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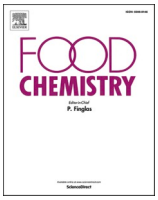

The effect of cations and epigallocatechin gallate on in vitro salivary lubrication

Georgios Agorastos [a,b,c,*], Eva van Uitert [c], Emo van Halsema [b], Elke Scholten [c], Aalt Bast [a], Peter Klosse [b]

[a] Faculty of Science and Engineering, Campus Venlo, Maastricht University, Nassaustraat 36, 5911 BV Venlo, the Netherlands
[b] T.A.S.T.E. Foundation, 6611 KS, Garstkampsestraat 11, Overasselt, the Netherlands
[c] Physics and Physical Chemistry of Food, Wageningen University, Bornse Weilanden 9, 6708 PG Wageningen, the Netherlands

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ABSTRACT

Ionic valency influences oral processing by changing salivary behavior and merits more attention since little is known. In this study, the influence of three ionic valences (monovalent, divalent and trivalent), ionic strength and epigallocatechin gallate (EGCG) on lubricating properties of saliva were investigated. Tribological measurements were used to characterize the lubrication response of KCl, $MgCl_2$, $FeCl_3$, and $AlCl_3$ in combination with EGCG to the ex vivo salivary pellicle. KCl at 150 mM ionic strength provided extra lubrication via hydration lubrication. Contrarily, trivalent salts aggregated together with the salivary mucins via ionic cross-link interactions, which led to a decrease in salivary lubrication. $FeCl_3$ and $AlCl_3$ affected the salivary lubrication differently, which was attributed to changes in the pH. Finally, in presence of EGCG, $FeCl_3$ interacted with EGCG via chelating interactions, preventing salivary protein aggregation. This resulted in less desorption of the salivary film, retaining the lubrication ability of salivary proteins.

1. Introduction

The perceived mouthfeel of beverages and foods has an influence on consumer acceptance and prospective consumption (Silletti et al., 2007). Although rather underestimated, saliva plays an essential role in mouthfeel sensations (Mosca & Chen, 2017). Saliva is a biolubricant that covers all surfaces of the oral cavity. Changes in salivary lubrication can result in different mouthfeel sensations. Astringency is such a mouthfeel attribute, which is linked to changes in salivary behavior, due to interactions with components present in food and beverages. In particular, the interactions between polyphenols and salivary proteins have been connected to astringency (Laguna et al., 2019).

Astringency is a drying and puckering sensation elicited by food products with high polyphenol content, such as wine, tea, beer, chocolate, olive oil, etc. (Hufnagel & Hofmann, 2008). In addition to polyphenols, multivalent cations can also cause an astringent mouthfeel (Biegler et al., 2016; Breslin et al., 1993; Lim & Lawless, 2005). The exact chemosensory mechanism of astringency still remains unknown. The two current hypotheses suggest that astringent sensation results either from the salivary lubrication loss by the aggregation of salivary proteins caused by polyphenols or multivalent cations (Canon et al., 2018; Gawel et al., 2018; Mosca & Chen, 2017) or by direct interaction (receptor-based) of the polyphenols and salts with oral receptors (Bajec & Pickering, 2008).

Several salivary proteins, such as proline-rich proteins (PRPs) or calcium-binding statherin (Soares et al., 2011) have been suggested as the main salivary proteins responsible for an astringent sensation. However, the role of mucoproteins has been underestimated in this respect. A study by Biegler et al. (2016) showed the importance of mucoproteins in salivary lubrication. Mucins are proteins with high molecular weight carrying highly dense oligosaccharide side chains. Mucins play an important role in oral lubrication and can be divided into 2 groups, based on their molecular weight; the high molecular-weight MUC5B (2000–4000 kDa), and the low molecular weight MUC7 (130–180 kDa) (Gibbins et al., 2014). Based on their amphiphilic character, mucins can attach to various surfaces and due to steric

Abbreviations: EGCG, Epigallocatechin gallate; MUC5B, Largest major saliva mucin; MUC7, Major saliva mucin; pI, Isoelectric point; PRPs, Proline-rich proteins; aPRPs, Acidic proline-rich protein; gPRPs, Basic proline-rich protein; SDS-PAGE, Sodium dodecyl sulphate polyacrylamide gel electrophoresis.

* Corresponding author at: Faculty of Science and Engineering, Campus Venlo, Maastricht University, Nassaustraat 36, 5911 BV Venlo, the Netherlands.
E-mail address: george.agorastos@maastrichtuniversity.nl (G. Agorastos).

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repulsion, the surface anchored glycan side chains stretch away from the protein core to form a "bottlebrush"-like superstructure (Bansil & Turner, 2006).

Mucins are known to provide oral lubrication via two mechanisms. The first is based on their high water-binding capacity. Due to the low isoelectric point between 2 and 3, mucins have a negative net charge at the physiological pH of saliva (around pH 7). Based on their negative charge, mucins are surrounded by a hydration shell of water molecules, which can provide oral lubrication (Ma et al., 2015; Yakubov et al., 2009). The second mechanism is related to the continuous de- and re-adsorption of mucins onto the oral mucosa (Crouzier et al., 2015). In addition, the mucin coating can provide steric repulsion between two surfaces, which gives lower friction (Yakubov et al., 2009). Based on those mechanisms, mucoproteins can provide lubrication to the oral surfaces and have a coating effect on mouthfeel.

A disruption of salivary lubrication can cause an astringent sensation. Examples of components that can cause an astringent sensation are salt ions and polyphenols. Different interactions between these components and salivary proteins cause protein aggregation. Whereas cations aggregate together with salivary proteins via electrostatic interactions, polyphenols interact via hydrophobic interactions and hydrogen bonds (Canon et al., 2018). This aggregate formation leads to loss of lubrication of the mucosal pellicle and elicits the astringency sensation. Such loss of lubrication by phenols and trivalent ions has been demonstrated by Rudge et al. (2021) where they used frictional measurements to determine the level of lubrication loss. Despite that trivalent salts are known to cause astringency, limited information is known about the effect of different metal valences on salivary lubrication, and how the ionic strength influences lubrication. Macakova et al. (2011) demonstrated the profound impact of ionic strength on the architecture of salivary pellicle which dramatically affects lubrication behavior. Nevertheless, this study focused on the ionic strength concentration that can be found in physiological conditions in human saliva and below, not on the effect of ionic strength values derived from food or beverage consumption.

Phenolic components such as catechins are commonly present in beverages such as wine, beer and tea. Epigallocatechin gallate (EGCG) has been shown to significantly increase the astringency perception compared to other catechins (Rossetti et al., 2009). However, studies regarding the presence of multiple components, such as cations and phenols, their interactions with salivary proteins and the impact on salivary lubrication are limited. One of the few studies on ionic strength was performed by Canon et al. (2013), who found that upon the addition of EGCG to purified human PRPs, aggregate formation was more profound at an ionic strength of 1 mM than at 2 mM. However, Canon et al. (2013), focused only on low ionic strength values and they did not perform any frictional measurements. Therefore, new insights are needed to clarify the effect of the interplay between salt ions and EGCG on salivary lubrication at different ionic strengths.

In this study, we hypothesize that different cationic valences and ionic strengths will affect saliva's lubrication behavior in different ways, while EGCG will further enhance the effect. The aim of this study is thus to understand the effect of varying cationic valences and ionic strengths on the lubrication behavior of saliva with and without the presence of EGCG. Changes in salivary lubrication were measured by a dynamic soft-tribological protocol and protein aggregation was determined by SDS-PAGE. The outcome of this research provides a better understanding of the effect of different beverage components on salivary lubrication and potential astringency sensation.

2. Materials and methods

2.1. Materials

The materials used to prepare the samples were potassium chloride (≥99.5%), magnesium chloride (≥98 %), iron(III)chloride (97 %), aluminium chloride (99 %), potassium hydroxide (≥85 %), and EGCG (≥97 %), which were all purchased from Sigma-Aldrich Corp. (St. Louis, USA). Sodium chloride (99 %) was purchased from Honeywell (Charlotte, USA). Lastly, hydrochloric acid (37–38 %) was purchased from Actu All chemicals (Randmeer, the Netherlands).

2.2. Saliva collection

Unstimulated fresh saliva was collected between 8 and 10 AM from three healthy female subjects (age 20–25, Caucasian). The saliva was collected in 50 mL plastic tubes and pooled. During the collection, the tubes were maintained in ice to ensure low temperature upon collection. This time frame was chosen to reduce differences in salivary composition during the day (Vijay et al., 2015). The volunteers did not eat, nor drink except for water for at least 1 h prior to collection. The collected saliva (around 25 mL) was centrifuged at 10,000 rpm (9520 g) for 10 min at 4 °C in a Hermle tabletop refrigerated centrifuge Z 383 K (Wehingen, Germany). After centrifugation, the supernatant, containing the PRPs and mucins was collected using a pipette. The supernatant was preserved in ice for a maximum of 4 h until measurements were performed. The saliva collection has been approved by the Ethical Review Committee at Maastricht University [ethics reference (ERCIC_335_23_03_2022)].

2.3. Preparation of salt, pH solutions, and EGCG

The salt solutions containing KCl, $MgCl_2$, $FeCl_3$ and $AlCl_3$ were prepared at 1, 10 and 25 mM using milli-Q water upon continuous stirring. Additionally, salt solutions were prepared to represent ionic strength values of 60 and 150 mM. EGCG solutions were prepared by dissolving the EGCG into milli-Q water in a glass bottle to obtain a concentration of 2 mM EGCG, which was then covered in aluminum foil. The EGCG samples were stored at 4 °C for a maximum of 24 h to prevent degradation (Krupkova et al., 2016). Solutions with both salt and EGCG were also prepared. A maximum of 1 h prior to measurements, the prepared salt solutions (KCl, $MgCl_2$, $FeCl_3$ and $AlCl_3$) at 1, 10 and 25 mM were mixed in a 1:1 ratio with the 2 mM EGCG-solution and subsequently stored at 4 °C in the dark. To analyze the effect of salts on the pH of the mixtures, a Schott Instruments micro pH meter (Mainz, Germany) was used. For the investigation of the pH effect on salivary lubrication, HCl model solutions were prepared to represent pH values of 6.8, 4.7 and 2.2 after mixing with saliva in a ratio of 1:1. pH values of the mixtures were measured at 20 °C.

2.4. Salivary protein composition

The composition of the initial saliva and after the addition of salt and ECGC was determined by electrophoresis (SDS-PAGE), according to a method in the NuPAGE® Technical Guide (Thermo Fisher) and described by Biegler et al. (2016). Prior to electrophoresis, the treated samples were prepared by the addition of 0.5 mL of the salt or EGCG solutions to 0.5 mL centrifuged saliva (section 2.2) in a 2.0 mL Eppendorf tube and subsequent mixing with an orbital shaker for 10 min at 20 °C. These samples were then centrifuged at 10,000 rpm (9520 g) for 10 min at 4 °C in a Hermle Table Top Refrigerated Centrifuge Z 383 K to remove formed aggregates by collecting only the supernatant. The supernatant was subsequently used for SDS-PAGE analysis.

Thirty-nine μL of the prepared samples was mixed with 6 μL reducing agent and 15 μL LDS sample buffer (SimplyBlue Safestain, Thermo Fisher, USA), and heated at 70 °C for 10 min. Before the samples were added to the SPS-page gel, the 1 mm thick 4–12 % BisTris NuPAGE® Gel (Thermo Fisher, USA) was rinsed with 5 % MES running buffer (Thermo Fisher, USA) and placed into the XCell SureLockTM Mini-Cell (Carlsbad, USA). The wells were then filled with 15 μL of the prepared SDS-PAGE samples and a marker (15 μL Mark12 Unstained Protein Standard 2.5–200 kDa, Thermo Fisher, USA) was added as a reference to identify

the size of the different proteins. The inner and outer chamber of the cell was filled with the 5 % MES running buffer. Then a constant voltage of 200 V was applied for 35 min. The gels were then rinsed and stained with SimplyBlueTM SafeStain. After staining, the gels were again rinsed with water and a 20 % (w/w) NaCl solution. The gels were stored in a NaCl solution for 7 to 14 days at 4 °C to increase the detection limit of the protein bands before they were scanned with a SDS-PAGE gel scanner (Biorad - GS900 Calibration densitometer, Taiwan) and analysed using ImageLab (version 6.0.1). The interpretation of the results and especially of the identification of the bands was according to previous studies of Biergler et al. (2016) and Ligtenberg et al. (2015).

2.5. Zeta potential and particle size distribution

Particle size distribution of the salivary proteins and aggregates was measured using dynamic light scattering (Malvern Instruments Zetasizer, UK), as previous reported by Rudge et al. (2021). The zeta potential of these samples was measured using electrophoretic light scattering. Before measurements, a 120 s equilibration time was used to ensure a constant temperature of 25 °C for each sample. The measurements were performed with a semi-micro cuvette (Brand®, Merck) in triplicate with a 0.001 absorption and a refractive index of water and the sample of 1.33 and 1.45, respectively. After the determination of the particle size distribution, a maximum voltage of 40 mV was applied to measure the zeta potential in folded capillary zeta cells (DTS1070, Malvern, UK). The cuvettes were cleaned in between each measurement with demi-water, ethanol, and again demi-water and subsequently dried with pressurized air. The cuvettes were disposed when the metal strips showed visible oxidation by turning black. Every measurement was performed in duplicate.

2.6. Tribology measurement

The lubrication properties were examined by measuring the friction coefficient using an Anton Paar rheometer with temperature control (MCR302) at 20 °C. A tribological configuration was mounted in the rheometer, which consisted of a glass ball as a probe, and a sample holder with 3 PDMS pins (BC12.7/SS – 52837). PDMS is one of the prevailing materials currently used in tribology as it mimics the tongue relatively well (Rudge et al., 2019). The sliding speed of the probe was set to 1 mm/s and the force exerted by the probe was 1 N. The normal force of 1 N was based on the procedure by Laguna et al. (2017). The sliding speed of 1 mm/s represents the boundary lubrication regime, which is considered most relevant for astringency perception (Brossard et al., 2016). Measuring at a constant velocity allowed close monitoring of any changes in the lubricating properties as a result of different interactions (Biegler et al., 2016; Laguna et al., 2017). Upon the start of the measurements, 0.6 mL of supernatant from centrifuged saliva samples was added to the sample holder and the measurement was started. The friction coefficient was measured at 20 °C for 5 min, in which a constant value was obtained, indicating the formation of a salivary protein film on the PDMS surface. After 5 min, 0.6 mL of the model salt and ECGC/salt solutions were then added to the sample holder with the probe still in place. This resulted in a 1:1 ratio of the model salt or EGCG/salt solutions and saliva. The measurement was continued for another 5 min to register changes in the friction coefficient, Δμ. The Δμ was calculated by subtracting the friction coefficient after (10 min) and before (5 min) the injection of the model salt and ECGC/salt solutions. Each measurement was performed in duplicate with the same set of PDMS pins.

2.7. Viscosity measurement

The viscosity of the saliva samples after the addition of the model solutions was measured with a double gap geometry (DG26.7/Ti – 21,833 and C-DG26.7/Ti – 10743) on a rheometer (Anton Paar Rheometer MCR301). Samples were prepared by mixing 3.8 mL of the salt and EGCG/salt solutions with centrifuged saliva in a 1:1 ratio. After combining the model solution with saliva, the mixture was transferred to the rheometer and left for 5 min at 20 °C to allow interactions to occur between the astringent agent and the salivary proteins. The viscosity was measured in duplicates at a shear rate ranging from 0.1 to 100 s^{-1} over a period of 5 min at 20 °C.

2.8. Statistical analysis

The results were analyzed using a multivariate analysis of variance (MANOVA) and a Pearson correlation. When the values from MANOVA were significantly different ($p < 0.05$), an additional Tukey-Kramer HSD (honestly significant difference) test was used to identify the differences between the parameters. To verify the assumptions of normal distribution and homogeneity of variances, Shapiro-Wilk and Levene's tests were used. All the statistical analyses were performed with the software R (R core team and foundation for statistical computing), R-studio version 4.0.3.

3. Results and discussion

3.1. Effect of cationic valency on salivary lubrication

The effect of monovalent, divalent, and trivalent cations on lubrication behavior was investigated in this study using KCl, $MgCl_2$, $FeCl_3$, and $AlCl_3$. The concentration range of the salts was selected based on previous studies investigating the effect of cations on taste of food and beverages (Lawless et al., 2003). Additionally, to take into account even extreme concentrations of trivalent salts that can be present in fortification food and beverage products, 25 mM was chosen as the highest concentration (Habeych et al., 2016). The reasoning was that iron fortification products may lead to different oral lubrication. It is acknowledged that 25 mM of iron is an extreme value that can only be used in experimental fortification studies since this value is higher than allowed limits in food or beverages. Additionally, such high values can impact the stability of food due to color, oxidation, etc.

The effect of the salts on salivary lubrication behavior was determined by measuring the changes in friction coefficient, Δμ (Fig. 1a). First, water was added to saliva to adjust the protein concentration as a reference sample. The value for Δμ was 0.004 after the addition of demi-water, indicating that the change in protein concentration had a limited effect on the lubrication behavior of saliva itself. Changes in friction upon the addition of salt or ECGC solutions should thus be a result of interactions between the components, and not because of a dilution effect.

The addition of monovalent or divalent salt did not significantly change the lubrication of saliva (Fig. 1a). Even though divalent salts are known to form salt bridges with compounds such as proteins, no effect was observed on the lubrication behavior. The trivalent salts were found to change the lubrication behavior of saliva significantly ($p < 0.001$). At a lower concentration of 1 mM, $FeCl_3$ and $AlCl_3$ did not significantly ($p > 0.05$) change the lubrication of saliva. However, especially at higher concentrations of 10 and 25 mM, both $FeCl_3$ and $AlCl_3$ significantly decreased the lubrication behavior of saliva as an increase in Δμ was found. A different effect was observed between $FeCl_3$ and $AlCl_3$. Overall, $FeCl_3$ showed higher friction, thus a larger Δμ, compared to $AlCl_3$ for both 10 and 25 mM. Additionally, the friction decreased for the $FeCl_3$ samples when the concentration increased from 10 to 25 mM, although the difference was not significant. For $AlCl_3$, we observed an increase in friction when the concentration increased from 10 to 25 mM, although also not significant. The given outcomes show that $FeCl_3$ leads to a larger effect on salivary lubrication than $AlCl_3$. This effect indicates that the two trivalent salts may affect the salivary proteins at a different level. To understand the origin of this difference, we investigated how the salts influenced the properties of saliva.

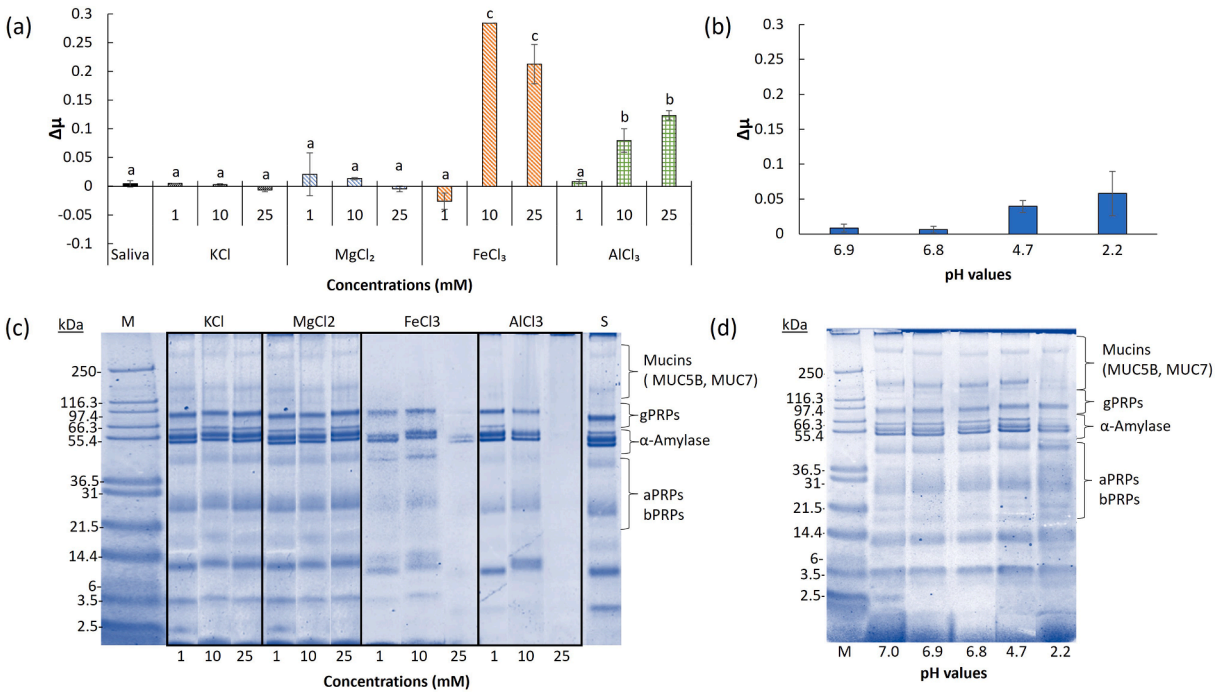

Fig. 1. (a) Friction coefficient changes (Δμ) for saliva, and the different salt solutions at different concentrations. The values are presented in mean ± standard error. The letters identify that the samples are significantly different according to Tukey's HSD test: $p < 0.05$. (b) Friction coefficient (Δμ) for saliva at different pH values. The values are presented in mean ± standard error. (c) SDS-page of remaining salivary proteins in the supernatant after addition of salt solutions and centrifugation. (d) SDS-page of the salivary proteins at different pH values.

First, the influence of the individual trivalent salts on the change in friction coefficient was measured without the presence of saliva (data not shown). It was observed that the salt itself did not change the friction coefficient. Changes in friction must therefore be related to the interactions between the salivary proteins and the salts. As both $FeCl_3$ and $AlCl_3$ are known to lower the pH and therefore alter the charge of the salivary proteins, different effects on the salt-protein interactions can be speculated. Therefore, to gain more insight into the differences between the trivalent salts, zeta potential and pH values were measured.

Saliva itself (diluted with demi water in a 1:1 ratio) had a zeta potential of around −15.1 mV at pH 7. This value is common in human saliva (Rykke et al., 1995) and is mainly due to the negatively charged mucins (pI around 2.5) (Veerman et al., 1992). Mucins are considered the most important protein group in saliva to determine the charge since they make up a large portion of the protein content in saliva (Çelebioğlu et al., 2020). Upon addition of both $FeCl_3$ and $AlCl_3$, the pH of saliva decreased to values of 2.2 and 3.1, respectively (Fig. S1, supplementary material). This drop in the pH affected the charge of the salivary proteins. In the case of $FeCl_3$, saliva had a positive charge with a zeta-potential of 16.8 mV. This can be explained by the fact that the pH of 2.2 was below the isoelectric point of the mucins, which is around 2.5 (Veerman et al., 1992). For $AlCl_3$, the pH of 3.1 was slightly above the pI of the protein, where the charge was slightly net positive with a low zeta-potential value of 3.1 mV. In this case, the effect of pH leads to aggregate formation since the pH is close to the isoelectric point of salivary mucins. The low values of zeta potential (charge decrease) at low pH indicate that the salivary proteins' charge leads to aggregate formation due to a change in the electrostatic interactions (less repulsion among mucins). The reduced electrostatic repulsion between the mucins leads to aggregation via hydrogen bonds (Frenkel & Ribbeck, 2015; Mehrotra et al., 1998). Due to the aggregation of the mucin, the "bottlebrush" like superstructure (Bansil & Turner, 2006) is not able to provide the salivary lubrication properties anymore. As a result, changes in pH can lead to aggregation of the proteins, which reduces the amount of saliva proteins available for lubrication. To gain insight into the effect of pH alone, we also measured the change in friction coefficient when the pH was altered by HCl, and the results are reported in Fig. 1b. However, the effect of pH alone (Fig. 1b) was different than the combined effect of pH and trivalent salts (Fig. 1a), indicating that the salt itself also has an influence.

The selected pH values of 6.8, 4.7, and 2.2 represent the changes in the pH observed upon the addition of the monovalent, divalent and trivalent salts. The effect of pH on changes in friction, Δμ, can be seen in Fig. 1b. At lower pH values of 2.2, the change in friction was higher than for higher pH values of 4.7 and 6.8. Even though there is not a significant difference between the friction values (Fig. 1b), we do observe an apparent trend. This confirms that changes in pH indeed lead to changes in saliva properties, and the changes in friction are most likely due to a higher degree of aggregation of the proteins. Since the pH values are close to the pI (low net charge) of salivary mucins the electrostatic repulsion is limited and mucins self-aggregate. The aggregation may also be enhanced by the additional salt bridges that the trivalent salts can make with negatively charged proteins (Rudge et al., 2021). Comparing the friction measurements from Fig. 1a and Fig. 1b we observed that the samples with the trivalent cations, at concentrations of 10 and 25 mM, had higher friction values than the samples without. Therefore, we concluded that trivalent salts can create salt bridges with the salivary proteins before the pH has completely dropped and while the proteins are still negatively charged.

To further understand which proteins are involved in the aggregation process when salts are added, SDS-PAGE was used to identify the remaining proteins which are available for lubrication after aggregation and centrifugation by the astringent agents. Fig. 1c shows the remaining salivary proteins in the supernatant after centrifugation. The samples to which KCl and $MgCl_2$ were added present the same composition as saliva (S, right column) itself, confirming that all salivary proteins were still present in saliva, and therefore did not aggregate together with these salts. However, the trivalent salts influenced the composition of the saliva, as less salivary proteins were present in the supernatant, thereby indicating that aggregate formation of proteins indeed occurred

(Fig. 1c). For both salts, mucins were not present in the SDS-PAGE gel, which shows that salivary mucins were involved in the aggregate formation. As mucins are negatively charged over a large pH range, they were able to form cross-links with the positive salts via ionic bonds (salt bridges). However, depending on the salt type and concentration, also other proteins were involved. At 25 mM concentration, for both trivalent salts, the PRPs were also not present anymore, indicating that the gPRPs were also present in the protein aggregates. However, these results do not explain the difference in the change in friction coefficients, as no visible difference in the amount of aggregated proteins could be observed for $FeCl_3$ and $AlCl_3$, indicating that the same proteins were involved in the aggregate formation (Fig. 1c). To identify which proteins are involved in the aggregation due to the presence of salts, and which proteins aggregate due to a change in pH, we also determined the saliva composition of the supernatant at different pH values. The samples were adjusted to pH values of 6.8, 4.7 and 2.2, which represent the pH after the addition of iron and aluminium and subsequently centrifuged. The supernatant was then analyzed with SDS-PAGE. Fig. 1d shows the protein composition of the supernatant for the different pH values. It can be observed that the mucin, especially MUC7, proteins were present in a limited amount at pH 2.2, whereas they were still present at higher pH. These results show that at low pH, the mucin MUC7 aggregate without the presence of salt. The aggregation of mucin proteins due to lower pH can be explained by the self-aggregation of mucins due to their pI of 2.5 (Frenkel & Ribbeck, 2015; Mehrotra et al., 1998). Since the pH drops close to their isoelectric point the net charge is not sufficient enough to maintain their repulsion which eventually leads to self-aggregation. Those results indicate that the aggregation of mucins, as shown in Fig. 1c, is due to both the effect of salt bridge formation and pH. In addition, mucins were the only proteins that were aggregated at salts concentrations of 10 mM (of trivalent salts), which makes them the most responsible for lubrication losses.

The aggregate formation due to trivalent salts increased the friction coefficient (Fig. 1a). As high molecular weight mucins are known to provide lubrication in the mucosal pellicle (Crouzier et al., 2015), it is logical that mucin aggregation leads to most lubrication losses. The degree of protein aggregation was also seen to depend on the type of salt and the pH of the solution. Based on those findings, we conclude that two effects are responsible for the changes in lubrication properties of saliva upon the addition of salts. Trivalent salts, at high concentrations, firstly aggregate mucins by cross-linking and secondly by lowering the pH values leading to the self-aggregation of mucins (mainly MUC7). The aggregate formation results in a decrease in lubrication behavior. These results are consistent with the result in the literature, in which they investigated the lubrication changes upon the addition of trivalent salts as well (Biegler et al., 2016). However, our research provides additional new information on the effect of salt bridging and pH and their influence on salivary lubrication.

3.2. Effect of ionic strength on the lubrication properties

In the first section, the effect of cationic valence on salivary lubrication was investigated for salts with a similar concentration range. The results show that the monovalent and divalent salt did not lead to changes in protein aggregation and subsequent lubrication losses. However, the ionic strength of those samples was much lower than in the case of the trivalent salts. Therefore an additional research was conducted to study the effect of monovalent, divalent and trivalent salts at the same ionic strength on salivary lubrication. The ionic strength values were 60 and 150 mM for all salts. These values are representative of the 10 and 25 mM concentrations of the trivalent salts used initially, and therefore higher concentrations for the monovalent and divalent salt were used. The concentrations of monovalent and divalent salt were calculated using the equation of ionic strength.

The Δμ values of the salt solutions based on ionic strength of 60 and 150 mM can be seen in Fig. 2a. In the case of KCl, the increase in ionic strength from 60 mM to 150 mM significantly ($p < 0.001$) decreased the Δμ from −0.03 to −0.1, respectively. Also in the case of $MgCl_2$, Δμ decreased significantly ($p < 0.001$), although the effect was less pronounced than for KCl. Those outcomes showed that monovalent and divalent salts at higher ionic strengths provide extra salivary lubrication. The differences in the results at the same ionic strength concentration reveal that different salt ions affect lubrication due to different mechanisms. More specifically, monovalent and divalent salts affect differently the salivary lubrication compared to trivalent salts at the same ionic strength which is in contrast with the equal molar solutions. At an ionic strength of 150 mM, trivalent salts disrupt the lubrication properties of saliva film, while monovalent and divalent salts can provide extra lubrication. These differences are most likely due to changes in interactions as a result of the different salts. An important observation is that salivary lubrication increases with the larger cationic size. More specifically, potassium has a larger radius compared to magnesium and iron which may explain the increased lubrication properties upon the addition of KCl. Similar trends were noticed by Yu et al. (2019), where they showed improved lubrication properties in zwitterionic phosphorylcholine-based brushes with larger sizes of anions. Even though the studied system by Yo et al. (2019) is not similar to the polymeric mucins a similar mechanism can be expected. More information regarding the effect of potassium on improved salivary lubricational properties will be discussed further below.

To get more insights into the interactions involved in the aggregation of salivary proteins with salt with a different valency, the viscosity was measured at an ionic strength of 150 mM. Saliva was also diluted with water as a reference. KCl resulted in a lower salivary viscosity, and even

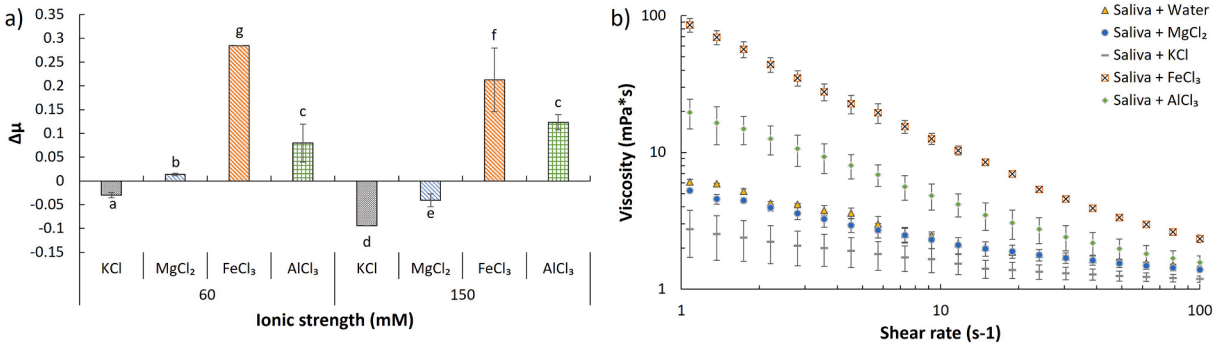

Fig. 2. (a) Δμ values of saliva upon addition of KCl, $MgCl_2$, Fe(III)Cl_3 and $AlCl_3$ solutions at ionic strengths of 60 and 150 mM. The values are presented in mean ± standard error. The letters in each bar identify that the samples are significantly different according to Tukey's HSD test: $p < 0.05$. (b) The viscosity as a function of shear rate when saliva was mixed with water (triangles), KCl (dashes), $MgCl_2$ (circles), $FeCl_3$ (squares), and $AlCl_3$ (diamond). The ionic strength of 150 mM was used for all salt solutions.

though there is not a statistically significant difference an apparent trend can be observed (Fig. 2b). The lower viscosity with the addition of KCl suggests that the interactions between mucins change since those proteins are responsible for the variations in viscosity (Coles et al., 2010; Gibbins & Carpenter, 2013). This change in interactions can be explained by a reduction in the electrostatic repulsion between the mucins, and a change in confirmation. Such change in confirmation has also been shown by others, where they demonstrated that the decrease in negative net charge caused the changes in salivary proteins conformation (Chaudhury et al., 2015). This is a result of the screening of the charges on salivary mucins by the monovalent cations, which leads to less internal repulsion between charged groups within mucin. $MgCl_2$ can also lead to screening effects, but no visible change in the viscosity of saliva was observed. This may be due to divalent ions being known to provide salt bridges, which may have contributed to a slight increase in viscosity, thereby cancelling out the decrease in viscosity by screening effects.

$FeCl_3$ and $AlCl_3$ both increased the viscosity of saliva, although $FeCl_3$ increased the viscosity to a larger extent (Fig. 2b). The increased salivary viscosity results from the formation of aggregates, as already discussed earlier for trivalent salts and mucins. Such an increase in viscosity in the presence of trivalent salts has also been reported by others which is consistent with the larger effect which has been observed for the $FeCl_3$ (Biegler et al., 2016).

These results show that no protein aggregation occurs with monovalent and divalent salts, whereas trivalent salts are expected to lead to aggregation. To verify this, the particle size distribution of saliva in the presence of the different salts was examined. In addition, SDS-PAGE was applied to evaluate which proteins were involved in the aggregation. Fig. 3a presents the particle size distribution of salivary proteins upon the addition of the different salts. Iron chloride was used as representative of the trivalent salt since it showed the largest impact compared to aluminum chloride. Both KCl and $MgCl_2$ decreased the size of the salivary proteins, indicating that indeed no aggregation took place. This was also confirmed by the SDS-PAGE results, as all proteins were still present in the supernatant after centrifugation of the saliva mixtures, indicating that no aggregate formation took place (Fig. 3b). The decrease in hydrodynamic radius also confirms that a decrease in electrostatic repulsion and a change in confirmation were obtained (Fig. 3a). This decrease in size and reduction of the electrostatic repulsion between the mucins can explain the better lubrication properties of these systems via a mechanism called hydration lubrication (Ma et al., 2015; Yakubov et al., 2009). Hydration lubrication originates from hydrated ions trapped between charged surfaces that repulse each other. This would lead to a more hydrated polymer that provides aqueous lubrication properties (Garrec and Norton, 2012). The lubrication ability has already been shown to depend on the type of salt, as the hydration ability is related to the hydration enthalpy (Garrec and Norton, 2012) according to the Hofmeister series. Therefore, in a system containing salivary proteins, only monovalent salt improves the salivary lubrication via hydration. Where hydrated potassium cations were placed among the negatively charged polymeric mucins providing better lubrication.

As expected, $FeCl_3$ and $AlCl_3$ did lead to aggregation of the salivary proteins (Fig. 3a). This is mainly due to the aggregate formation of MUC5B and MUC7, as the PRPs and other proteins are still present in the supernatant (Fig. 3b). Especially, for 60 mM $AlCl_3$ the majority of all the proteins seem to be aggregated. However, the different ionic strength values did significantly change the lubrication behavior for $FeCl_3$ but not for $AlCl_3$. The changes in mucins state seem to be the main reason for the loss of lubrication by the trivalent salts.

Concluding, two main effects are likely to influence the lubrication properties of saliva in the presence of salts with different cationic valences. First, monovalent and divalent salts at high ionic strength values provide extra lubrication via hydration lubrication. Second, trivalent salts lead to loss of lubrication due to aggregation of the salivary mucins. An illustration of the two new different mechanisms of the monovalent and trivalent salts in salivary is given in Fig. 4.

3.3. Effect of cations and phenols on salivary lubrication

Astringency in alcoholic or non-alcoholic beverages is mostly associated with plant polyphenols. However, how different salts influence the interactions between polyphenols and salivary proteins is still not known, nor how this affects the lubrication properties. To provide new insights into the role of cations in phenol interactions, a polyphenol was added to the different salt solutions. EGCG was selected as it is known for its astringent sensation (Biegler et al., 2016). A concentration of 2 mM was selected, which is similar to the concentration in green tea (Almajano et al., 2008).

The addition of EGCG alone increased the friction of saliva by approximately 0.15 Δμ (Fig. 5a). This increase in friction is an effect of the aggregate formation of salivary proteins and EGCG due to hydrogen-bond and hydrophobic interactions (Biegler et al., 2016). The addition of monovalent and divalent salts did not affect the lubrication properties of saliva in combination with EGCG (data not shown). Only the presence of trivalent salts in the EGCG solution influenced salivary lubrication. $FeCl_3$ was selected as a representative of a trivalent salt, since this salt showed a larger effect on salivary lubrication than $AlCl_3$. The effect of EGCG on Δμ values of the $FeCl_3$ series can be seen in Fig. 5a. Δμ values for the salt solutions were added as references. As expected, the addition of a mixture of EGCG and $FeCl_3$ to saliva significantly increased the friction of the system for all $FeCl_3$ concentrations. However, the absolute increase depended on the specific combination of the salt and EGCG. At a concentration of 1 mM $FeCl_3$, the presence of EGCG increased Δμ.

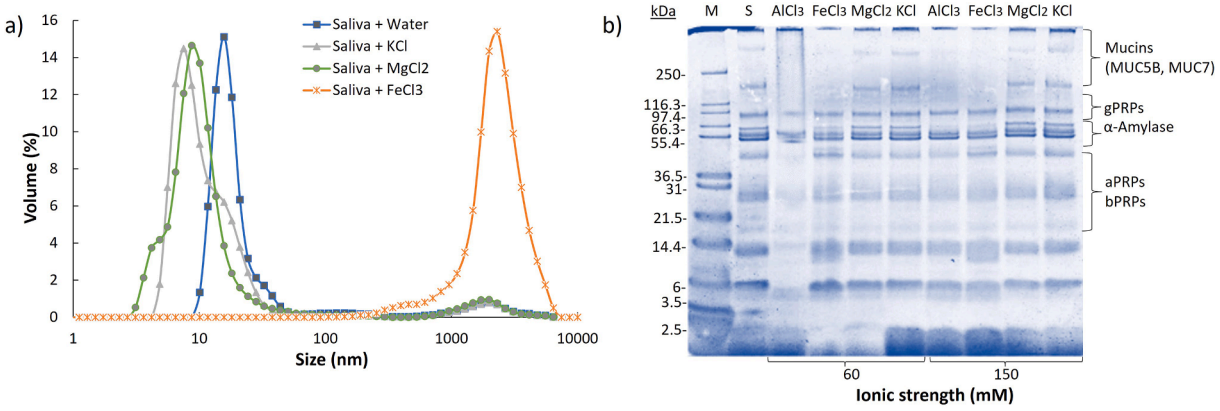

Fig. 3. (a) Particle size of salivary proteins in mixtures with water (squares), KCl (triangles), $MgCl_2$ (circles). and $FeCl_3$ (crosses) at an ionic strength of 150 mM. (b) SDS-page of remaining salivary proteins in the supernatant after addition of salts at an ionic strength of 60 and 150 mM and centrifugation.

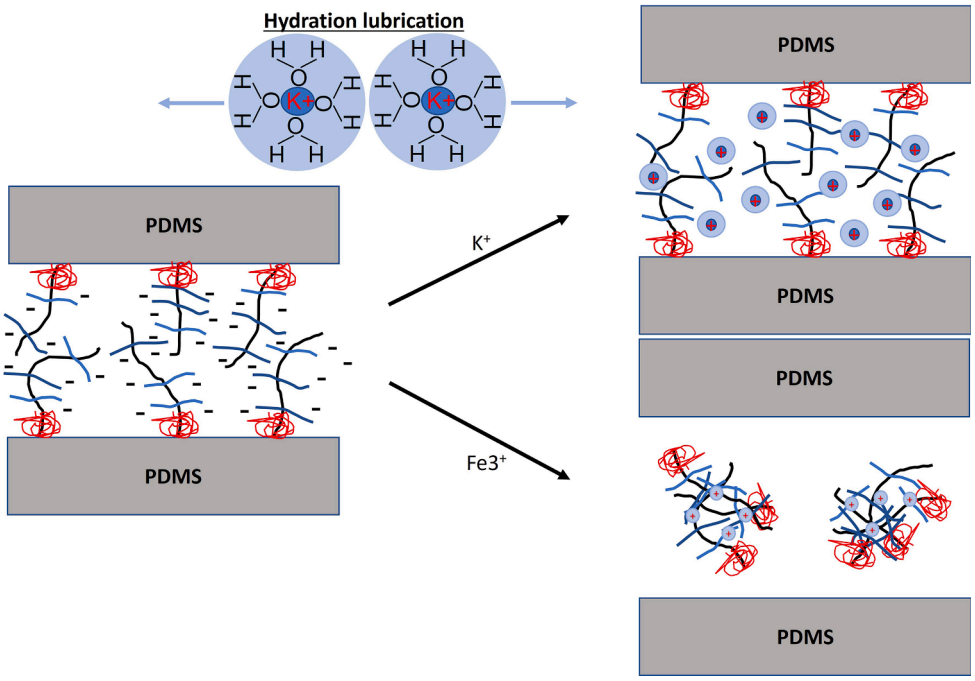

Fig. 4. Schematic overview of the lubrication mechanisms based on different ion valences (monovalent and trivalent). The red lines represent the hydrophobic part of the mucins. The blue lines represent the amphiphilic part of the mucins. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

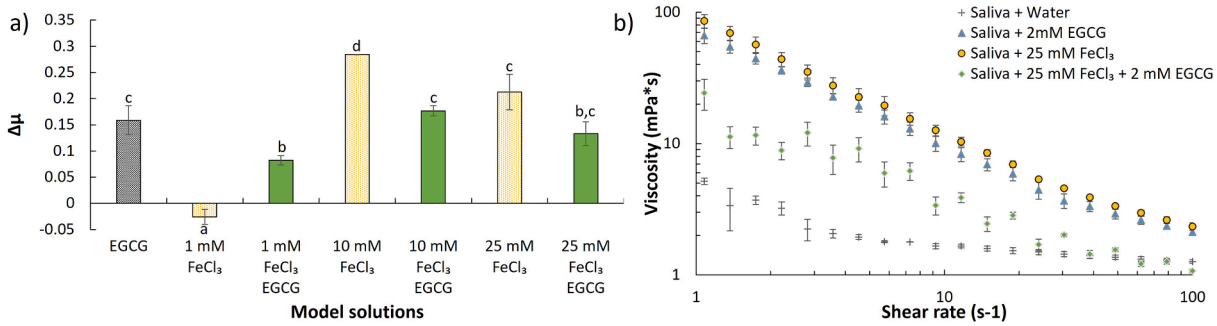

Fig. 5. (a) Change in friction coefficient (Δμ) for mixtures of saliva with EGCG solution (2 mM) and $FeCl_3$ solutions at different concentrations with and without the addition of 2 mM EGCG. The values are presented in mean ± standard error. The letters in each bar identify that the samples are significantly different Tukey's HSD test: $p < 0.05$. (b) Viscosity of saliva- water (crosses), saliva-$FeCl_3$ (circles), saliva-$FeCl_3$-EGCG (diamonds) and saliva – EGCG (triangles).

However, the change in Δμ was lower than when only EGCG was added. However, in the case of 10 and 25 mM, Δμ values for solutions with EGCG were lower than the samples with only salt. Similarly, to the other concentrations (10 and 25 mM) of $FeCl_3$, the addition of EGCG prevents the lubrication loss. These results show that the presence of EGCG in iron chloride solution prevents iron to decrease salivary lubrication due to the interaction of EGCG-iron. This can be explained by the fact that EGCG and iron cations interact with each other, which leaves less EGCG or salt available to interact with the salivary proteins. For instance, at lower concentrations of iron, EGCG is the most responsible astringency agent for the aggregate formation of salivary proteins, since the iron is trapped by the EGCG. While at high concentrations of iron, EGCG interacts with the iron and the remaining "free" iron is responsible for salivary protein aggregation and therefore lubrication loss. Those changes in Δμ between EGCG and $FeCl_3$ concentrations indicate the interaction of EGCG-$FeCl_3$ and that there is a critical concentration where ECGC prevent lubrication loss by iron or vice versa.

The different interactions occurring in the mixtures could also be seen in the color changes of the mixtures. Dissolving $FeCl_3$ into demi water resulted in a transparent orange solution due to the presence of Fe^{3+} ions. Upon the addition of EGCG, the color changed to a more blue/black color. This color change is due to the interaction between Fe^{3+} and EGCG, which has also been reported by others. The cation can chelate to hydroxyl groups on the EGCG to form dipolar interactions (Inoue et al., 2002). The color depended on the concentration of $FeCl_3$. In the case of 1 mM $FeCl_3$, the color was light blue, while a darker blue/black color was seen for concentrations of 10 mM $FeCl_3$, and an orange/black color for 25 mM $FeCl_3$ (Fig. 2S, supplementary material). These differences can be explained by the number of Fe^{3+} ions that can bind to one EGCG molecule or partly due to the oxidation of phenol by the presence of iron. It is known from the literature that two Fe^{3+} ions can bind to one EGCG molecule (Ryan & Hynes, 2007). For low $FeCl_3$ concentration, all Fe^{3+} ions (1 mM) could theoretically bind to EGCG (2 mM), and therefore limited Fe^{3+} ions were present to provide an orange color, and the EGCG/FE complexes provided a blue color, as EGCG itself does not provide any color. In the case of a higher concentration of 10 mM, all EGCG was bound to the Fe^{3+} ions, while Fe^{3+} was present in excess, which increased the darkness of the blue color. For an even higher concentration of 25 mM $FeCl_3$, more free Fe^{3+} ions were in excess, which provided again a more orange color. These EGCG binding properties

have also been reported for Al^{3+} (Inoue et al., 2002), and therefore we expect similar results for $AlCl_3$.

The interaction between EGCG and iron cations prevents the loss of salivary lubrication compared to iron cations alone. The ratio between $FeCl_3$ and EGCG determines how much $FeCl_3$ is used for chelating, which then determines how much remaining $FeCl_3$ can aggregate with saliva proteins together. For instance, at 1 mM of $FeCl_3$ there is more EGCG for aggregation, while between 1 and 10 mM the presence of more salt ions leads to salivary aggregation. To prove our hypothesis about the changes in salivary protein aggregate formation, we measured the viscosity of solutions containing $FeCl_3$ and salivary proteins and solutions with $FeCl_3$ with EGCG and salivary proteins as well.

Fig. 5b shows the viscosity of the saliva with demi water and saliva with EGCG over a shear rate range of 1 to 100 s^{-1}. The graph shows that EGCG increased the viscosity of saliva, which indicates the formation of salivary protein aggregate together with EGCG, as also discussed by others (Biegler et al., 2016). Similarly, $FeCl_3$ increased the viscosity to the largest extent, where a larger aggregate formation can be observed compared to EGCG. Even though there is no statistically significant difference between the sample with $FeCl_3$ and $FeCl_3$-EGCG, an apparent trend is noticed among the shear rate values. The observed lower viscosity of the sample containing both EGCG (2 mM) and $FeCl_3$ (25 mM) suggests the formation of fewer aggregates with the salivary proteins compared to $FeCl_3$ (25 mM) alone. Therefore, the lower friction coefficient values for the mixture of EGCG (2 mM) and $FeCl_3$ (25 mM) than for 25 mM $FeCl_3$ alone can be explained by less bridging of "free" $FeCl_3$ to salivary proteins due to EGCG-$FeCl_3$ interaction.

These results provide new insights related to the interactions between phenols and trivalent cations and accompanying changes in salivary lubrication. To our knowledge, there are no studies in the literature which compare the combined interaction of phenols and minerals. Trivalent cations can interact with salivary mucins due to salt bridging, but also affect the pH which further leads to the aggregation of mucins. This aggregate formation leads to a loss of salivary lubrication. Similarly, EGCG is known to result in salivary lubrication loss due to the hydrogen bonds and hydrophobic interactions with salivary proteins. Interestingly, this study shows that in the presence of EGCG, the Fe^{3+} can interact with EGCG via a chelating interaction. This results in less binding ability of salivary proteins with the two astringent agents (ECGC and iron). The current outcomes reveal a prevention mechanism for salivary lubrication loss coming by the interaction of EGCG-Iron. As changes in salivary lubrication have been shown to associate with astringency perception, the current results are expected to match differences in astringency perception. A sensory study would be required to further confirm the relations between the concentrations of iron and EGCG. However, future attention should be given to the interaction of minerals with larger phenolic compounds like tannins (polyphenols), and it should be investigated whether metallic ions at concentration levels present in food will still have an effect on different types of astringent compounds. The current findings show that changes in composition concerning minerals and phenol content, and based on their interaction, lead to different oral sensations. These results are interesting for the beverage industry since the loss of lubrication or astringency can be avoided to some extent by the addition of salts.

4. Conclusions

In summary, we demonstrated that different cationic valences and ionic strengths affected differently the salivary lubrication in presence of EGCG and without. Different new mechanisms were detected for the three different valences (monovalent, divalent and trivalent salt) and their association with frictional measurements. Monovalent (KCl) and divalent salts ($MgCl_2$) did not influence salivary lubrication at low molar concentrations. However, a new mechanism for the monovalent salts was profound at high ionic strength values. KCl was found to provide extra lubrication via hydration lubrication. The hydrated mucins, provide aqueous lubrication which results in lower friction. The trivalent salts ($FeCl_3$ and $AlCl_3$) resulted in lubrication loss. This was mainly due to the interactions of mucins with the salt ions. Compared to $AlCl_3$, $FeCl_3$ led to greater salivary lubrication loss due to the effect of pH on salivary mucins (especially MUC7). Additionally, this study provides new insights into salt-phenol interactions which are related to salivary lubrication. Although not confirmed yet, our results suggest that trivalent cations may have the potential to limit the induced increase in friction by EGCG. EGCG-$FeCl_3$ interactions resulted in lower binding and aggregation of salivary mucins with either an excess ECGC or salt, which lead to less lubrication loss. Therefore, the addition of salts can reduce the aggregation of salivary proteins in presence of EGCG and vice versa. This study provides insights into the effect of mineral salts present in beverages and foods. The new outcomes suggest that interactions between salt ions and phenol need to take into account since affecting oral lubrication. Viewing the omnipresence of salts in foods, these insights are important for understanding the changes in salivary properties and consequently the taste preferences of individuals.

CRediT authorship contribution statement

Georgios Agorastos: Investigation, Conceptualization, Formal analysis, Writing – original draft. **Eva van Uitert:** Investigation, Methodology. **Emo van Halsema:** Conceptualization, Writing – review & editing, Project administration. **Elke Scholten:** Supervision, Conceptualization, Writing – review & editing. **Aalt Bast:** Supervision, Writing – review & editing. **Peter Klosse:** Supervision, Writing – review & editing, Funding acquisition, Project administration.

Declaration of Competing Interest

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

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.136968.

References

Almajano, M. P., Carbó, R., Jiménez, J. A. L., & Gordon, M. H. (2008). Antioxidant and antimicrobial activities of tea infusions. *Food Chemistry, 108*(1), 55–63. https://doi.org/10.1016/j.foodchem.2007.10.040

Bansil, R., & Turner, B. S. (2006). Mucin structure, aggregation, physiological functions and biomedical applications. *Current Opinion in Colloid & Interface Science, 11*(2–3), 164–170. https://doi.org/10.1016/j.cocis.2005.11.001

Bajec, M. R., & Pickering, G. J. (2008). Astringency: Mechanisms and perception. *Critical Reviews in Food Science and Nutrition, 48*(9), 858–875. https://doi.org/10.1080/10408390701724223

Biegler, M., Delius, J., Käsdorf, B. T., Hofmann, T., & Lieleg, O. (2016). Cationic astringents alter the tribological and rheological properties of human saliva and salivary mucin solutions. *Biotribology, 6*, 12–20. https://doi.org/10.1016/j.biotri.2016.03.002

Breslin, P. A. S., Gilmore, M. M., Beauchamp, G. K., & Green, B. G. (1993). Psychophysical evidence that oral astringency is a tactile sensation. *Chemical Senses, 18*(4), 405–417. https://doi.org/10.1093/chemse/18.4.405

Brossard, N., Cai, H., Osorio, F., Bordeu, E., & Chen, J. (2016). "Oral" Tribological study on the astringency sensation of red wines. *Journal of Texture Studies, 47*(5), 392–402. https://doi.org/10.1111/jtxs.12184

Canon, F., Neiers, F., & Guichard, E. (2018). Saliva and flavor perception: Perspectives. *Journal of Agricultural and Food Chemistry, 66*(30). https://doi.org/10.1021/acs.jafc.8b01998

Canon, F., Paté, F., Cheynier, V., Sarni-Manchado, P., Giuliani, A., Pérez, J., … Cabane, B. (2013). Aggregation of the salivary proline-rich protein IB5 in the presence of the tannin EgCG. *Langmuir, 29*(6), 1926–1937. https://doi.org/10.1021/la3041715

Çelebioğlu, H. Y., Lee, S., & Chronakis, I. S. (2020). Interactions of salivary mucins and saliva with food proteins: A review. *Critical Reviews in Food Science and Nutrition, 60*(1), 64–83. https://doi.org/10.1080/10408398.2018.1512950

Chaudhury, N. M. A., Shirlaw, P., Pramanik, R., Carpenter, G. H., & Proctor, G. B. (2015). Changes in saliva rheological properties and mucin glycosylation in dry mouth. *Journal of Dental Research, 94*(12), 1660–1667. https://doi.org/10.1177/0022034515609070

Coles, J. M., Chang, D. P., & Zauscher, S. (2010). Molecular mechanisms of aqueous boundary lubrication by mucinous glycoproteins. *Current Opinion in Colloid & Interface Science, 15*(6). https://doi.org/10.1016/j.cocis.2010.07.002

Crouzier, T., Boettcher, K., Geonnotti, A. R., Kavanaugh, N. L., Hirsch, J. B., Ribbeck, K., & Lieleg, O. (2015). Modulating mucin hydration and lubrication by deglycosylation and polyethylene glycol binding. *Advanced Materials Interfaces, 2*(18), 1500308. https://doi.org/10.1002/admi.201500308

Frenkel, E. S., & Ribbeck, K. (2015). Salivary mucins in host defense and disease prevention. *Journal of Oral Microbiology, 7*(1). https://doi.org/10.3402/jom.v7.29759

Garrec, D. A., & Norton, I. T. (2012). Boundary lubrication by sodium salts: A Hofmeister series effect. *Journal of Colloid and Interface Science, 379*(1), 33–40. https://doi.org/10.1016/j.jcis.2012.04.049

Gawel, R., Smith, P. A., Cicerale, S., & Keast, R. (2018). The mouthfeel of white wine. *Critical Reviews in Food Science and Nutrition, 58*(17), 2939–2956. https://doi.org/10.1080/10408398.2017.1346584

Gibbins, H. L., & Carpenter, G. H. (2013). Alternative mechanisms of astringency – What is the role of saliva? *Journal of Texture Studies, 44*(5). https://doi.org/10.1111/jtxs.12022

Gibbins, H. L., Yakubov, G. E., Proctor, G. B., Wilson, S., & Carpenter, G. H. (2014). What interactions drive the salivary mucosal pellicle formation? *Colloids and Surfaces B: Biointerfaces, 120*. https://doi.org/10.1016/j.colsurfb.2014.05.020

Habeych, E., van Kogelenberg, V., Sagalowicz, L., Michel, M., & Galaffu, N. (2016). Strategies to limit colour changes when fortifying food products with iron. *Food Research International, 88*, 122–128. https://doi.org/10.1016/j.foodres.2016.05.017

Hufnagel, J. C., & Hofmann, T. (2008). Orosensory-directed identification of astringent mouthfeel and bitter-tasting compounds in red wine. *Journal of Agricultural and Food Chemistry, 56*(4). https://doi.org/10.1021/jf073031n

Inoue, M. B., Inoue, M., Fernando, Q., Valcic, S., & Timmermann, B. N. (2002). Potentiometric and ^{1}H NMR studies of complexation of Al^{3+} with (−)-epigallocatechin gallate, a major active constituent of green tea. *Journal of Inorganic Biochemistry, 88*(1), 7–13. https://doi.org/10.1016/S0162-0134(01)00323-3

Krupkova, O., Ferguson, S. J., & Wuertz-Kozak, K. (2016). Stability of (−)-epigallocatechin gallate and its activity in liquid formulations and delivery systems. *The Journal of Nutritional Biochemistry, 37*, 1–12. https://doi.org/10.1016/j.jnutbio.2016.01.002

Laguna, L., Álvarez, M. D., Simone, E., Moreno-Arribas, M. V., & Bartolomé, B. (2019). Oral wine texture perception and its correlation with instrumental texture features of wine-saliva mixtures. *Foods, 8*(6). https://doi.org/10.3390/foods8060190

Laguna, L., Bartolomé, B., & Moreno-Arribas, M. V. (2017). Mouthfeel perception of wine: Oral physiology, components and instrumental characterization. *Trends in Food Science & Technology, 59*, 49–59. https://doi.org/10.1016/j.tifs.2016.10.011

Lawless, H. T., Rapacki, F., Horne, J., & Hayes, A. (2003). The taste of calcium and magnesium salts and anionic modifications. *Food Quality and Preference, 14*(4), 319–325. https://doi.org/10.1016/S0950-3293(02)00128-3

Lim, J., & Lawless, H. T. (2005). Oral sensations from iron and copper sulfate. *Physiology & Behavior, 85*(3), 308–313. https://doi.org/10.1016/j.physbeh.2005.04.018

Ligtenberg, A. J., Brand, H. S., van den Keijbus, P. A., & Veerman, E. C. (2015). The effect of physical exercise on salivary secretion of MUC5B, amylase and lysozyme. *Archives of Oral Biology, 60*(11), 1639–1644. https://doi.org/10.1016/j.archoralbio.2015.07.012

Ma, L., Gaisinskaya-Kipnis, A., Kampf, N., & Klein, J. (2015). Origins of hydration lubrication. *Nature Communications, 6*(1), 6060. https://doi.org/10.1038/ncomms7060

Macakova, L., Yakubov, G. E., Plunkett, M. A., & Stokes, J. R. (2011). Influence of ionic strength on the tribological properties of pre-adsorbed salivary films. *Tribology International, 44*(9), 956–962. https://doi.org/10.1016/j.triboint.2010.12.006

Mehrotra, R., Thornton, D. J., & Sheehan, J. K. (1998). Isolation and physical characterization of the MUC7 (MG2) mucin from saliva: Evidence for self-association. *Biochemical Journal, 334*(2). https://doi.org/10.1042/bj3340415

Mosca, A. C., & Chen, J. (2017). Food-saliva interactions: Mechanisms and implications. *Trends in Food Science & Technology, 66*. https://doi.org/10.1016/j.tifs.2017.06.005

Rudge, R. E., Scholten, E., & Dijksman, J. A. (2019). Advances and challenges in soft tribology with applications to foods. *Current Opinion in Food Science, 27*. https://doi.org/10.1016/j.cofs.2019.06.011

Rossetti, D., Bongaerts, J. H. H., Wantling, E., Stokes, J. R., & Williamson, A. M. (2009). Astringency of tea catechins: More than an oral lubrication tactile percept. *Food Hydrocolloids, 23*(7), 1984–1992. https://doi.org/10.1016/j.foodhyd.2009.03.001

Ryan, P., & Hynes, M. J. (2007). The kinetics and mechanisms of the complex formation and antioxidant behaviour of the polyphenols EGCg and ECG with iron(III). *Journal of Inorganic Biochemistry, 101*(4), 585–593. https://doi.org/10.1016/j.jinorgbio.2006.12.001

Rykke, M., Smistad, G., Rölla, G., & Karlsen, J. (1995). Micelle-like structures in human saliva. *Colloids and Surfaces B: Biointerfaces, 4*(1). https://doi.org/10.1016/0927-7765(94)01152-U

Silletti, E., Vingerhoeds, M. H., Norde, W., & van Aken, G. A. (2007). The role of electrostatics in saliva-induced emulsion flocculation. *Food Hydrocolloids, 21*(4), 596–606. https://doi.org/10.1016/j.foodhyd.2006.07.004

Soares, S., Vitorino, R., Osório, H., Fernandes, A., Venâncio, A., Mateus, N., … de Freitas, V. (2011). Reactivity of human salivary proteins families toward food polyphenols. *Journal of Agricultural and Food Chemistry, 59*(10). https://doi.org/10.1021/jf104975d

Veerman, E. C. I., van den Keybus, P. A. M., Valentijn-Benz, M., & Nieuw Amerongen, A. V. (1992). Isolation of different high-Mr mucin species from human whole saliva. *Biochemical Journal, 283*(3). https://doi.org/10.1042/bj2830807

Vijay, A., Inui, T., Dodds, M., Proctor, G., & Carpenter, G. (2015). Factors that influence the extensional rheological property of saliva. *PLOS ONE, 10*(8). https://doi.org/10.1371/journal.pone.0135792

Yakubov, G. E., McColl, J., Bongaerts, J. H. H., & Ramsden, J. J. (2009). Viscous boundary lubrication of hydrophobic surfaces by mucin. *Langmuir, 25*(4), 2313–2321. https://doi.org/10.1021/la8018666

Yu, Y., Yao, Y., van Lin, S., & de Beer, S. (2019). Specific anion effects on the hydration and tribological properties of zwitterionic phosphorylcholine-based brushes. *European Polymer Journal, 112*, 222–227. https://doi.org/10.1016/j.eurpolymj.2019.01.013