (OD). However, its therapeutic effect among different phenotypes of OD patients has not yet been compared. We aimed to assess the therapeutic effect and α-amylase resistance of a mixed gum/starch thickener [Fresubin Clear Thickener® (FCT)] on four phenotypes of OD patients: G1) 36 older; G2) 31 head/neck cancer (HNC); G3) 30 Parkinson's disease; and G4) 31 chronic post-stroke. Therapeutic effect of FCT was assessed during videofluoroscopy using the Penetration-Aspiration Scale (PAS), for 5/20 mL boluses, at four levels of shear-viscosity (<50, 250, 1000 and 2000 mPa·s). The effect of α-amylase was assessed after 30 s of oral incubation. Patients had high prevalence of VFS signs of impaired efficacy (98.44%) and safety (70.31%) of swallow with a severe PAS score (4.44 ± 0.20). Most severe OD was in HNC (80.6% unsafe swallows). FCT showed a strong therapeutic effect on the safety of swallow at a range between 250–1000 mPa·s (74.19–96.67%, safe swallows in G1, G3, G4, and 58.06% in G2), without increasing pharyngeal residue. Viscosity was unaffected by α-amylase. Increasing shear-viscosity with FCT causes a strong viscosity-dependent therapeutic effect on the safety of swallow. This effect depends on the phenotype and is similar among older, Parkinson's and post-stroke patients.

Keywords: viscosity; rheology; amylase resistance; aspirations

1. Introduction

Oropharyngeal dysphagia (OD) is a deglutition disorder, which has been classified in the last editions of the International Classification of Diseases ICD-9 and ICD-10 (787.2, R13) of the World Health Organization [1]. Prevalence is increasing with the aging of the population and it is a common condition among four main phenotypes of patients: older patients, patients treated for head and neck cancer and patients with neurological and neurodegenerative diseases [2]. OD has been recently recognized as a geriatric syndrome by two European Societies [3] and affects up to 27% of independently

living and 51% of institutionalized older people [4,5] (1;2), 38.5% of patients with head and neck cancer [6], over 40% of post-stroke patients [7–9] and 82% in Parkinson's disease [10]. OD causes two main groups of complications: impaired efficacy of swallow, which can lead to malnutrition and dehydration, and impaired safety of swallow with bolus penetrations into the laryngeal vestibule and tracheobronchial aspirations, leading to aspiration pneumonia and high mortality rates [11,12]. Videofluoroscopy (VFS) is the gold standard method to diagnose the biomechanical alterations of the oropharyngeal swallow response (OSR) [13]. It consists of a dynamic radiological exploration that evaluates the safety and efficacy of deglutition, characterizes the major signs of oropharyngeal dysfunction, quantifies the OSR and assesses the short-term effect of therapeutic strategies on patients with OD [13–15]. Delayed laryngeal vestibule (LV) closure has been recognized as the main mechanism of impaired airway protection in patients with OD leading to unsafe swallow [11].

Treatment of OD is currently mainly compensatory with fluid adaptation (volume and viscosity with thickening agents), texture-modified foods and the use of postures and maneuvers [16,17]. The use of thickening agents aims at maintaining the hydration status of patients with dysphagia, although evidence on the therapeutic effect of these compounds is weak [17]. The white paper published in 2016 by the European Society for Swallowing Disorders (ESSD) concluded that "there is evidence for increasing viscosity to reduce the risk of airway invasion and that it is a valid management strategy for OD. However, new thickening agents should be developed to avoid the negative effects of increasing viscosity on residue, palatability, and treatment compliance. New controlled trials should establish the optimal viscosity level for each phenotype of dysphagic patients and descriptors, terminology and viscosity measurements must be standardized" [16]. The thickeners' composition is progressively changing from starches to gums. It is well known that standard starch-based thickeners greatly increase post-deglutitive oropharyngeal residue, especially in patients with deficient bolus propulsion such as older patients [11,16], increasing the risk of post-swallow aspirations [18]. Another disadvantage of starch-based thickeners is their poor acceptance by patients [19] causing a low compliance on prescriptions and treatments [20]. Moreover, in less than 30 s in the oral phase of swallow, the viscosity of starch-based thickeners can be dramatically reduced by oral salivary α-amylase as this breaks down starch molecules [21], reducing its therapeutic effect [22,23]. A new generation of thickening agents, such as xanthan gum or mixtures of gums and α-amylase-resistant modified starches, has shown better therapeutic properties for these patients [24–27] than the starch-based thickeners. The first generation of thickening agents marketed in EU was made of starch or starch derivatives (maltodextrins). These initial products offered a high protection but also a high oral and pharyngeal residue. New generation of thickening agents are composed by xanthan gum, which provide better properties on the safety, efficacy and a higher resistance to amylase. Differences on these groups of thickening agents are due to the mechanism of action: gum-based thickeners form hydrocolloids with water and remain stable on time, starch-based thickeners absorb water and swell, leading [26] to an inconstant viscosity behaviour on time and a strong affection of salivary amylase which hydrolyses the O-glycoside bonds of starch chains. Another important property that can affect safety of thickening agents is the shear-thinning behaviour, defined as a reduction of apparent viscosity under shear strain caused by bolus velocity in non-Newtonian fluids [28]. Bolus velocity through the gastrointestinal tract is maximal in the pharynx and can reach up to 30 cm/s [29]. It has been shown that bolus flow in the oral cavity occurs at shear rates of 50 s^{-1} approximately. However, during swallowing, the shear rate in the mesopharynx is over 300 s^{-1} and it can reach up to 900 s^{-1} in the hypopharynx [21,29].

Our aim was to assess and compare the therapeutic effect of a new thickening agent (Fresubin Clear Thickener® [FCT], Fresenius Kabi), formulated with xanthan gum and modified starch, on the safety and efficacy of swallow plus kinematics of the swallow response. This involved four groups of patients with OD from several etiologies (aging, head and neck cancer, stroke and Parkinson's disease). Moreover, we studied bolus flow resistance to α-salivary amylase and the shear-thinning behaviour of simulated boluses at three different levels of shear-viscosity (250 mPa·s, 1000 mPa·s and 2000 mPa·s, at 50 s^{-1}).

2. Materials and Methods

2.1. Study Design

This was a prospective, single-center clinical study on patients with OD caused by the four main etiologies (aging, head and neck cancer, stroke and Parkinson's disease) to evaluate and compare the therapeutic effect and rheological characteristics of the thickening agent Fresubin Clear Thickener® [FCT], (Fresenius Kabi Deutschland GmbH, Bad Homburg, Deutschland). First, patients were clinically assessed for OD with the Volume-Viscosity Swallowing Test (V-VST) [30] and if positive (with clinical signs of impaired efficacy and/or safety of swallow) they underwent VFS using four different viscosities (liquid <50, 250, 1000 and 2000 mPa·s) and 2 volumes (5 mL and 20 mL) for each viscosity to evaluate the therapeutic effect of the thickener and the kinematics of the swallow response. The general study design is presented in Figure 1. We also designed an in vitro study to assess the effect of α-amylase during the oral phase and the effect of shear-thinning during the oral and pharyngeal phase of this new thickener.

The following data were also collected: sociodemographic characteristics of the population, functional capacity according to the Barthel Index [31], force with a hand dynamometer (Takei Analogue Dynamometer 5001, Takei Scientific Instruments, Japan), quality of life according to the EUROQoL-5D [32] and nutritional status according to body mass index (BMI) and the short form of the Mini Nutritional Assessment (MNAsf) [33].

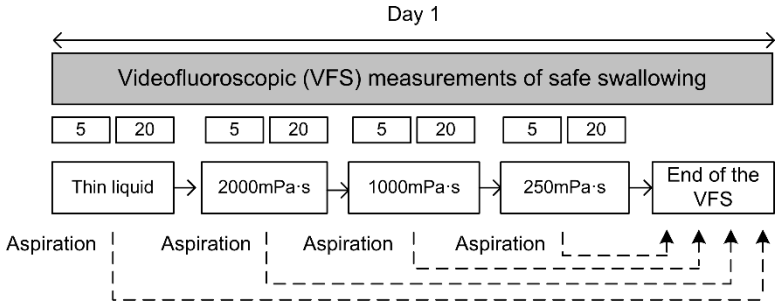

Figure 1. Study design.

2.2. Study Population

We studied 128 patients with OD divided in four groups according to OD etiology: a) 36 older patients, b) 31 patients following treatments for head and neck cancer (HNC), c) 31 post-stroke patients, and d) 30 Parkinson's disease patients (Figure 2). Study population was prospectively recruited from the Gastrointestinal Physiology Unit of the Hospital de Mataró (Spain) between February 2016 and February 2018. Inclusion criteria were: patients over 18 years old, presenting OD according to the V-VST caused by any of the following etiologies: aging (>70 years), following treatment for head and neck cancer, stroke and Parkinson's disease. Exclusion criteria were: pregnancy or lactation, unstable cardiopulmonary status, unstable medical conditions or major respiratory disease needing oxygen therapy. All of the participants were informed about the study and signed the informed consent form. Study protocol was approved by the ethical committee of the Hospital de Mataró with code 57/15 and was conducted according to the principles and rules laid down in the Declaration of Helsinki and its subsequent amendments and following the EU rules for clinical trials on humans (EU Clinical Trial Regulation (EU-CTR, EU No 536/2014)).

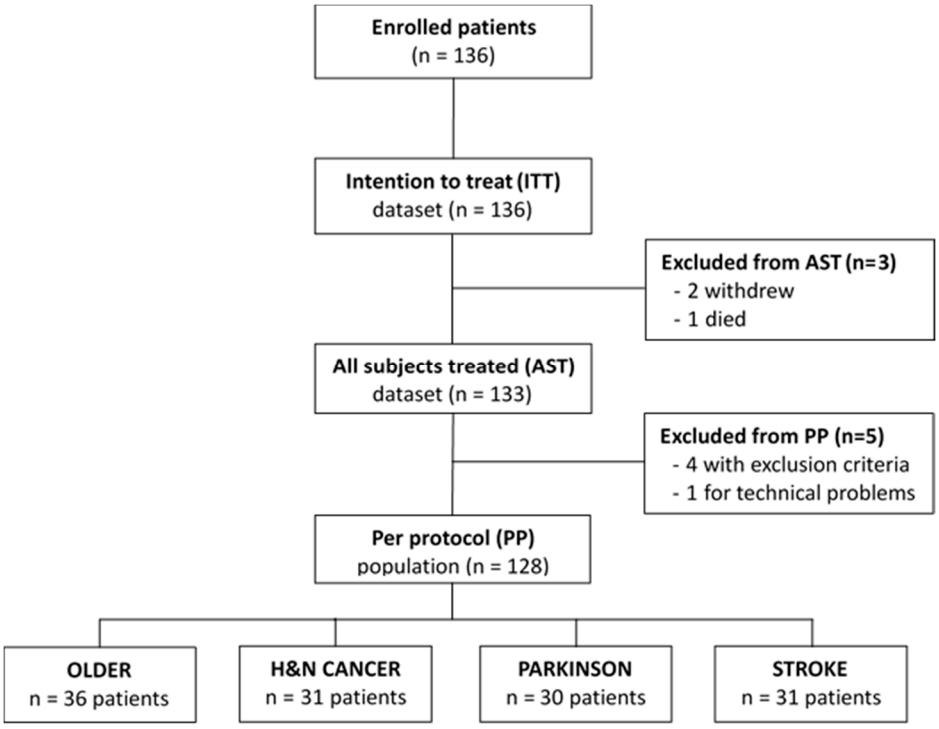

Figure 2. Study flow chart. Patients included in the study.

2.3. Study Product and Bolus Rheology

We used the Fresubin Clear Thickener® (Fresenius-Kabi Deutschland GmbH, Bad Homburg, Deutschland) composed of xanthan gum, modified starch, maltodextrin, modified cellulose and flavouring. The solutions used for the in vivo study were prepared by adding the thickener to a given volume of water plus an X-ray contrast agent, and stirring thoroughly for 20 s as recommended by the manufacturer. Then, shear-viscosities (250 mPa·s, 1000 mPa·s; and 2000 mPa·s) were obtained by adding 0.7 g, 2.3 g, and 4.2 g, to 50 mL of liquid obtained by mixing 1:1 mineral still water and the X-ray contrast Omnipaque® (GE Healthcare, La Florida, Spain) respectively, at room temperature (20 °C). For the in vitro rheological studies to assess the effect of salivary amylase and the effect of shear-thinning, boluses were prepared with 100 mL mineral still water and 1.75 g, 5.25 g, and 10.5 g respectively of FCT to achieve each level of viscosity.

2.4. Swallowing Evaluation Measurements

2.4.1. Screening (V-VST)

The V-VST was used to clinically screen patients for dysphagia. This test uses three volumes and three viscosities together with a pulse oximeter to assess clinical signs of OD. The procedure, algorithm and the psychometric characteristics of this tool have been described elsewhere [30,34].

2.4.2. Instrumental Evaluation (Videofluoroscopy)

VFS was performed to assess the severity of OD, the metrics of the OSR and the therapeutic effect of the thickener. Patients were studied in lateral projection while seated and exploration included the oral cavity, pharynx, larynx, and cervical esophagus. Videofluoroscopic recordings were obtained with a Super XT-20 Toshiba Intensifier (Toshiba Medical Systems Europe, Zoetermeer, The Netherlands) and recorded at 25 frames/s using a Canon DM-XM2 E video camera (Canon Inc. Japan). The study algorithm was designed as an effort test to protect the patient from aspiration. It consisted of a series of 5 and 20 mL boluses of the viscosities selected. It began with thin liquid viscosity (<50 mPa·s) and continued with 2000 mPa·s followed by 1000 mPa·s and 250 mPa·s. As a safety rule for patients, if

the patient presented an aspiration (PAS>5) at 5 mL thin liquid bolus, the 20 mL liquid bolus was skipped and the patient continued the exploration with the 5 mL 2000 mPa·s bolus [30]. If the patient presented an aspiration at 20 mL thin liquid, the exploration was continued with 5 mL 2000 mPa·s viscosity. Finally, if the patient presented an aspiration at any of the other boluses (after 20 mL thin liquid), the exploration was stopped as a safety rule to avoid further aspirations (Figure 3).

Videofluoroscopic signs of impaired safety and efficacy of deglutition were identified accordingly to previous studies [11,35]. Signs of impaired safety of swallow were assessed in each deglutition and included laryngeal vestibule penetrations and tracheobronchial aspirations, classified according to the PAS [36]. Unsafe swallow was defined as presenting a PAS score greater than two [36,37]. OSR was measured with the 5 mL bolus at each of the studied viscosities. Biomechanics of OSR were described as in previous studies [11,35], included the time to LVC, to upper esophageal sphincter opening (UESO), final kinetic energy (KE) of the bolus, bolus propulsion force, and mean bolus velocity. OSR was measured with specific software (Swallowing Observer; Image & Physiology SL, Barcelona, Spain) [25,27].

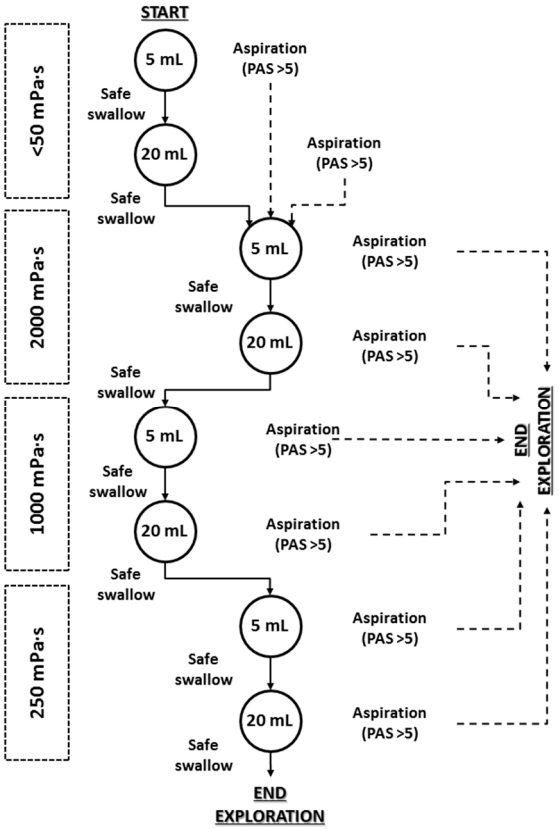

Figure 3. Study algorithm and safety stop rule for the videofluoroscopic exploration of the therapeutic effect of the different levels of viscosity. PAS: penetration-aspiration scale.

2.5. Rheological Characterization

We used a rotational viscometer (Thermo Fisher Scientific, Haake Viscotester® 550, Germany) to analyze shear viscosity and shear-thinning behaviour at 25 °C, with shear rates from 1 to 1000 s^{-1}. Viscosity values were measured at a shear rate of 50 s^{-1} and 300 s^{-1} respectively. An NV-rotor was used to analyze 250 mPa·s viscosity and an SV-DIN rotor to analyze higher viscosities (i.e., 1000 and 2000 mPa·s). RheoWin software, version 4.61 (Thermo Fisher Scientific, Waltham, MA, USA), was used for data processing. To assess the effect of salivary α-amylase during the oral phase of swallow, 15 mL boluses (250, 1000 and 2000 mPa·s) were given to the patients in a randomized order. Participants

spit the bolus after 30 s of oral incubation and viscosities at both shear rates (50 and 300 s^{-1}) were determined [29].

2.6. Palatability of the Product

Subjective palatability of the product was measured after each of the swallowed boluses at every volume and viscosity during VFS. Patients were asked to answer the following: What is your perception about the palatability of the given bolus? Each bolus was evaluated with a visual-analogue scale (VAS). The VAS scale was presented in a numerical form with values from 0 to 10 presented in a straight horizontal line of fixed length oriented from right to left. The descriptive term used for 0 was "bad" and for 10 was "excellent". No descriptive terms were used for the rest of the numbers.

2.7. Adverse Events

Adverse events were reported and their relationship with the study product assessed from the initiation of any study procedure to the end of the study treatment follow-up according to the WHO and the Uppsala Monitoring Centre (WHO-UMC) category guideline [38].

2.8. Data and Statistical Analysis

Qualitative data are presented as relative and absolute frequencies and analyzed by the Fisher's exact test (sex, VFS signs of impaired efficacy and safety of swallow) or the Chi-square test (MNA-sf categories, PAS score categories). The viscosity levels were compared with thin liquid by applying the McNemar's test (VFS signs of impaired efficacy and safety of swallow between viscosities). Continuous data is presented as mean ± standard error of the mean (SEM) and compared using the *t*-test (intergroup comparisons) or paired *t*-test (intragroup comparisons); for those variables that did not follow a normal distribution, we used the nonparametric Mann–Whitney U test (intergroup comparisons), the Wilcoxon-paired test (intragroup comparisons) or the Kruskal–Wallis test for multiple comparisons with Dunn's multiple comparison test. To assess normality, we used the D'Agostino and Pearson omnibus normality test. The main variable was prevalence of patients with safe swallowing at each one of the viscosities. These data were handled as binary by dividing the patients into two categories: a) patients who could swallow safely (PAS 1-2) vs. patients who could not swallow safely (PAS 3-8, including patients who discontinued the study due to the safety rule) over the ''per protocol" population. We compared prevalence of patients that could vs. could not swallow safely for each of the thickened viscosities compared with thin liquid. Safety of swallow of each patient for the whole VFS exploration or at a particular viscosity or level was expressed as the worst PAS score. Effect on penetrations (PAS 3-5) and aspirations (PAS 6-8) were also assessed. The efficacy of swallowing was also handled as binary data for oral and pharyngeal residue: if residue was observed in the oral cavity or at any of the three pharyngeal locations (pharyngeal wall, vallecular and pyriform sinus) the residue was present (yes), if no residue was observed at any of the locations the residue was absent (no). Results were interpreted according to the obtained *p*-value, the magnitude of the observed effect and their clinical and biological plausibility. Statistical significance was accepted if *p*-values were less than 0.05. Statistical analysis was performed with GraphPad Prism 6 (San Diego, CA, USA).

3. Results

3.1. Demographic Characteristics of the Population

We found many demographic differences among the study population groups (age, sex, functionality, BMI, force and health status self-perception) (Table 1). As a summary, older and stroke patients were the oldest and weakest (handgrip) groups, while those with HNC were the youngest and with the best functional capacity. In the older group, unlike the others, the majority of patients were women. Regarding nutritional status, MNA-sf evaluation showed high and similar percentages of malnourished or at risk of malnutrition patients (from 63.34% to 40%) in all groups.

Patients with HNC had the lowest BMI. Finally, health status self-perception was quite low except for the HNC group (Table 1).

Table 1. Demographic characteristics of the study population (continuous variables expressed as mean ± SEM).

	OLDER	HNC	PARKINSON	STROKE	*p*-Value
N	36	31	30	31	-
Age	82.96 ± 1.24	68. 29 ± 1.39 ****	72.34 ± 1.92 ****	79.42 ± 1.36 #### ŦŦŦ	<0.0001
Sex (female, %)	66.67 (24)	32.26 (10) **	20.00 (6) ***	35.48 (11) *	0.0008
Barthel (%)	78.33 ± 4.25	96.50 ± 1.68 *	77.33 ± 4.48 #	74.83 ± 5.31#Ŧ	0.0007
Optimum (100) (%)	38.89 (14)	67.34 (21) *	23.33 (7) ###	43.33 (13)	0.005
Sub-optimum (< 100) (%)	61.11 (22)	32.26 (10)	76.67 (23)	56.67 (17)	
MNA-sf (%)	10.97 ± 0.38	11.50 ± 0.35	11.7 ± 0.47 #	10.6 ± 0.51##	
Well-nourished (12–14)	47.22 (17)	48.57 (17)	60.00 (18)	36.67 (11)	0.326
At risk (8–11)	44.44 (16)	37.14 (13)	33.33 (10)	56.67 (17)	0.610
Malnourished (0–7)	8.33 (3)	2.86 (1)	6.67 (2)	6.67 (2)	
BMI (kg/m2)	27.59 ± 0.88	23.97 ± 0.69 *	27.50 ± 0.85	27.78 ± 0.74	0.002
Handgrip Force (kg)	16.33 ± 1.42	22.78 ± 1.97	25.38 ± 1.78 **	17.77 ± 1.48Ŧ	0.0009
Health status self-perception (0-100)	63.57 ± 3.32	70.83 ± 3.85	56.25 ± 3.74#	58.67 ± 5.29	0.055

MNA-SF indicates mini nutritional assessment short form; BMC, body mass index; HNC, head and neck cancer. * *p*-value <0.05, ** <0.01, *** <0.001, ****<0.0001 vs. Older; # *p*-value <0.05, ## <0.01, ### <0.001, #### <0.0001 vs. HNC; Ŧ *p*-value < 0.05, ŦŦŦ <0.001 vs. Parkinson.

3.2. Swallowing Evaluation by Videofluoroscopy (VFS)

3.2.1. Videofluoroscopic Signs

(a) Effect of patient phenotype

VFS evaluation showed an overall study population with a high prevalence VFS signs of impaired safety (70.31%) and efficacy (98.44%) of swallow and residue and a severe PAS (4.44 ± 0.20). However, there was a different pattern of swallowing impairment depending on OD phenotype. Regarding impaired safety of swallow, we found a high prevalence of penetrations (46.67%–74.19%) and aspirations (13.33%–41.94%) in all groups and high mean PAS score (3.80–5.36) at thin liquid (<50 mPa·s). Patients with HNC had the most severe impairment of safety with a mean PAS score of 5.36 ± 0.41, 41.94% of them presenting aspirations and up to 25.81% of them, silent aspirations (Table 2). Older patients presented the highest prevalence of oral residue (91.67%) and those with stroke the lowest (61.29%, $p < 0.01$). HNC had the highest prevalence of pharyngeal residue (96.80%) while older patients had the lowest (66.67%, $p < 0.01$) (Table 2).

Table 2. Videofluroscopic signs of impaired efficacy and safety of swallow in the study groups (continuous variables expressed as mean ± SEM) for any viscosity level.

	ALL	OLDER	HNC	PARKINSON	STROKE	*p*-Value
N	128	36	31	30	31	
Impaired Efficacy (%)	98.44 (126)	100.00 (36)	100.00 (31)	100.00 (30)	96.67 (29)	nc
Oral Residue	82.81 (106)	91.67 (33)	80.65 (31)	76.67 (23) ##	61.29 (19) ** ###	0.0002
Pharyngeal Residue	80.47 (103)	66.67 (24)	96.80 (30) **	86.67 (26) ####	74.19 (23) # Ŧ	<0.0001
Impaired Safety (%)	70.31 (90)	63.89 (23)	83.87 (26)	56.67 (17) #	77.42 (24)	0.076
Penetrations	61.72 (79)	58.3 (21)	74.19 (23)	46.67 (14) #	67.74 (21)	0.135
Aspirations	28.91 (37)	25.00 (9)	41.94 (13)	13.33 (4) #	35.48 (11)	0.071
Silent Aspirations (PAS = 8)	14.84 (19)	11.11 (4)	25.81 (8)	13.33 (4)	9.67 (3)	0.256
Higher PAS score	4.44 ± 0.20	4.08 ± 0.39	5.36 ± 0.41	3.80 ± 0.40#	4.55 ± 0.41	0.038

PAS indicates penetration-aspiration scale; HNC, head and neck cancer; Impaired efficacy: fractional swallow, oral residue and pharyngeal residue. * *p*-value <0.05, ** <0.01 vs. Older; # *p*-value <0.05, ## <0.01, ### <0.001, #### <0.0001 vs. HNC; Ŧ *p*-value <0.05 vs. Parkinson; nc: not calculable.

(b) Therapeutic effect of bolus viscosity with FCT

Safety of swallow: We found a strong shear viscosity-dependent therapeutic effect on the safety of swallow, with a maximal significant therapeutic effect at 1000 mPa·s (80.56%, 96.67%, and 74.19% of safe swallows) for older, Parkinson's and stroke patients, respectively. This therapeutic effect was greatly reduced in HNC patients, at 58.06%. (Figure 4 and Supplementary Figure S1). Further increase of viscosity up to 2000 mPa·s did not cause a significant increase in safety in any study group. We also found that the therapeutic effect of FCT depended on the phenotype of patient assessed: therapeutic effect was significantly reduced in HNC vs. all the other groups together ($p < 0.05$ <50 mPa·s; $p < 0.01$ 250 mPa·s; $p < 0.01$ 1000 mPa·s; and $p < 0.001$ 2000 mPa·s) (Figure 5). However, it was similar between the three other groups, achieving 82.47% of patients with safe swallow at 1000 mPa·s, and 95.88% of patients with safe swallow at 2000 mPa·s (Figure 5). We also found an important reduction in the severity of OD measured with the PAS with significant changes when comparing <50 mPa·s vs. 1000 mPa·s and 2000 mPa·s in all groups of patients and 250 mPa·s vs. 2000 mPa·s in older, Parkinson's and stroke groups, but not between 1000 and 2000 mPa.s (Figure 6), even when grouped together (Figure 7). The therapeutic range of FCT was defined between 250 and 1000 mPa·s. On the other hand, 250 mPa·s was selected as the minimum level of viscosity presenting a significant therapeutic effect (compared to thin liquid), and 1000 mPa·s as the maximal significant therapeutic effect compared to 2000 mPa·s.

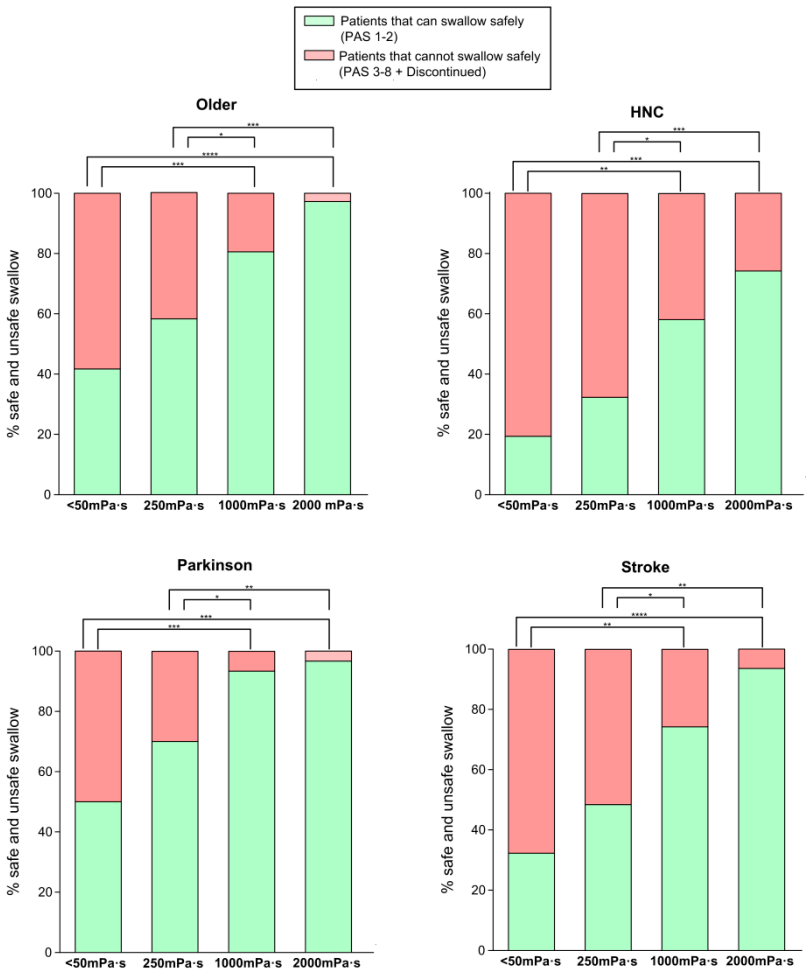

Figure 4. Percentage of patients who could swallow safely vs. those who could not swallow safely at each viscosity level. * p-value <0.05, ** < 0.01, *** <0.001, **** <0.001. HNC: head and neck cancer.

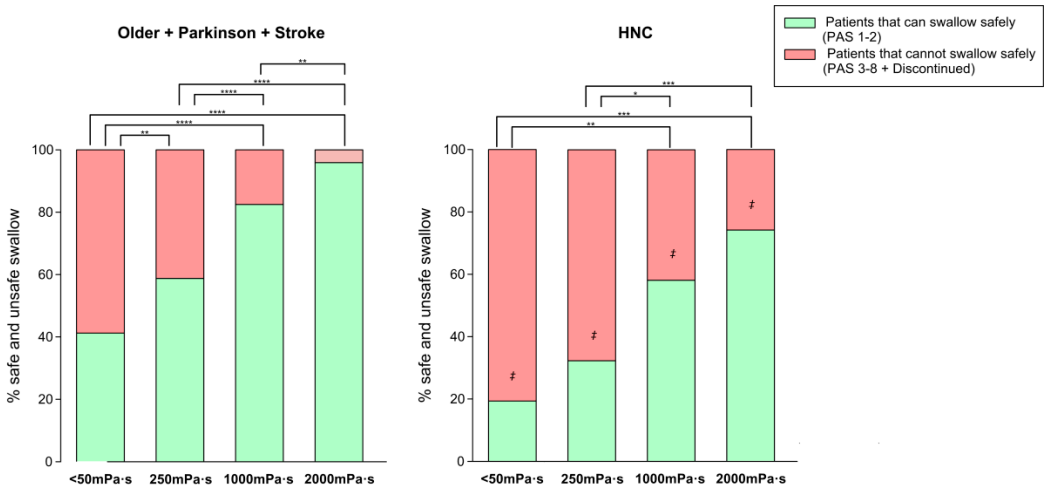

Figure 5. Percentage of patients who could swallow safely vs. those that cannot swallow safely comparing three groups together against HNC. Left: older, Parkinson's and stroke patients; right: patients with head and neck cancer. * p-value <0.05, ** <0.01, *** <0.001, **** <0.001. ‡ p-value <0.05 Older + Parkinson's + Stroke. HNC: Head and neck cancer.

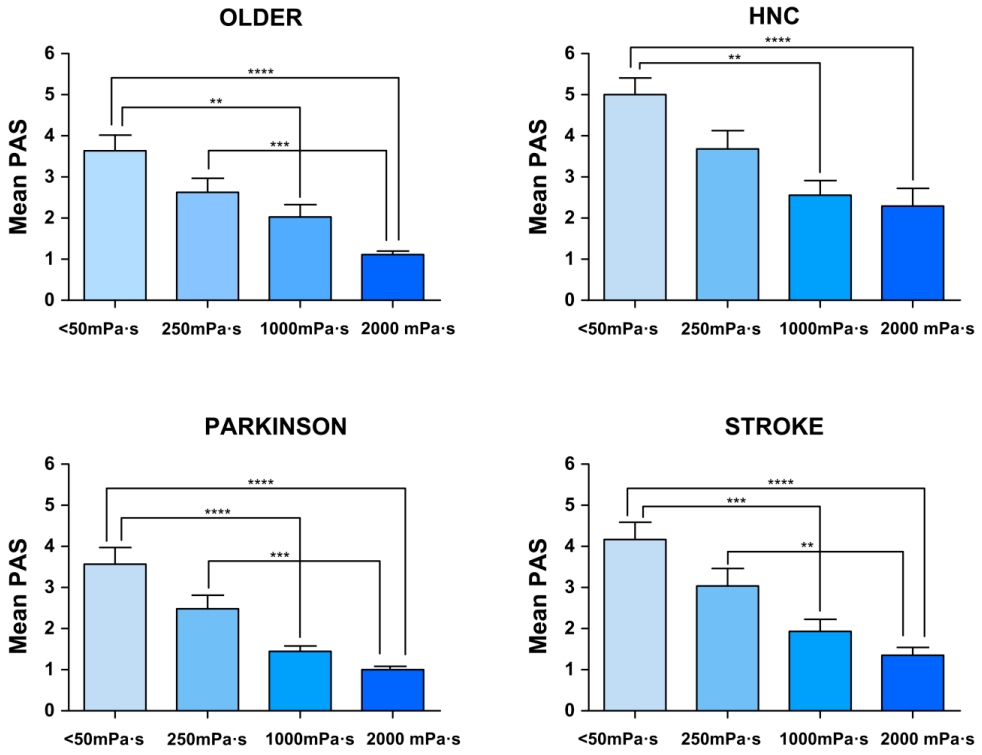

Figure 6. Mean penetration-aspiration scale (PAS) score in each viscosity of each of the study groups. * p-value <0.05, ** <0.01, *** <0.001, **** <0.001. HNC indicates head and neck cancer.

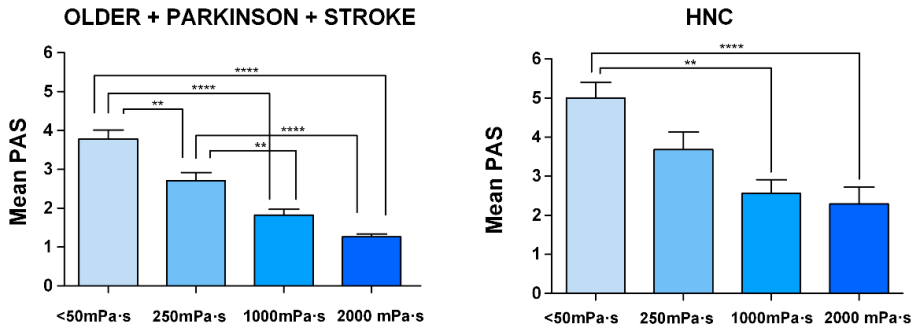

Figure 7. Mean penetration-aspiration scale (PAS) score in each viscosity comparing 3 groups together vs. HNC. Left: older, Parkinson's and stroke patients; right: patients with head and neck cancer. * *p*-value <0.05, ** <0.01, *** <0.001, **** <0.001. HNC: Head and neck cancer.

Efficacy of swallow: We found a significant increase in the prevalence of oral residue when we compared the study viscosities with thin liquid in all phenotypes (Supplementary Table S1). Older patients that had the highest prevalence of oral residue and also presented significant differences in oral residue when comparing 2000 mPa·s vs. 250 and 1000 mPa·s (Supplementary Table S1 and Figure 8). On the other hand, there were no differences regarding pharyngeal residue when we compared the tested viscosities in any of the studied phenotypes (Supplementary Table S2). The highest prevalence of pharyngeal residue was in HNC patients vs. the rest of the groups together: $p < 0.001$ <50 mPa·s; $p < 0.01$ 250 mPa·s; $p < 0.05$ 1000 mPa·s; and $p = 0.068$ 2000 mPa·s in all the tested viscosities (Figure 8).

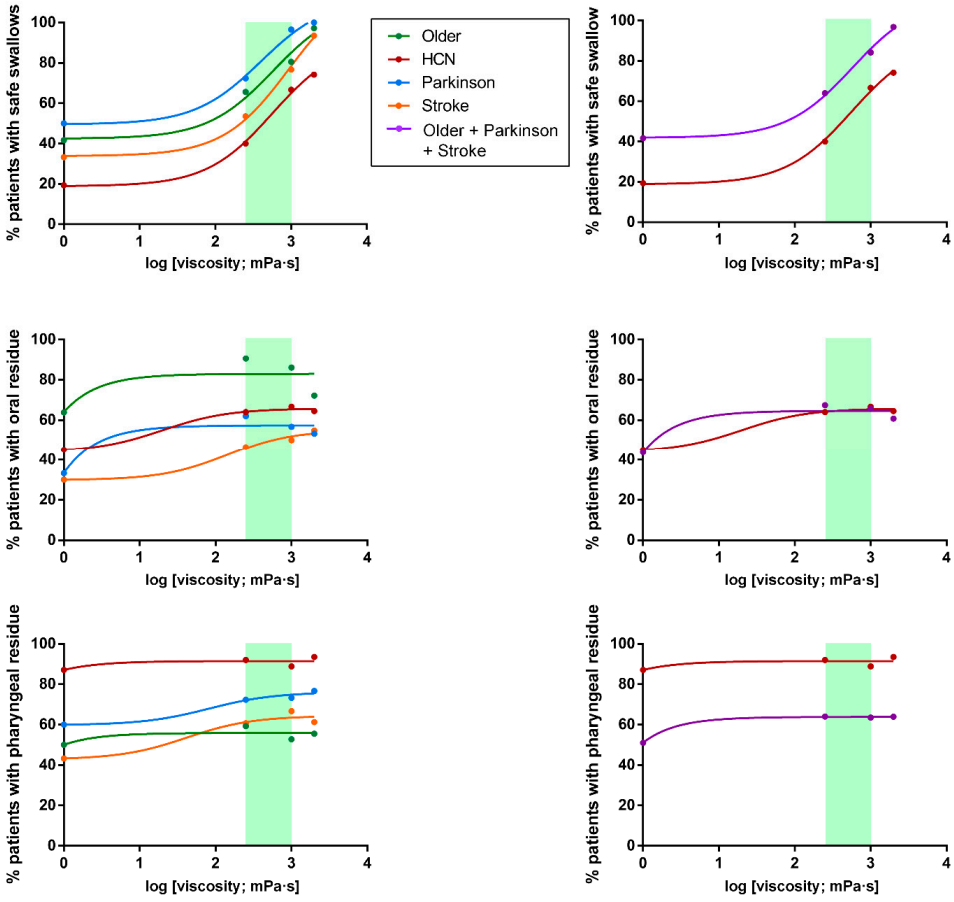

Figure 8. Viscosity-dependent effect of FCT on the safety and efficacy of swallow (oral and pharyngeal residue). Left: all groups of patients; right: older, Parkinson's and stroke patients merged vs. patients with head and neck cancer. The therapeutic range has been marked with a green rectangle.

(c) Effect of bolus volume

We found no statistically significant differences in the prevalence of signs of impaired safety of swallow (PAS 3-8) when we compared 5 mL vs. 20 mL at any of the tested viscosities over the whole study population nor for each specific phenotype. However, we found statistically significant differences in all the viscosities when comparing the effect of volume on impaired efficacy of swallow (oral + pharyngeal residue), with a significant increase in residue at 20 mL (5 mL vs. 20 mL < 50 mPa·s: 61.90% vs. 90.38%, $p < 0.0001$; 250 mPa·s: 83.33% vs. 93.20%, $p < 0.05$; 1000 mPa·s: 87.70% vs. 97.37%, $p < 0.01$; and 2000 mPa·s: 81.10% vs. 95.16%, $p < 0.001$). Regarding the effect of FCT on the efficacy of swallow (oral + pharyngeal residue) for each specific phenotype, we found statistically significant differences when we compared 5 vs. 20 mL in <50 mPa·s in the older ($p < 0.05$), Parkinson's ($p < 0.01$) and stroke groups ($p < 0.01$); and at 2000 mPa·s in the older group ($p < 0.05$). Taken together, our results show that increasing bolus volume from 5 mL to 20 mL has no impact on impaired safety of swallow but significantly increases oropharyngeal residue in all groups of patients together at all the tested viscosities.

(d) Dose-responses curves

FCT presented a strong therapeutic effect depending on the shear viscosity. The minimum viscosity tested (250 mPa·s) showed a significant therapeutic effect vs. thin liquid for all the phenotypes studied and it increased until the maximum viscosity assessed (2000 mPa·s) achieving >90% of safe swallows in older, Parkinson's and stroke groups and 74.19% in HNC patients (Figure 8). However, no significant differences were seen between 1000 mPa·s and 2000 mPa·s for each group compared individually (80.56%, 96.67%, 74.19% and 58.06 of patients with safe swallow for each group at 1000 mPa·s, respectively). Accordingly, the threshold viscosity of safety was 250 mPa·s and maximal viscosity, 1000 mPa·s. Regarding dose-response curves for oral residue, we found a significant increase when comparing differences between <50 mPa·s and the rest of viscosities in all groups (Supplementary Table S1), but no differences between 250-2000 mPa·s and a higher prevalence of residue in the older group. Finally, the dose-response curve of pharyngeal residue was constant between viscosities for each phenotype but with a significant increase in prevalence in the HNC group compared to the rest of the study groups (Figure 8).

3.2.2. Oropharyngeal Swallow Response (OSR)

OSR at 5 mL liquid bolus was severely impaired in all studied groups when compared with our earlier studies on healthy volunteers (HV) [11]. Time to LVC was severely delayed ranging from 360 to 428 ms among the different groups (HV < 160 ms) and time to UESO was delayed and ranged from 240 to 281.33 ms (HV 200 ms). Regarding bolus kinematics, bolus propulsion force was weak (11.90 to 18.57 mN vs. HV 22 mN) leading to decreased bolus velocity from 0.24 to 0.33 m/s [11] (Table 3). Although patients with HNC presented the most delayed LVC (428.00 ± 35.11), there were no significant differences between groups. This impaired OSR, especially the severe delay in protection of the airway (LVC time), puts our patients at great risk of aspiration and residue through weak propulsion.

When we assessed the effect of viscosity on the OSR within groups, we found a reduction in the time to LVC in two groups of patients, older ($p < 0.05$, 2000 mPa·s vs. <50 mPa·s) and Parkinson's

($p < 0.05$, 1000 mPa·s and 2000 mPa·s vs. <50 mPa·s). The other OSR parameters affecting oropharyngeal reconfiguration or bolus propulsion forces were not affected by increasing bolus viscosity with FCT (Table 3).

Table 3. Comparison between viscosities of oropharyngeal swallow response among the study groups (continuous variables expressed as mean ± SEM).

		<50 mPa·s (5 mL)	250 mPa·s (5 mL)	1000 mPa·s (5 mL)	2000 mPa·s (5 mL)	*p*-Value
ALL	LVC (ms)	387.00 ± 13.32	359.00 ± 11.68	315.80 ± 9.59 *** #	316.20 ± 10.92 **** ##	<0.0001
	UESO (ms)	259.50 ± 9.79	257.20 ± 9.40	260.00 ± 8.61	290.10 ± 15.34	0.066
	KE (mJ)	0.96 ± 0.08	0.90 ± 0.06	0.83 ± 0.06	0.85 ± 0.10	0.112
	Force (mN)	14.87 ± 1.01	14.43 ± 1.08	13.19 ± 0.97	13.31 ± 1.42	0.228
	x̄ bolus vel. (m/s)	0.26 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.24 ± 0.01	0.230
OLDER	LVC (ms)	360.00 ± 20.85	336.25 ± 14.90	298.89 ± 14.78	284.57 ± 12.70 *	0.006
	UESO (ms)	260.00 ± 18.69	238.75 ± 13.85	232.22 ± 8.72	262.86 ± 14.21	0.304
	KE (mJ)	0.90 ± 0.11	0.90 ± 0.11	0.92 ± 0.10	0.80 ± 0.15	0.408
	Force (mN)	14.64 ± 1.63	15.24 ± 1.65	14.73 ± 1.23	12.48 ± 1.83	0.228
	x̄ bolus vel. (m/s)	0.26 ± 0.02	0.27 ± 0.01	0.27 ± 0.01	0.25 ± 0.02	0.541
HNC	LVC (ms)	428.00 ± 35.11	370.00 ± 30.48	338.46 ± 26.37	360.00 ± 32.72	0.222
	UESO (ms)	240.00 ± 21.07	257.60 ± 19.47	280.00 ± 19.80	282.80 ± 25.26	0.153
	KE (mJ)	1.28 ± 0.25	0.82 ± 0.12	0.62 ± 0.07	0.81 ± 0.16	0.160
	Force (mN)	18.57 ± 3.08	12.13 ± 1.57	9.50 ± 1.11	12.28 ± 2.22	0.136
	x̄ bolus vel. (m/s)	0.33 ± 0.03	0.28 ± 0.02	0.25 ± 0.01	0.27 ± 0.02	0.157
PARKINSON	LVC (ms)	373.33 ± 21.81	342.07 ± 26.68	293.33 ± 17.82 *	289.33 ± 12.08 *	0.009
	UESO (ms)	257.33 ± 20.18	249.66 ± 24.20	237.33 ± 16.49	241.33 ± 15.16	0.862
	KE (mJ)	0.89 ± 0.11	1.08 ± 0.18	0.99 ± 0.16	1.23 ± 0.32	0.963
	Force (mN)	14.18 ± 1.61	17.80 ± 3.30	16.50 ± 3.16	19.33 ± 4.78	0.946
	x̄ bolus vel. (m/s)	0.27 ± 0.02	0.29 ± 0.02	0.29 ± 0.02	0.30 ± 0.03	0.920
STROKE	LVC (ms)	392.00 ± 27.55	390.67 ± 21.92	338.06 ± 18.35	335.48 ± 23.14	0.148
	UESO (ms)	281.33 ± 18.14	284.00 ± 16.98	296.77 ± 21.37	326.45 ± 23.18	0.421
	KE (mJ)	0.74 ± 0.07	0.77 ± 0.10	0.73 ± 0.10	0.58 ± 0.07	0.317
	Force (mN)	11.90 ± 1.14	12.17 ± 1.44	11.34 ± 1.47	9.26 ± 1.13	0.325
	x̄ bolus vel. (m/s)	0.24 ± 0.01	0.24 ± 0.02	0.24 ± 0.02	0.21 ± 0.01	0.3367
OLDER	LVC (ms)	374.20 ± 13.47	356.40 ± 12.59	309.70 ± 9.88 *** #	302.5 ± 9.78 *** ##	<0.0001
	UESO (ms)	265.80 ± 11.00	256.40 ± 10.87	254.40 ± 9.51	276.70 ± 10.82	0.170
	KE (mJ)	0.85 ± 0.06	0.92 ± 0.08	0.88 ± 0.07	0.87 ± 0.12	0.220
	Force (mN)	13.66 ± 0.87	15.07 ± 1.31	14.22 ± 1.19	13.63 ± 1.72	0.147
	x̄ bolus vel. (m/s)	0.26 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.26 ± 0.01	0.267

x̄ indicates mean; HNC, Head and neck cancer; PK, Parkinson; STR, Stroke; LVC, Laryngeal vestibule closure; UESO, upper esophageal sphincter opening; KE, kinetic energy; HNC, Head and neck cancer. * *p*-value <0.05, *** <0.001, **** <0.0001 vs. <50 mPa·s; # <0.05, ## <0.01 vs. 250 mPa·s.

3.3. In Vitro Studies

(e) α-amylase effect and shear-thinning viscous behaviour

FCT presented strong resistance to salivary α-amylase effect. Viscosity was not affected by oral incubation at any of the viscosity levels tested compared with control samples without saliva for any phenotype assessed (Table 4). Surprisingly, we only found a slight but significant increase of viscosity in the 1000 mPa·s thickened fluid in patients with OD and stroke (Table 4; Supplementary Figure S2). FCT thickened fluids presented a non-Newtonian viscous behaviour (shear-thinning type) when submitted to shear. We found a similar percentage of viscosity reduction for all the samples between shear rates from 50 s^{-1} to 300 s^{-1}, that was unaffected by salivary α-amylase in any group. Linear regression curves performed between a shear rate from 0 to 1000 s^{-1} are presented for each level of tested viscosity (Table 4).

Table 4. Viscosity percentage change between comparisons of control vs. samples after oral incubation (amylase effect) and shear thinning effect represented with the linear regression from 0 to 1000 s^{-1} shear rate. Positive values indicate increase and negative values decrease of viscosity.

	% Change (Viscosity at 50 s^{-1} before and after Oral Incubation)	*p*-Value	Shear-Thinning Effect (Linear Regression from 0 to 1000 s^{-1})	Correlation Coefficient'r'
ALL	+7.85	0.637	fx = −0.91x + 4.18	0.99
	+7.16	0.135	fx= −0.93x + 4.71	0.99
	−2.96	0.560	fx= −0.91x + 4.94	0.99
OLDER	+30.47	0.062	fx= −0.83x + 4.067	0.99
	−4.01	0.471	fx= −0.83x + 4.39	0.99
	−2.29	0.659	fx= −0.81x + 4.69	0.99
HNC	+9.30	0.459	fx= −0.90x + 4.15	0.99
	+4.17	0.531	fx= −0.91x + 4.64	0.99
	−1.86	0.706	fx= −0.91x + 4.91	0.99
PARK.	+9.47	0.447	fx= −0.92x + 4.2	0.99
	+11.64	0.061	fx= −0.93x + 4.68	0.99
	−5.22	0.424	fx= −0.88x + 4.86	0.99
STROKE	−10.17	0.327	fx= −0.90x + 4.09	0.99
	+14.11	0.025	fx= −0.92x + 4.69	0.99
	−2.62	0.683	fx= −0.91x + 4.93	0.99

HNC: Head and neck cancer; PARK: Parkinson's.

3.4. Palatability of the Product

Palatability was not significantly affected by increasing bolus viscosity. Palatability with the study product measured on a 10-point scale was similar in all the viscosities tested (6.73 ± 0.48 with liquid; 6.22 ± 0.48 with 250 mPa·s; 5.63 ± 0.43 with 1000 mPa·s; and 5.48 ± 0.42 with 2000 mPa·s). In addition, we did not find any difference in this item between volumes and viscosities in any of the study groups nor between groups (Supplementary Figure S3).

3.5. Adverse Events

No adverse events or serious adverse events related or not related to the study product were reported during the study period.

4. Discussion

The main result of this study was that increasing bolus viscosity with FCT had a strong shear viscosity-dependent therapeutic effect by improving safety of swallow, with a threshold level of 250 mPa·s and maximal protection level at 1000 mPa·s^{-1}. The therapeutic effect of FCT was very high and similar in older patients, patients with Parkinson's disease and post-stroke patients compared with HNC patients, as FCT enabled safe swallow in 96% of patients of these three phenotypes. In contrast, the high severity of OD in HNC patients reduced this therapeutic effect at the same viscosity range. Finally, FCT is safe, well tolerated and not affected by salivary amylase in any of the study groups.

This study includes four of the most representative phenotypes of patients with OD: older, post-stroke, and patients with Parkinson's disease and HNC. We have observed some similarities, but also very important differences, among these phenotypes of patients with OD. All of the patients presented high and similar prevalence of malnutrition, and a severe pattern of swallowing dysfunction with high prevalence of signs of impaired safety and efficacy of swallow and thus, were at great risk of developing further nutritional and/or respiratory complications [39]. Although HNC patients were the youngest with the best functionality and QOL, they had the most severe OD, with the highest prevalence of penetrations, aspirations, PAS and pharyngeal residue, and presented the lowest viscosity-dependent therapeutic effect. This is probably because HNC patients present OD as a

consequence of structural changes secondary to the surgical process or radiochemotherapy (fibrosis, mucositis, etc.), in contrast to the other phenotypes that present OD mainly associated with impaired swallow physiology.

Older and stroke patients recruited in this study were demographically similar to those studied previously by our group [25–27,40]. Regarding the swallowing status of our phenotypes, older and stroke presented severe OD in terms of prevalence of signs of impaired safety (63.89% in older and 77.42% in stroke), impaired efficacy of swallow (100% in older and 96.67% in stroke), and PAS (mean PAS score of 4.08 ± 0.39 in older and 4.55 ± 0.41 in stroke). In addition, our patients also presented similar impaired OSR with a delayed time to LVC (336.25 ms in older and 390.67 ms in stroke) [11,40,41]. In a study by Vilardell et al. they found that time to LVC ≥340 ms predicted unsafe swallow in a group of chronic post-stroke patients with high diagnostic accuracy [40]. Patients from our study, including those with post-stroke OD, had a delayed time to LVC and thus a high prevalence of unsafe swallows. We also found that older patients presented the highest prevalence of oral residue. Oral residue is related to impaired bolus propulsion force due to weakness of pharyngeal muscles related to sarcopenia, a prevalent condition among older patients [42]. This specific phenotype conferred the lowest force measured with a handgrip dynamometer, but we did not find correlation between handgrip force and tongue bolus propulsion force in these patients. In contrast, the youngest and most functional patient group of the study, HNC patients, was that with the highest OD severity (highest prevalence of impaired safety of swallow and mean PAS score) related to the fact that they present severe pharyngeal anatomical alterations caused by surgery and concomitant treatments. In general, VFS signs for older, Parkinson's and stroke were quite similar when comparing with those of the HNC phenotype and so the results for the three phenotypes were merged to discuss some results of the study vs. HNC values. Regarding the OSR, we only found a significant reduction in the time to LVC at 2000 mPa·s in older and Parkinson's group. It is known now that older and stroke patients with OD present decreased pharyngeal sensitivity with impaired conduction and cortical integration of pharyngeal sensory inputs [43–45]. The reduction in the time to LVC at the higher viscosity could indicate that, at 2000 mPa·s, the bolus is more perceived in the pharynx increasing the sensory input and triggering a faster swallow response. We did not find any other effect of FCT in any other of the OSR parameters, showing that this therapy does not improve swallowing physiology but has a compensatory effect [11,16,25].

We observed that the therapeutic effect of increasing shear-viscosity with FCT is linked to the specific pathophysiology of OD in each phenotype, but it also presented a shear viscosity-dependent behaviour. We found a significant therapeutic effect with the lowest viscosity level assessed (250 mPa·s) as threshold viscosity. The percentage of patients with safe swallow increased for all groups until the highest viscosity level (2000 mPa·s), which presented high levels of protection. However, no significant differences were seen between 1000 mPa·s vs. 2000 mPa·s for each phenotype assessed individually. We also analyzed the differences between prevalence of aspirations regarding viscosity levels and we found no significant differences on the reduction of aspirations for any phenotype except for the older group and the number of aspirations increased for the highest viscosity in comparison to 1000 mPa·s even for the HNC phenotype. Differences that appeared for the older group at 2000 mPa·s were not clinically significant when compared with 1000 mPa·s when analyzing the percentage of safe swallows and the mean PAS value. Therefore, our results show a therapeutic range of 250–1000 mPa·s for FCT in the four phenotypes of patients.

To complete the characterization of this thickener, we carried out a rheological analysis. FCT presented very good rheological properties, as it was resistant to α-salivary amylase, which demonstrated that viscosity remained constant in the oral cavity, increasing the therapeutic value of FCT compared with thickeners composed just by starch. The low adherence of patients to fluid modification is due to the low palatability of those products at high viscosity levels [46]. The basal palatability of this product was quite good and surprisingly, the palatability analysis performed showed no significant differences between liquid (<50 mPa·s) and the thickened viscosities (250-2000 mPa·s).

These result shows that this product is well accepted by patients and it might present high adherence results among all phenotypes assessed in the present study.

One of the limitations of the study comes from the screening (V-VST); although it offers a high sensitivity and specificity, some patients suffering from dysphagia can be missed. The main limitation of the study arose from the safety rule established during VFS algorithm, as some patients did not receive all the tested viscosities in order to avoid risks. This is an ethical decision that cannot be easily solved, as we are not able to push patients towards a risk of aspiration in order to respond to a research question. As previous studies have shown, there is higher risk of aspiration as we decrease the viscosity [24]. Another limitation is that we have used a palatability score for an acute situation, and that would not reflect the daily life acceptability of a patients that has to take this product in a chronic way. Thus, we believe that a longitudinal study should be done in order to know the exact palatability and acceptance of FCT in daily clinical practice.

We can conclude that the strong therapeutic effect of FCT is viscosity and phenotype-dependent and would work very well with those older, Parkinson's and stroke patients, offering a therapeutic range of 250–1000 mPa·s and maximal protection with 1000 mPa·s. However, those patients with HNC, which present more severe anatomical and physiological alterations, showed a more reduced therapeutic effect of increasing shear-viscosity with FCT on the safety of swallow.

5. Conclusions

Increasing the bolus viscosity with Fresubin Clear Thickener® had a strong viscosity-dependent therapeutic effect on patients with OD by improving the safety of swallow with a high protection level at 1000 mPa·s. The therapeutic range of Fresubin Clear Thickener® in OD was established from 250 to 1000 mPa·s. This therapeutic effect is phenotype-dependent, the greatest therapeutic effect being in older, Parkinson's and stroke patients. The high severity of OD in HNC patients reduces this therapeutic effect of increasing shear-viscosity at the same therapeutic range. In addition, Fresubin Clear Thickener® is resistant to salivary α-amylase.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/12/6/1873/s1, Figure S1. Penetration Aspiration Scale (PAS) score frequency between viscosities and patients. PAS 1-2 indicates safe swallow and PAS 3-8, unsafe swallow; HNC, head and neck cancer. Figure S2. Effect of saliva (samples with saliva) on viscosity measured with a rotational viscometer within groups. Figure S3. Palatability of the study product presented for all patients valuated with a visual analogue scale (0-10). Table S1. Comparison between viscosities of oral residue among the study groups. HNC: Head and neck cancer. Table S2. Comparison between viscosities of pharyngeal residue among the study groups. HNC: Head and neck cancer.

Author Contributions: Conceptualization, O.O. and P.C.; methodology, O.O., V.A., W.V.N. and P.C.; formal analysis, O.O., M.B.-P. and P.C.; investigation, O.O., M.B.-P., V.A., W.V.N., N.T. and P.C.; data curation, O.O. and M.B.-P.; writing—original draft preparation, O.O., M.B.-P. and P.C.; writing—review and editing, O.O., M.B.-P. and P.C.; supervision, P.C.; funding acquisition, C.G., E.B.-d.L.F., P.C. All authors have read and agreed to the published version of the manuscript.

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