Effect of α-amylase and pH on the rheological properties of thickened liquids containing starch in in vitro conditions relevant to oral processing and swallowing

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Effect of α-amylase and pH on the rheological properties of thickened liquids containing starch in *in vitro* conditions relevant to oral processing and swallowing

Abstract

Gelatinized starch, frequently used as a thickener, is hydrolyzed in the mouth by salivary α-amylase, which can modify bolus rheology and influence texture perception, aroma release, and swallowing safety.

The objective of this study was to investigate quantitatively the impact of saliva on the rheological properties of thickened drinks (IDDSI Level 3) with different pH. Oral digestion was simulated and followed using a rheometer. An insalivation ratio measured from spitted boli, was used in the *in vitro* oral digestion experiments, comparing unstimulated human saliva to an artificial saliva.

The initial viscosity of thickened water samples (pH 5.3 and 7.4) was reduced by 80% after only 5 s of *in vitro* oral digestion. A similar viscosity decay was observed with the artificial saliva. This decrease in viscosity was attributed to the breakdown of the starch granule structure by α-amylase and in a lesser extent to a dilution effect. In contrast, the rheological properties of thickened lemon drink (pH = 2.7) and thickened orange juice (pH = 4.0) were not influenced significantly by human salivary amylase. These results suggest that at these pH, starch-based thickened drinks can maintain their initial IDDSI level, despite a strong dilution with saliva, which could help in the management of dysphagia. Clinical trials should be performed to confirm this hypothesis.

Only human salivary α-amylase should be used to study products between pH 3 and 5 to imitate the structural and rheological breakdown happening before swallowing, while α-amylase from *Bacillus sp.* could also be used outside this range. The method developed in this study can be used to quantify the impact of food oral processing and evaluate rheological properties relevant for swallowing in the presence of saliva.

Keywords

Thickener, saliva, amylase, viscosity, pH, test *in vitro*, deglutition, deglutition disorders

Introduction

Many common semi-solid foods, like purees, sauces, soups, custards, or puddings, contain gelatinized starch providing their specific texture and mouthfeel. Modified starches are also frequently used as thickeners to adjust the rheological properties of drinks for patients suffering from swallowing disorders [1–3].

In the mouth, foods and drinks are mixed with saliva to form a bolus and initiate digestion. Saliva contains α-amylase which is an endo-splitting enzyme, cleaving randomly α-1,4-glycosidic bonds in amylose and amylopectin [4]. Gelatinized starch granules are therefore rapidly hydrolyzed by salivary α-amylase into oligosaccharides such as maltose, maltotriose, maltotetraose, etc [5]. This structural breakdown may modify the bolus rheology, influence texture perception, aroma release, and swallowing safety [6–8].

Enzymatic degradation of gelatinized starch during the oral phase of digestion depends on the properties of the ingested foods and drinks. First, the microstructure of the food may protect starch granules from the enzymatic attack and delay their hydrolysis [9]. Then, minor components such as phytates, phenolic compounds, saponins, or lectins can also act as amylase inhibitors [9,10]. Finally, the food pH can influence the amylolytic process since the human salivary α-amylase optimum pH is between 6-7 [4,10–12] and its activity decreases above and below this pH. This effect should not be underestimated since many popular drinks that may be mixed with starch-based thickeners for dysphagic patients are acidic, like fruit juices or syrups, sodas, and coffee for example [13].

The impact of saliva on the rheological properties of semi-solid starchy foods is fast and very challenging to measure *in vivo*. To overcome this difficulty, changes in products have been measured *in vitro* by mimicking the oral conditions during food oral processing. However, different studies considered different conditions (temperature, food:saliva ratio, shear rate, etc.), enzymes (human saliva or artificial saliva with different compositions and activities), and instruments (Rapid Visco Analyzers, controlled stress rheometers, or custom-built devices), making comparison between studies difficult [6].

The objective of this study was to investigate quantitatively the impact of salivary α-amylase on the rheological properties of thickened liquids containing starch. Different thickening methods were compared, and drinks with different acidic pH were considered. The hypothesis of this study was that it is possible to quantify the impact of food oral processing on the rheological properties of starchy semi-solid foods, using a simple test *in vitro* and artificial saliva, and use this method to ascertain the important role of pH, in reducing the destructuration of starch-based product by α-amylase.

Materials & Methods

Materials

This study considered fluids with different pH: water, a pure orange juice (Helior, LSDH, Saint Denis de l'Hôtel, France), and a lemon drink prepared by diluting the concentrated lemon juice in mineral water (Vittel) with a ratio 1:7, according to the recommendations of the supplier ("Pulco Citron à diluer", Suntory Beverage & Food France, Neuilly-sur-Seine, France).

These liquids were thickened using either a powdered commercial food thickener containing modified maize starch (Ressource ThickenUp, Nestlé Health Science, Nestlé, Vevey, Switzerland) or a stabilized and cross-linked waxy maize starch slurry (acetylated adipate distarch, C* Tex 06205, Cargill, Baupte, France) prepared by heating the native starch in deionized water (5.4 % w/v) under constant stirring to 70°C for 10 min. This starch slurry was then cooled down to room temperature in an iced water bath.

The lemon drink and the orange juice were thickened with ThickenUp (TU) by adding 5.4 g of powder directly to 100 mL of the liquid and stirring with a spatula for 30 s. Samples thickened with the waxy maize starch (WMS) were obtained by mixing liquid samples with a thicker cooled starch slurry (6.4 %w/v) with a ratio 1:7.

After the addition of the thickener, all fluids classified as Level 3 according to the International Dysphagia Diet Standardization Initiative (IDDSI) framework. All samples were prepared and tested on the same day, and stored at room temperature before measurements; pH of the different thickened samples are presented in Table 2.

Artificial saliva

The simulated salivary fluid (SSF) was prepared according to the recommendations of the Infogest network [14]. It contained potassium chloride (15.1 mM), potassium phosphate (3.7 mM), sodium bicarbonate (13.6 mM), magnesium chloride hexahydrate (0.15 mM), ammonium carbonate (0.06 mM), and calcium chloride dehydrate (1.5 mM). The pH of this electrolytes solution was adjusted to 7 using hydrochloric acid (HCl).

The enzyme α-amylase from *Bacillus sp.* (A6380, type II-A, 843 U/mL, Sigma-Aldrich) was added just before each test to the SSF to obtain the artificial saliva. Enzyme activity was determined using the amylase activity assay recommended by Brodkorb et al. 2019. Briefly, the rate at which maltose is released from starch is measured by its ability to reduce 3,5-dinitrosalicylic acid (Bernfeld, 1955). One unit releases 1.0 mg of maltose equivalent from starch in 3 min at pH 6.9 and 20 °C. The amount of α-amylase in the artificial saliva was adjusted to obtain an enzyme activity of 75 U/mL in the final mixture with the thickened fluid.

Saliva

Saliva was collected from two healthy donors (women between 20 and 35 years old) in the morning, prior to experimentation. Volunteers did not consume any food after brushing their teeth 2 h before saliva recollection. Saliva secretion was mechanically stimulated by chewing a piece of Parafilm (5x5 cm), and 15 mL of saliva was collected from each donor over a period of 30 min. Saliva samples were stored at room temperature for less than 3 h until use.

Characterizing the evolution of the viscosity during in vitro oral digestion

The insalivation ratio, food:saliva ratio of 5:1, was determined experimentally during preliminary tests. The incorporation of saliva was evaluated according to Drago et al. [15]. Briefly, healthy volunteers took a teaspoon of water thickened with TU (IDDSI level 3), kept the product in mouth for 30 s, and spat it in a container. The ratio of saliva added in the bolus with respect to the wet food sample (h_w) was 0.22 ± 0.05, meaning that approx. 0.2 g of saliva were incorporated /g of thickened water.

Oral digestion was stimulated using a Modular Compact Rheometer 102 (Anton Paar GmbH, Graz, Austria) fitted with a starch stirrer cell. The evolution of the apparent viscosity of each sample was recorded at 37°C under a shear rate of $\dot{\gamma}$ = 250 s^{-1}. This shear rate was chosen to obtain good mixing between sample, saliva, and α-amylase inhibitor during the test, it does not represent the shear rate to which thickened liquids are subjected to in the mouth. Conversely, the shear rate of thickened bolus flowing in the pharynx has been estimated as high as 300 s^{-1} [16].

First, 20 g of the fluid were added to the starch pasting cup, and sheared for 5 min. Then, 4 mL of saliva, artificial saliva or SSF (control without enzyme) were added during the test on top of the sample with a pipette.

The evolution of the viscosity of the product was recorded over 2 min, after which 60 µL of hydrochloric acid (6 M) were added to the starch pasting cup to decrease the sample pH under 2.5, and stop the enzymatic reaction [10]. This incubation time of 2 min is recommended by the Infogest network to simulate the oral phase of digestion of solid foods [14].

Finally, the digested sample was retrieved from the starch pasting cup, stored briefly at room temperature, before further measurements.

The evolution of viscosity with time, due to the action of α-amylase were modeled using the following exponential decay function:

$$d\eta_r/dt = -\lambda(\eta_r)^n \quad (1)$$

The integrated form of this equation is:

$$\eta_r(t) = \eta_{r_0} e^{-\lambda/t} \text{ (for n=1)} \quad (2)$$

Where: $\eta_r(t)$ is the relative viscosity at time t, calculated as a ratio of the absolute viscosity and η_{r_0}, the initial relative viscosity (at time t = 0), and λ is the rate constant. Derived parameters from this model were the decay rate k (3), and the half life $t_{1/2}$ (4):

$$k = \frac{1}{t_1} \quad (3)$$

$$t_{1/2} = t_1 \ln(2) \quad (4)$$

Steady shear tests

The shear viscosity of the thickened samples before and after *in vitro* oral digestion was assessed with a double gap measuring system on the same rheometer, at 37°C. Three repetitions were performed for each experimental condition. Flow curves in a range of shear rates between 1 and 500 reciprocal seconds were obtained, and fitted with a Power law model:

$$\eta_a = K \dot{\gamma}^{n-1} \quad (5)$$

Where: n is the Power law index, and K the consistency index of the Power law model.

IDDSI flow test

The IDDSI level of each fluid before and after *in vitro* oral digestion was evaluated at room temperature. A standard luer slip tip syringe was first filled up to the 10 mL mark with the sample. Then, the liquid was allowed to flow for 10 s. Based on the remaining volume left in the syringe, liquid samples were categorized in four levels of increasing thickness: Level 0 (less than 1 ml remaining), Level 1 (1–4 ml remaining), Level 2 (4–8 ml remaining), or Level 3 (8–10 ml remaining) (IDDSI, 2019).

Statistical analysis

Data were first tested for normality and since they were not normally distributed, non-parametric statistical tests were selected. Kruskall-Wallis tests were used to study differences among samples. Conover-Iman tests were then used to determine the significant differences among samples ($p < 0.05$). All analyses were performed with XLSTAT statistical software (version 2020.3.1.27, Microsoft Excel, Adinsoft, Paris, France).

Results & Discussion

Viscosity decay kinetics

Figure 1 shows the changes in viscosity of the thickened water samples occurring during the first 30 s of the oral digestion *in vitro*. Similar results were observed for the mineral water thickened with TU (Fig. 1a), and the deionized water thickened with the WMS (Fig. 1b). This was expected since both are made of modified maize starch. The different pH of the water samples (cf. Table 2) did not influence the enzymatic reaction.

When the artificial saliva without α-amylase was added to the thickened water samples viscosity decreased during the first 2 s of the test, and rapidly reached a plateau. This is attributed to a dilution effect. Conversely, when saliva was added to thickened water samples a more important, but slower decay in viscosity was measured. After only 5 s, initial viscosity value was reduced by 80%. No further changes were observed after 20 s. This decrease is due to the breakdown of the starch granule structure by the salivary α-amylase, coupled to the dilution effect of saliva.

A similar viscosity decay was observed with the artificial saliva, meaning that in this range of pH (5.3 to 7.4) it was possible to reproduce the kinetics of structure breakdown occurring with human saliva using α-amylase from *Bacillus* sp. with an enzyme activity of 75 U/mL in the final mixture as recommended by the INFOGEST network (Table 1). In this study, a relatively high shear rate of $\dot{\gamma} = 250\ s^{-1}$ was chosen to optimize mixing, which is not representative of the food mixture with saliva in the mouth, although similar shear rates are reached during swallowing.

In previous studies measuring the rheological properties of starchy semi-solid foods under oral conditions *in vitro*, the decrease in viscosity has been reported within the first 10 to 30 s of contact between sample and saliva [17–23]. However, it must be noted that different samples (starch slurries, custards, purees, etc.), conditions (temperature, food: saliva ratio, shear rate, etc.), enzymes (human saliva or artificial saliva with different compositions), and devices (Rapid Visco Analyzers, controlled stress rheometers, or custom-built devices) have been used in each study, making comparison among them difficult.

In vivo studies of this phenomenon are scarcer. Suiter et al. [24] investigated the changes in viscosity of water samples thickened to nectar consistency with a corn starch-based thickener that were kept in mouth up to 30 s by a panel of 10 healthy women. They reported a significant reduction in viscosity after only 10 s dwell time.

In this study, the initial viscosity of the samples was reduced by 80% after only 5 s, consistently with Figure 1. Such rapid changes seem relevant from a food oral processing point of view (i.e., mouthfeel, flavor perception, and swallow). Foods such as custard, jelly, mousse, or puddings generally require 3 to 5 s before being swallowed by healthy adults [25], which is enough to detect sensory attributes related to the surface and bulk

properties of the food such as thickness, creaminess, or melting for example [8,19]. The structural breakdown due to α-amylase also accelerates the release of volatile compounds from the matrix of the food, which may influence flavor perception. Ferry et al. [17] reported an increase in the number of volatiles released from basil flavored starch pastes after 6 to 12 s of incubation with α-amylase.

Furthermore, individuals suffering from swallowing difficulties may retain a bolus in the oral cavity for longer, in some cases up to 60 s [18]. The rheological properties of the bolus swallowed may therefore be different from the intended rheological properties of the drink (i.e., in the cup), which could lead to clinical complications [26,27].

Rheological properties of the thickened liquids after 2 min of enzymatic hydrolysis

Figure 2 shows the impact of the oral digestion *in vitro* on the thickened water samples. Similar results were observed for mineral water + TU (Fig. 2a), and deionized water + WMS (Fig. 2b).

Before *in vitro* oral digestion, thickened water samples were shear thinning liquids with a shear viscosity of approx. 850 and 450 mPa.s at $\dot{\gamma}$ = 50 s^{-1} for mineral water + TU, and deionized water + WMS, respectively. In control samples (i.e., without α-amylase) the shear viscosity at $\dot{\gamma}$ = 50 s^{-1} decreased by approx. 50% compared to the initial shear viscosity of the thickened water samples (Appendix 1). This diminution is again attributed to the dilution of the sample by saliva. The characteristic shear thinning behavior of gelatinized starch suspensions was maintained, meaning that starch granules were still intact. As expected, this diminution in viscosity decreased the amount of product left in the syringe during the evaluation of the IDDSI level of the fluid (Table 2). Depending on the initial viscosity of samples, dilution by saliva was enough to go from an IDDSI level 3 to an IDDSI level 2, which may be relevant clinically.

In samples containing saliva, the structure formed by starch granules was completely destroyed. After *in vitro* oral digestion, samples were Newtonian fluids with a shear viscosity of approx. 1 mPa.s, down to IDDSI Level 0 (Table 2), and similar to water before thickening. Hanson et al. [18] and Lee et al. [21] also reported a reduction of 99% of the initial viscosity of water samples thickened with starch-based thickeners after mixing with saliva for 60 s or more, even if they used different experimental conditions *in vitro*, and in particular a lower sample to saliva ratio (10:1).

This phenomenon has also been studied *in vivo*. Vallons et al. [7] determined the bolus viscosity of water samples thickened to honey consistency with a starch-based thickener, before and after being held in the mouth by 35 healthy adult volunteers. They measured a decrease in viscosity of approx. 55% and 70% after 10 and 20 s of oral processing, respectively. More recently, Bolivar-Prados et al. [26] measured the viscosity of thickened

water samples after oral incubation in a group of 5 healthy young volunteers. Samples containing modified maize starch were held in the mouth for 30 s and spat out for analysis. Authors reported a reduction in viscosity after oral incubation that ranged from 96.78 to 99.26% of their initial viscosity.

The same tendency was observed in samples containing the artificial saliva, meaning that it was possible to reproduce the structure breakdown occurring with human saliva in this type of samples. These results confirmed our previous observations during the first seconds of the oral digestion test in the starch stirring cell.

Effect of pH

Figure 3 shows the impact of the oral digestion *in vitro* on thickened drinks with different acidic pH. Neither the saliva nor the artificial saliva had an effect on the shear viscosity of the lemon drink (Fig. 3a). Only a dilution effect was observed, similar to the control without enzyme, meaning that starch was not hydrolyzed by α-amylase at pH 2.7. The artificial saliva SSF is therefore imitating well the effect of human saliva at this low pH too.

The addition of saliva to the orange juice (pH = 4) did not led to a significant decrease in shear viscosity compared to the control sample without α-amylase (Fig. 3b). This suggests a significant inhibition of the enzymatic activity due to the acidic pH of the thickened drinks and the relatively short incubation time used in this study (i.e., 2 min). Indeed, human salivary α-amylase optimum pH is between 6 and 7, and its activity is approximately reduced by half around pH 4, and under pH 3 to 3.5 it is completely inhibited [11]. Comparable results were obtained *in vitro* by Hanson et al. [18] with an orange juice having an acidic pH of 3.8. However, they used a larger food:saliva ratio (10:1), and measured the viscosity of their samples at 25°C, and only at $\dot{\gamma}$ = 40 s^{-1}. In another study from the same group [28], authors studied the effect of salivary α-amylase on the viscosity of drinks thickened with starch, simulating the clinical scenario where a cup is contaminated with the patient's saliva. They showed that lowering the pH of the drink systematically slowed the action of the salivary α-amylase, and that at pH ≤ 3.6 it completely stopped. Unfortunately, this effect has not been yet studied *in vivo*.

The artificial saliva (SSF) had an impact on the rheological properties of the thickened orange juice. A shear thinning behavior was still observed, but the shear viscosity of the sample at $\dot{\gamma}$ = 50 s^{-1} was reduced by 98% (Fig. 3b) and the liquid classified IDDSI Level 0 after the oral digestion (Table 2). This is probably due to a different optimum pH of the enzymes between saliva and artificial saliva. The α-amylase from Bacillus sp., used in the formulation of the artificial saliva (SSF) is active from pH 3.5 to 7.5 and has an optimum pH of 4.5 [29], very close to the pH of the orange juice used in this study. Consequently, starch hydrolysis and structure breakdown

in oral conditions are overestimated using α-amylase from Bacillus sp in this pH range (i.e., from 3 to 5) and human salivary α-amylase cannot be substituted.

Conclusions

In this study, a simple test *in vitro* was used to investigate quantitatively the impact of salivary α-amylase on the rheological properties of thickened liquids containing starch. An insalivation ratio of 5:1 (food: saliva), measured from spitted boli was used, and unstimulated human saliva was compared to an artificial saliva. Experiments provided new insights on the impact of food oral processing on the rheological properties of neutral and acidic starchy semi-solid foods.

The shear viscosity of thickened liquids between pH 5.3 and 7.4 was rapidly reduced by α-amylase. After only 5 s of contact with saliva or artificial saliva, the initial viscosity of the thickened water samples was reduced by 80%. This decrease was attributed to the breakdown of the starch granule structure by α-amylase, and in a lesser extent to the dilution effect of saliva. The kinetics of this reaction are relevant for food oral processing and swallowing, particularly for individuals suffering from swallowing difficulties who may keep a bolus in the oral cavity for more than 5 s.

On the contrary, the rheological properties of thickened drinks at low pH (2.7 to 4.0) were not influenced by human saliva: the thickened lemon drink and orange juice maintained their initial IDDSI level, despite a strong dilution with saliva. Clinical studies should be performed to confirm whether starch based thickened drinks (IDDSI level 1 to 3) with acidic pH (lower than 4), could be a cost-effective solution to manage dysphagia and a viable alternative to the more expensive XG-based formulations.

The method developed in this study can be used to quantify the impact of food oral processing, but only human salivary α-amylase should be used to study products between pH 3 and 5 to imitate the structural and rheological breakdown happening before swallowing, while α-amylase from *Bacillus sp.* could also be used outside this range of pH.

Conflict of interest

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339

340 **Tables**

341 Table 1: Parameters obtained from the exponential decay model.

	k (s^{-1})	$t_{1/2}$ (s)	R^2
Mineral water + TU			
+ Saliva	0.59 ± 0.07 a	1.18 ± 0.14 a	0.98 ± 0.01
+ Artificial saliva	0.55 ± 0.10 a	1.28 ± 0.26 a	0.97 ± 0.02
Deionized water + WMS			
+ Saliva	0.45 ± 0.11 a	1.59 ± 0.35 a	1.00 ± 0.00
+ Artificial saliva	0.56 ± 0.11 a	1.27 ± 0.27 a	0.96 ± 0.01

342

343 Table 2: Results from the IDDSI syringe test.

Sample	Condition	Volume remaining in the	IDDSI level

		syringe (mL)	
Deionized water +WMS (pH = 7.4)	Before oral digestion	9.0 ± 0.0	3
	+ Saliva	0.0 ± 0.0	0
	+ Artificial saliva (SSF)	0.0 ± 0.0	0
	+ SSF wo/ alpha-amylase	7.8 ± 0.3	2
Mineral water +TU (pH = 5.3)	Before oral digestion	9.5 ± 0.0	3
	+ Saliva	0.0 ± 0.0	0
	+ Artificial saliva (SSF)	0.0 ± 0.0	0
	+ SSF wo/ alpha-amylase	8.8 ± 0.7	3
Lemon drink +TU (pH = 2.7)	Before oral digestion	9.5 ± 0.0	3
	+ Saliva	9.0 ± 0.3	3
	+ Artificial saliva (SSF)	8.8 ± 0.8	3
	+ SSF wo/ alpha-amylase	8.5 ± 0.5	3
Orange juice +TU (pH = 4.0)	Before oral digestion	9.5 ± 0.0	3
	+ Saliva	8.0 ± 0.5	3
	+ Artificial saliva (SSF)	0.0 ± 0.0	0
	+ SSF wo/ alpha-amylase	8.8 ± 0.3	3

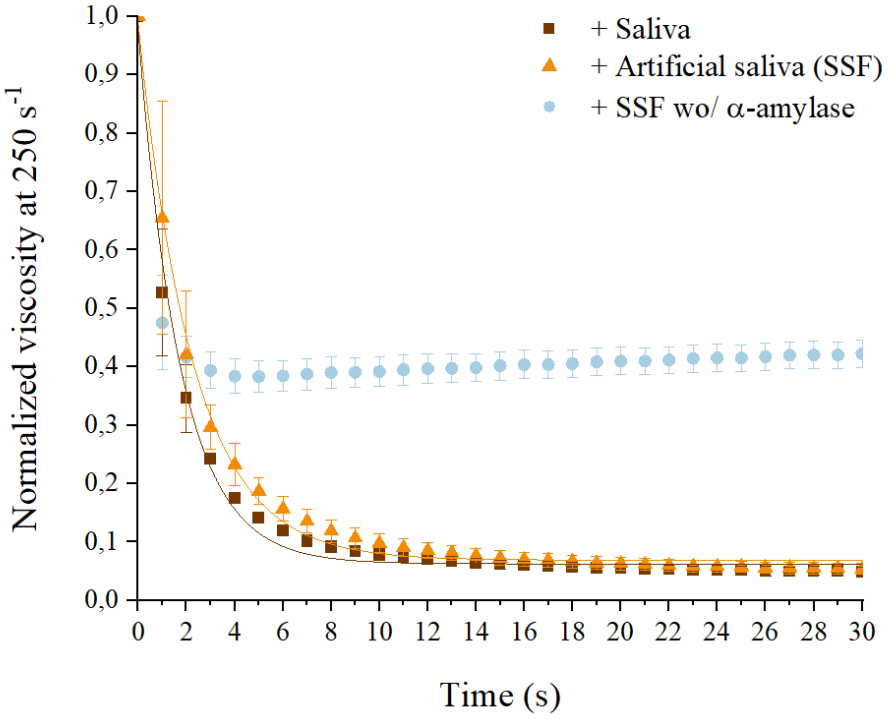
Legends

Figure 1: Evolution of the viscosity of aqueous solutions of (a) TU, or (b) WMS after addition of saliva, artificial saliva (SSF), or SSF without α-amylase (control). Continuous lines represent the exponential decay model.

Figure 2: Flow curves of aqueous solutions of (a) TU or (b) WMS, before and after *in vitro* oral digestion. Dashed lines represent the power law model.

Figure 3: Flow curves of (a) lemon drink (pH = 2.7) and (b) orange juice (pH = 4.0) samples thickened with TU, before and after *in vitro* oral digestion. Dashed lines represent the power law model.

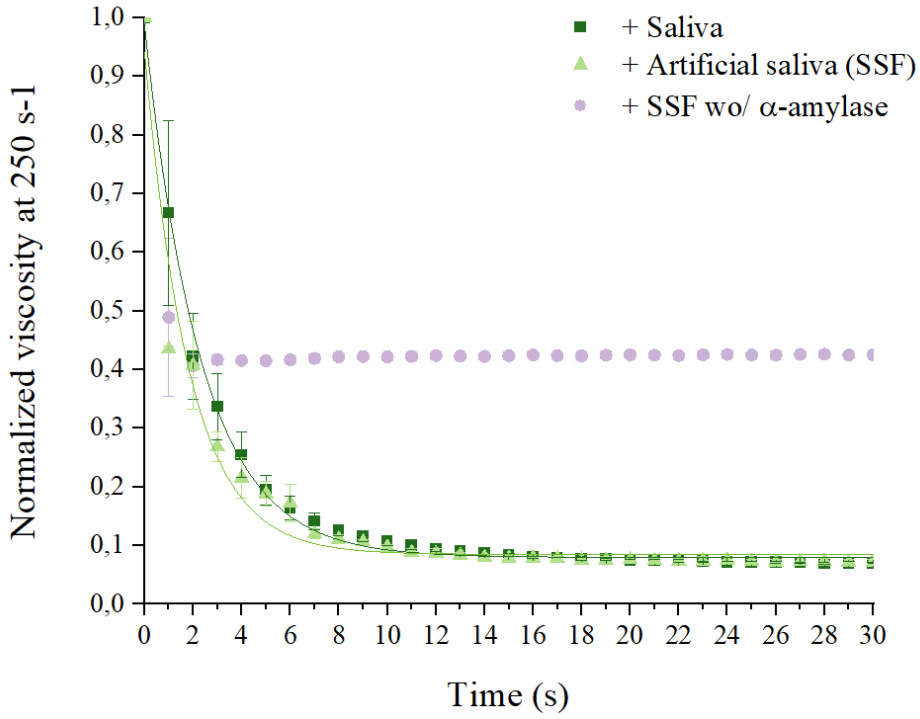
Figures



355 Fig. 1a

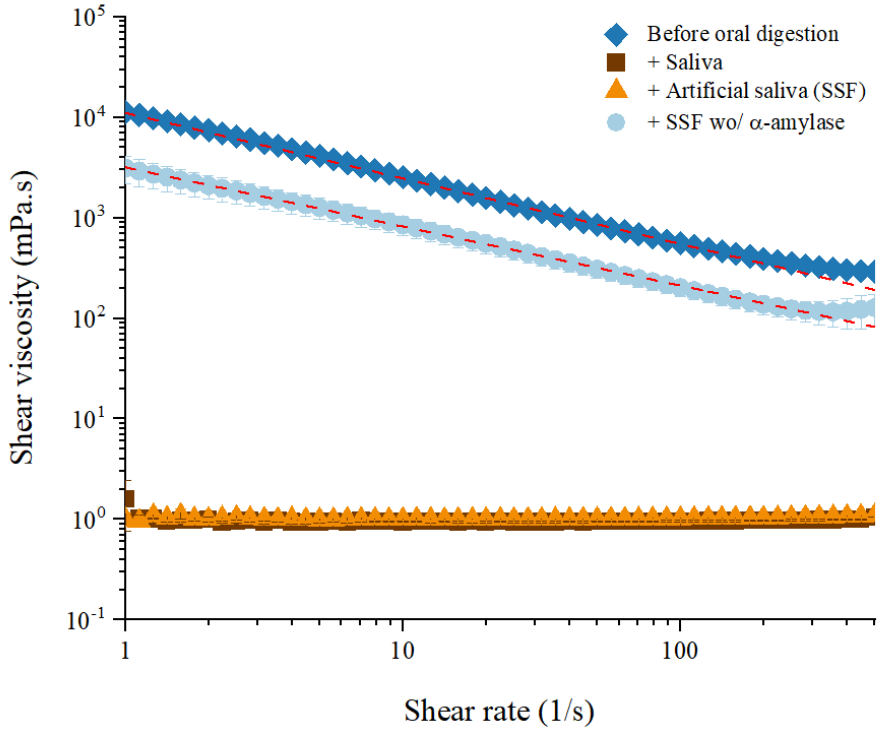
356

357 Fig. 1b



358

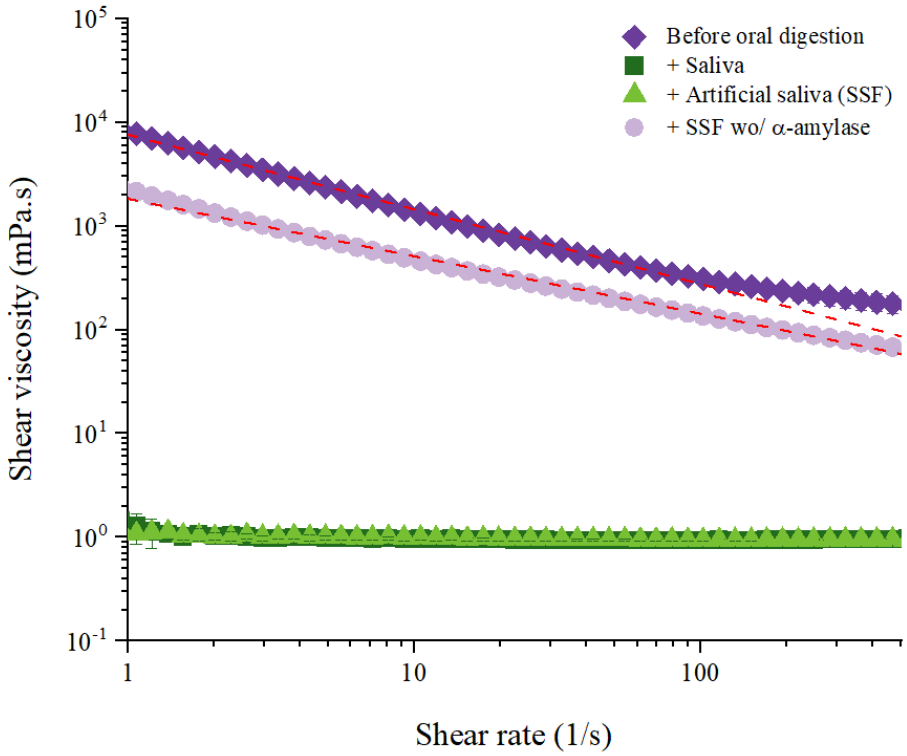
359 Fig. 2a



360

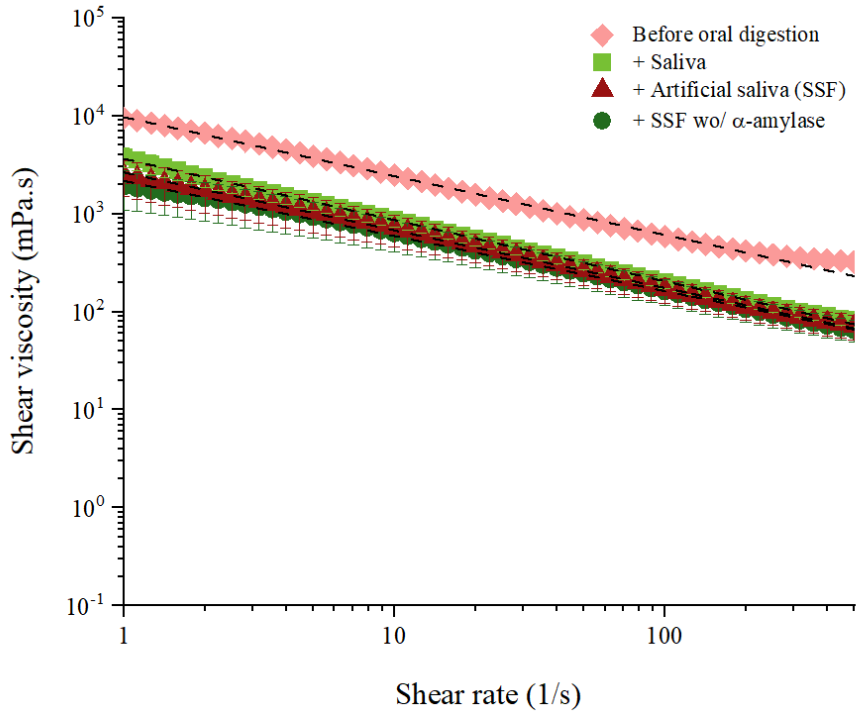
361 Fig. 2b

362

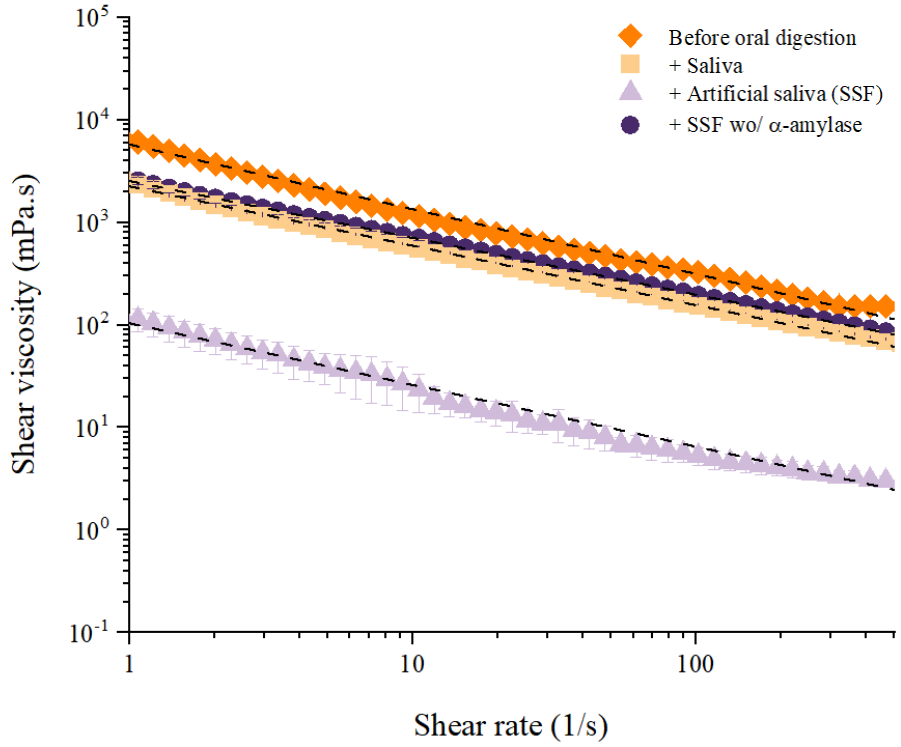


363

364 Fig. 3a



365



366 Fig. 3b

367

368