Impact of saliva incorporation on the rheological properties of in vitro gastric contents formulated from sour cream

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RESEARCH NOTE

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Impact of saliva incorporation on the rheological properties of in vitro gastric contents formulated from sour cream

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Abstract

Rheological properties of gastric contents depend on the food ingested, and on the volume and composition of secretions from the host, which may vary. This study investigates the impact of saliva regular incorporation in the stomach after a meal on the rheological properties of gastric contents, considering two levels of salivary flow (low = 0.5 and high = 1.5 mL/min). In vitro chymes were obtained by mixing sour cream, simulated gastric fluid, two different volumes of oral fluid (at-rest human saliva, SSF for Simulated Salivary Fluid or water) and adjusting pH at 3. Chymes samples were characterized at 37°C for their particle size and rheological properties. Overall, particle size distribution was not different between samples: incorporating a larger volume of saliva resulted in more heterogeneity, but the surface area moment D[3,2] and volume moment D[4,3] did not differ significantly with the oral fluid type. Shear viscosity of chyme samples was higher when saliva was incorporated, in comparison with water or SSF. In addition, as shown from data extracted at $\dot{\gamma} = 20\,s^{-1}$ the higher the fluid volume the lower the shear viscosity, which is attributed to a dilution effect. However, this dilution effect was attenuated in the case of saliva, most likely due to its composition in organic compounds (e.g., mucins) contributing to the rheological properties of this biological fluid. In these in vitro conditions, both saliva and the salivation rate had a significant but slight impact on the rheological properties of gastric contents (of the order of 1–5 mPa s at $\dot{\gamma} = 20\,s^{-1}$).

KEYWORDS
chyme, dairy, mucins, particle size, saliva, viscosity

1 | INTRODUCTION

The gastric phase of digestion performs most of the mechanical breakdown of particles present in food boli and initiates the enzymatic hydrolysis of macronutrients that will be completed further in the intestine. In addition, the stomach plays an important role in digestive kinetics through the rate of gastric emptying, with potential physiological impacts on satiety (Janssen et al., 2011) or glycemia (Marathe et al., 2015; Repin et al., 2017). The rate of gastric emptying depends on several interconnected factors: the volume and energy content of the food ingested (Calbet & MacLean, 1997; Hunt & Stubbs, 1975), the secretion of gastrointestinal hormones such as cholecystokinin, glucagon-like peptide 1, peptide YY or ghrelin among others (Camilleri, 2019), the physical properties of the food particles such as size, density, texture, and microstructure (Kong & Singh, 2008), and the rheological properties of gastric contents (Guerin et al., 2001).

Concerning the rheological properties of gastric contents, they are guided mainly by the food ingested. For example, pig gastric chymes from brown rice showed higher shear stress values than those from white rice containing less insoluble fibers (Bornhorst et al., 2013). A series of works have shown the impact on the rheological properties of gastric contents of different types of dietary fibers such as amylose and amylopectin (Patarin et al., 2015),

yellow mustard mucilage, soluble flaxseed gum, and fenugreek gum (Repin et al., 2017), or pectin (Wu et al., 2016). Rheology of gastric contents can also be influenced by the structure of casein clots, which itself can be modified by milk processing conditions (Li et al., 2022).

However, besides the ingested food itself, secretions from the host also contribute to the rheology of gastric chymes. First, the stomach responds to food ingestion by the secretion of gastric fluids leading to a rapid dilution of the gastric content. The volume of these secretions seems to vary depending on the properties of the food to achieve some sort of rheological homeostasis (Marciani et al., 2000; Wu et al., 2016). On the other hand, viscous components such as mucins are secreted in the stomach. Wu et al. (2016) observed that in vivo gastric contents obtained from pigs had higher viscosity and modulus than in vitro equivalents (made by diluting the food in water and adjusting the pH to reach the same conditions), because of the absence of gastric mucins. Human gastric aspirates (HGA) from fasting subjects were also compared to a simulated gastric fluid (containing pepsin, sodium taurocholate, phospholipids, and sodium chloride at pH 1.6), and to a hydrochloric acid (HCl) aqueous solution at pH 1.2. Despite a high inter-individual variability, most HGA exhibited a higher apparent viscosity and a shear-thinning behavior, which was not observed in the simulated gastric fluid and the HCl solution (Pedersen et al., 2013). This effect was attributed by the authors to the presence of gastric mucins in HGA.

Another potential source of mucins in gastric contents is the mucins secreted in saliva. Thus, during eating, saliva is first incorporated into the food bolus that reaches the stomach when swallowed. However, salivation is not limited to the time of eating. Throughout the day and apart from the sleep period, saliva is continuously secreted into the oral cavity and swallowed. As a consequence, after a meal, salivary mucins are also progressively incorporated in the gastric contents during the time of food residency in the stomach, which could influence the rheological properties of the chyme. According to Repoux et al. (2012), resting and stimulated salivary flows may vary from 0.1 to 1.2 mL/min, and from 0.7 to 5.3 mL/min, respectively. Another article estimated that, excluding pathological conditions, the total output of saliva varied from 0.5 to 1.5 L per day (Humphrey & Williamson, 2001). Considering an 8 h sleep period, this would result in an average flow of 0.5–1.6 mL/min. Such inter-individual variability in salivation rates could lead to different dilution levels and/or salivary proteins (including mucins) concentrations in gastric contents, but it has been overlooked so far in digestion studies. Furthermore, mucins are able to induce droplet flocculation in emulsions (Chang & McClements, 2016; Vingerhoeds et al., 2005) and influence their rheological properties differently depending on the type of emulsion studied (Silletti et al., 2008).

In this context, the present study aimed at investigating the impact of two different levels of salivary fluid incorporation on the rheological properties of gastric contents, containing a semi-solid dairy emulsion (i.e., sour cream), and produced in vitro using saliva or model digestive fluids.

2 | MATERIALS AND METHODS

2.1 | Materials

Sour cream was selected among semi-solid dairy emulsions because of its composition (high fat and low protein content) and because of its low pH (approx. 4.5) to avoid major changes in the structure of the product due to the coagulation of milk proteins in the acidic environment of the stomach. The sour cream used in this study was from a local supermarket ("crème fraîche épaisse entière Carrefour Classic," 2.91 kcal/mL, containing 30% lipids, 2.9% carbohydrates, and 2.4% proteins). Simulated Salivary Fluid (SSF) and Simulated Gastric Fluid (SGF) were used to simulate digestive fluids and were prepared according to the recommendations of the INFOGEST network (Brodkorb et al., 2019). Neither enzymes nor mucins were added to the digestive fluids. In order to minimize variations in saliva composition, fresh at-rest saliva was collected from a single donor, a 22-year-old male healthy subject. The donor was instructed to let saliva accumulate naturally in the mouth and to spit it out regularly into a cup placed on ice for 20 min. Saliva collection started at 9 AM, each day prior to experimentation. Saliva was stored on ice for a maximum of 6 h and used whole. Saliva was not clarified by centrifugation in order to retain the original content in salivary mucins, which are important contributors to the rheological properties of saliva. The choice of using at-rest saliva was guided by the relatively short time during which saliva flow or proteome composition is modified after stimulation by a pure tastant (Bader et al., 2018; Neyraud et al., 2009) or after ingestion of various snack foods (Simões et al., 2021). Although precise durations are not known and would depend on the stimulus or food considered, saliva is probably not altered profoundly for more than a few minutes, i.e., far less than the gastric emptying half-time of a typical meal.

2.2 | Preparation of the simulated gastric contents

Cream (20 mL) and SGF (20 mL) were preheated to 37°C and mixed before adding either fresh saliva, SSF, or distilled water. The 1:1 (v/v) dilution of food and SGF is as recommended in the INFOGEST protocol (Brodkorb et al., 2019). Two different rates of salivation, chosen based on the daily saliva output reported in Humphrey and Williamson (2001) were simulated: (1) a low salivary flow of 0.5 mL/min, or (2) a high salivary flow of 1.5 mL/min. The gastric emptying half-time ($T_{0.5}$ in min) for 20 mL of sour cream was calculated following the equation from Hunt and Stubbs (1975):

$$T_{0.5} = V \times (0.1797 - 0.1670e^{-k})$$

where V is the volume of food ingested (mL), k the calorie density of the food (kcal/mL).

The volume of oral fluid added to the gastric content sample was then $T_{0.5} \times 0.5$ or $T_{0.5} \times 1.5$ to simulate a low or high salivary flow, respectively. According to these calculations, 1.71 mL or 5.12 mL of

oral fluid was therefore added to the gastric content samples. Finally, the pH was adjusted to 3 with approx. 200 µL of HCl (5 M) under stirring (300 rpm). Three replicates were prepared for each condition. This protocol produces model gastric contents that can be considered as a proxy of chymes at one time point, namely gastric emptying half-time.

2.3 | Rheological properties

The rheological properties of the samples were measured with a Modular Compact Rheometer Physica 301 (Anton Paar GmbH, Graz, Austria) equipped with a Peltier plate, and a concentric cylinder system (CC17). The shear viscosity of the samples was measured at 37°C, 10 ± 1 min after the addition of the oral fluid. Flow curves in a range of shear rates ($\dot{\gamma}$) between 10 and 200 reciprocal seconds (s^{-1}) were obtained, and fitted in a range of $\dot{\gamma}$ of 10–100 s^{-1} with the Herschel-Bulkley model:

$$\tau = \tau_0 + K \cdot \dot{\gamma}^n$$

where τ and γ are the shear stress (Pa) and the shear rate (s^{-1}), respectively. τ_0 is the yield stress (Pa), K is the consistency index (Pa s) corresponding to the viscosity at $(\dot{\gamma}) = 1\ s^{-1}$, and n is the flow index (dimensionless) representing the behavior of the fluid (if $n < 1$ the fluid is shear thinning, if $n = 1$ the fluid is Newtonian, and if $n > 1$ the fluid is shear thickening). Three repetitions were performed for each experimental condition.

2.4 | Particle size distribution

The particle size distribution of the samples was measured by laser diffraction using a MasterSizer 2000 (Malvern Instruments, Malvern, UK) immediately after launching the rheological data acquisition. The refractive indexes used were 1.33 for the dispersant (water), and 1.458 for the particles (cow milk fat). Gastric contents measurements were done at room temperature in Milli-Q water (pH 7). The cream was also measured in Milli-Q water adjusted at pH 3 with hydrochloric acid at room temperature, and at 37°C. Additionally, sodium dodecyl sulfate (SDS), an anionic surfactant, was added to the cream (1% v/v) and measured at pH 7 and room temperature. Three repetitions were performed for each experimental condition. Results obtained were the size distribution in volume, the surface area moment mean D[3,2] which better represents the presence of fine particles, and the volume moment mean D[4,3] more representative of the presence of large particles.

2.5 | Statistical analysis

Results are shown as mean ± standard deviation. Statistical significance of the results ($p \leq .05$) was tested using Kruskal–Wallis test, and Tukey's multiple pairwise comparisons between groups. Data analyses were performed using the R software, version 4.2.2.

3 | RESULTS

3.1 | Rheological properties of the simulated gastric contents and the unstimulated saliva

Flow curves obtained in steady shear are presented in Figure 1a, and the shear viscosities of the samples at $\dot{\gamma} = 20\ s^{-1}$ are detailed in Figure 1b, as well as the parameters obtained with the Herschel-Buckley (HB) model in Table 1. All the samples showed a light shear thinning behavior across the range of shear rates studied (10–200 s^{-1}) with some differences (Figure 1). The fresh unstimulated saliva had the lowest shear viscosity, and was very close to the behavior of a Newtonian fluid, with $\tau_0 < 10$ mPa, $K < 5$ mPa s, and $n \approx 0.9$. These results are consistent with data reported in the literature (Schipper et al., 2007). The shear viscosity of the chyme samples with saliva was higher than the others up to $\dot{\gamma} = 50\ s^{-1}$ (Figure 1a). When comparing parameters from the HB model (Table 1), the yield stress (τ_0) was significantly higher with saliva than with the other fluids (approx. 75 mPa with saliva vs <40 mPa with water or SSF), but no significant differences were observed in the consistency index (K) among samples. Concerning the flow index (n), a significant difference was observed

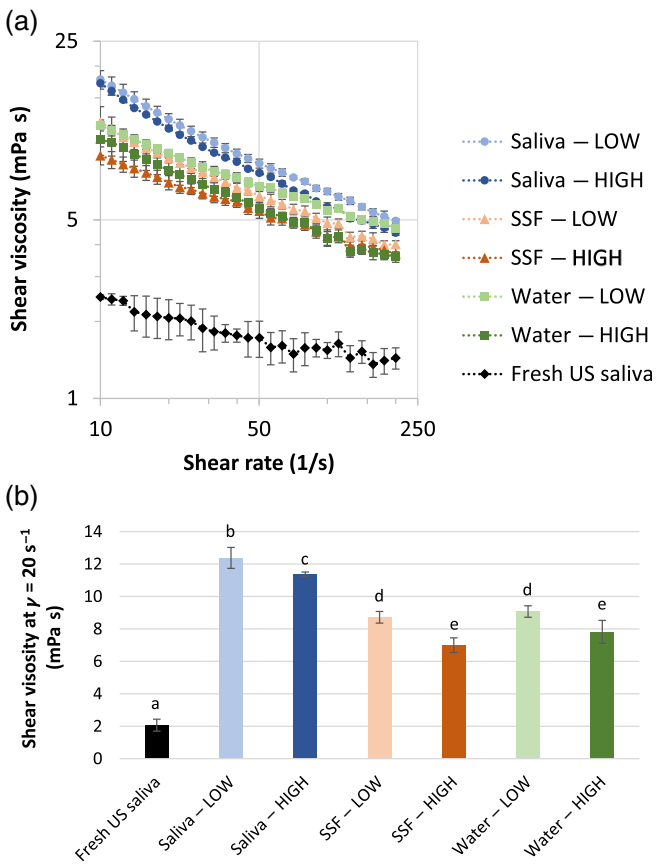

FIGURE 1 Steady shear viscosity (a) and shear viscosities at $\dot{\gamma} = 20\ s^{-1}$ (b) of the fresh unstimulated (US) saliva and the different simulated gastric contents ($n = 3$ for each experimental condition). HIGH, simulated high level of salivary flow; LOW, simulated low level of salivary flow; SSF, simulated Salivary Fluid. The same letter is used when the difference is not significant ($p \geq .05$).

TABLE 1 Parameters obtained from the Herschel Bulkley model ($n = 3$ for each experimental condition).

	τ_0 (mPa)	K (mPa s)	n	R^2
Fresh saliva	6.71 ± 5.65 a	2.82 ± 1.53 a	0.89 ± 0.11 a	.99
Saliva	76.61 ± 13.97 b	17.53 ± 2.55 b	0.74 ± 0.03 b	.99
SSF	21.28 ± 13.25 c	16.09 ± 3.28 b	0.73 ± 0.03 b	.99
Water	31.71 ± 11.44 c	13.47 ± 3.01 b	0.78 ± 0.03 c	.99

Note: Data from samples containing the same type of salivary fluid were averaged. The same letter is used in a column when the difference is not significant ($p \geq .05$).
Abbreviations: K, consistency index; n, flow index; SSF, Simulated Salivary Fluid; τ_0, yield stress.

between samples containing water and SSF ($n \approx 0.8$ and ≈ 0.7, respectively), showing that chyme samples with SSF had a slightly stronger shear thinning behavior. Then, differences in shear viscosity were observed among the chyme samples depending on the volume of oral fluid added (Figure 1b). At $\dot{\gamma} = 20\,s^{-1}$, all the samples with high levels of incorporated fluids (HIGH) had significantly lower shear viscosities than the corresponding samples with low levels of incorporated fluids (LOW), but the decrease depended on the type of oral fluid used (−8%, −14%, and −20% for saliva, water, and SSF respectively, Figure 1b). No significant differences in parameters from the HB model were observed according to the level of incorporated fluid (data not shown).

3.2 | Particle size distributions in the simulated gastric contents and sour cream

The surface area moment mean D[3,2] and volume moment mean D[4,3] corresponding to particle sizes in the different chyme samples are detailed in Table 2, and selected particle size distribution curves are presented in Figure 2. Similar particle size distributions were observed for all the experimental conditions studied with one relatively large peak around 25 µm, and a few particles measured around 3 µm (cf. water – LOW on Figure 2); except for saliva-HIGH in which smaller (<1 µm) or larger (>250 µm) particles appeared on some of the replicates (Figure 2) resulting in very high standard deviation values for D[3,2] and D[4,3] (Table 2). No significant differences were observed in D[3,2] and D[4,3] values depending on the type of oral fluid used. Regarding the level of fluid incorporation, significantly smaller particles were observed when increasing the volume of water in the sample (i.e., water LOW vs HIGH), but it was not the case with SSF or saliva (no significant differences observed).

Particle size distribution in the sour cream used in this study was also characterized by different conditions of pH, temperature, and surfactant. The pH modification (from 7 to 3) did not influence particles size in cream (D[3,2] ≈ 15 µm, and D[4,3] ≈ 25 µm), but raising the temperature of the Milli-Q water did: particle size distributions shifted toward larger size values, suggesting that the size of lipid

TABLE 2 Surface area moment mean D[3.2] and volume moment mean D[4.3] extracted from particle size distributions measured in the different chyme samples ($n = 3$ for each experimental condition).

	D[3,2]	D[4,3]
Saliva – LOW	22.85 ± 0.52 a	36.65 ± 2.44 a
Saliva – HIGH	16.52 ± 14.80 a	34.99 ± 20.14 a
SSF – LOW	18.65 ± 2.83 a	28.33 ± 4.21 a
SSF – HIGH	21.83 ± 0.23 a	32.07 ± 0.94 a
Water – LOW	21.60 ± 2.20 a	32.33 ± 4.40 a
Water – HIGH	17.22 ± 2.54 b	25.20 ± 3.08 b

Note: No significant differences were measured between samples of different fluid types ($p \geq .05$), so data were compared pairwise according to the level of oral fluid incorporated (the same letter is used when the difference is not significant, $p \geq .05$).
Abbreviations: D[3,2], surface area moment; D[4,3], volume moment; HIGH, simulated high level of salivary flow; LOW, simulated low level of salivary flow; SSF, Simulated Salivary Fluid.

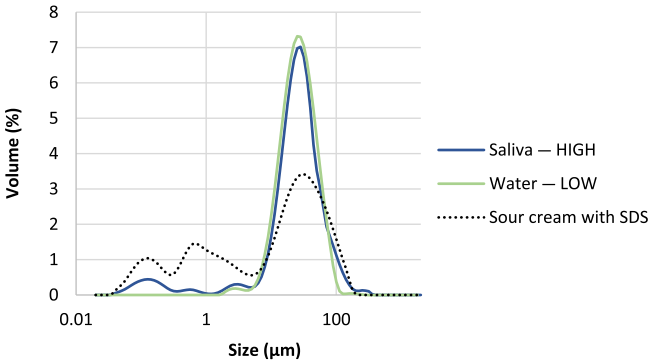

FIGURE 2 Particle size distributions in representative samples ($n = 3$ for each experimental condition). HIGH, simulated high level of salivary flow; LOW, simulated low level of salivary flow; SDS, sodium dodecyl sulfate (anionic surfactant).

droplets in the cream increased with temperature (D[3,2] ≈ 24 µm, and D[4,3] ≈ 38 µm, at 37°C and pH 3). The addition of an anionic surfactant (SDS) to the cream resulted in a modification of the particle size distribution measured and particles were observed around 0.15, 0.9, 3, and 36 µm (Figure 2).

4 | DISCUSSION

In this study, we investigated the impact of two different levels of salivary fluid incorporation on the shear viscosity of in vitro gastric contents formulated from sour cream, using fresh at-rest human saliva, SSF, or water. We also measured particle size distributions in the samples to evaluate the stability of the emulsion.

Sour cream is a thick fermented dairy product containing approx. 30% fat, and 2% proteins, with an acidic pH around 4.5. The microstructure of sour cream is made of lipid droplets covered by proteins and bacteria, both interacting with the casein network formed at

acidic pH (Lopez et al., 2015). The size of lipid droplets in this type of product may vary from 0.2 to 30 μm depending on the process used to produce the sour cream (e.g., homogenization, heat treatment, etc.) (Lopez et al., 2015). Most of the particles observed in this study, in the in vitro gastric contents, were in this range of sizes. The larger particles detected probably corresponded to aggregated structures of proteins and lipid droplets.

Overall, the type of oral fluid used did not influence particle size distributions in the chyme samples. Differences observed in terms of rheological properties are therefore probably not related to lipid droplet size distributions in the diluted emulsions formed between the sour cream and the digestive fluids. However, the level of fluid incorporation had a different impact on particle sizes depending on the type of fluid studied. No effect was observed with SSF, but the addition of a larger volume of water led to smaller particle sizes. This may be related to a decrease of the ionic strength in the continuous phase of the emulsion, as a result of diluting with water, modifying lipid droplet interactions with each other and/or with protein aggregates. On the other hand, the addition of a larger volume of saliva resulted in more heterogenous gastric content samples, probably because of a partial emulsion destabilization. A similar effect was observed when adding SDS, an anionic surfactant, to the cream. This observation is in line with various studies that have shown droplets flocculation in emulsions mixed with simulated oral fluids containing mucins (Liang et al., 2018; Lv et al., 2019; Yang & McClements, 2013), which are also negatively charged. These differences in particle sizes did not, however, influence the rheological properties of the gastric content samples.

Since shear rates in the gastrointestinal tract probably vary considerably depending on location and motility (Dikeman et al., 2006), the shear viscosity of the samples was measured across a large range of shear rates. However, using computational fluid dynamics, Kozu et al. (2010) calculated that the shear rate due to gastric fluid motion and wall deformation could reach a maximum of approximately 20 s^{-1} in the antrum for single-phase liquid systems. Therefore, the value of the shear viscosity at $\dot{\gamma} = 20\,s^{-1}$ was used to compare the rheological properties of the different gastric contents formulated in this study.

The rheological properties of samples containing fresh saliva were different compared to samples with SSF or water (higher shear viscosity and higher yield stress), which is probably related to differences in the initial properties of these fluids. Whole at-rest saliva is a viscoelastic non-Newtonian fluid with specific shear thinning and extensional properties (Gittings et al., 2015; Haward et al., 2011; Schipper et al., 2007; Stokes & Davies, 2007; Wagner & McKinley, 2017). On the other hand, SSF and water are Newtonian fluids (i.e., viscosity of approx. 1 mPa s at 37°C across a large range of shear rates), with no extensional properties.

Differences in the rheological properties of the gastric content samples were mostly observed at low shear rates (<50 s^{-1}) relevant to the gastric phase of digestion. The low and high levels of oral fluids incorporated in this study led to differences in the shear viscosity of the gastric contents: the higher the fluid volume the lower the shear viscosity, which is attributed to a dilution effect. Interestingly, this dilution effect was limited when fresh saliva was used but not when SSF was used. It seems that the organic constituents of saliva were the ones that partly counter the dilution effect and that it was not related to the ionic content of the fluids. The organic fraction of saliva is composed mainly of proteins, which are extremely diverse in nature and relative abundance (Denny et al., 2008). However, only a few classes of proteins have been related to the viscoelastic properties of saliva, for example mucins and statherin (Levine, 1993). Mucins, particularly the higher molecular weight mucins MUC5B, dominate the literature in the field. Besides the intrinsic rheological characteristics of saliva, when in contact with food, interactions between salivary proteins and various dietary constituents may occur. First, and of special relevance to sour cream, interactions between dairy proteins and salivary proteins have been described in recent reviews (Brown et al., 2021; Çelebioğlu et al., 2020). Again, the focus was mainly on mucins, and both articles concluded that interactions may take place mainly through electrostatic interactions, with an impact on mouthfeel perception. It is not excluded that such interactions may also impact the rheological properties of the chymes, although in the context of digestion, interactions would occur with milk protein aggregates formed at pH 3 rather than with individual proteins. Second, mucins could also interact with lipid droplets leading to flocculation (Bourlieu et al., 2015; Liang et al., 2018; Wang et al., 2021), which can also influence the rheological properties of emulsions (Silletti et al., 2008). Finally, the addition of these mucin oligomers in the continuous phase of the emulsion (i.e., the cream diluted in digestive fluids) may increase its viscosity (Barnes, 1994). To conclude, the specific rheological properties of the chyme samples containing saliva are therefore probably related to the presence of high-molecular-weight glycoproteins such as mucins in saliva.

One limitation of this study is that the rheological properties of the samples were characterized at 37°C and at pH 3, but without gastric enzymes that may modify the viscosity of gastric contents during digestion as evidenced by Devle et al. (2012). It would be interesting to monitor viscosity changes in the presence of gastric enzymes to verify if the addition of saliva is impacting the rheological properties of the chyme for the entire duration of the gastric phase or not. Also, this work used a static approach and neither the rate of gastric emptying nor the rate of gastric secretions were considered.

In spite of those limitations, this study demonstrates that both saliva and the salivation rate can have a significant impact on the rheological properties of gastric contents. However, only slight differences in the shear viscosity of the gastric contents were observed (of the order of 1–5 mPa s at $\dot{\gamma} = 20\,s^{-1}$) and it is unclear whether these differences can impact on digestive processes such as gastric emptying, gastric mixing, or enzyme diffusion. Nevertheless, it has been shown that small variations in the viscosity of aspirated and simulated human gastric fluids can significantly influence drug dissolution from solid oral dosage forms (Pedersen et al., 2022). Mucin concentration in saliva and the salivation rate of individuals may therefore have an effect on drug delivery. Further research is needed to understand if gastric digestion of foods such as emulsions could be affected in individuals with very low salivary secretions (i.e., suffering from xerostomia).

AUTHOR CONTRIBUTIONS

Anaïs Lavoisier: Investigation; writing – original draft; methodology; formal analysis; data curation. **Tino Jamme:** Investigation. **Florence Rousseau:** Investigation; supervision; methodology. **Martine Morzel:** Conceptualization; writing – review and editing; supervision; project administration.

CONFLICT OF INTEREST STATEMENT

The authors declare that they do not have any conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

ETHICS STATEMENT

This study does not involve any human or animal testing.

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