Difference in astringency of the main pea protein fractions

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ABSTRACT

Interactions between food and saliva govern complex mouthfeel perceptions such as astringency. Herein, we present a study of the interactions of salivary proteins with the main pea protein fractions that are obtained by isoelectric and salt precipitation (legumin-rich, vicilin-rich and albumin-rich fractions). The sensory evaluations performed on protein solutions by trained panelists evidenced that all three protein fractions exhibit a basal level of astringency, but that the albumin fraction was perceived as the most astringent one. All three fractions induced significant but comparable loss of salivary lubrication. Yet, when compared to the other fractions, the albumin fraction showed the formation of a thicker and more rigid film on salivary conditioning film-coated sensors as measured using a quartz crystal microbalance with dissipation monitoring (QCM-D). We also present proteomics studies on the precipitates obtained from the mixtures of saliva and pea protein fractions. Protein identification finds a pool of salivary proteins involved in non-specific interactions with all the three pea protein fractions. Yet, 13 pea proteins specific to the albumin fraction were identified as being involved in specific interactions with salivary proteins. Several of these proteins are part of the plant defense mechanisms and are likely to interact with many salivary proteins. This could explain the higher number of salivary proteins found in the precipitate induced by the albumin fraction when compared to the other two. These quantitative results increase the understanding of the complex links between plant protein-salivary protein interactions and astringency.

1. Introduction

Plant-based ingredients have become of paramount importance for food industries to replace ingredients derived from animals and improve the sustainability and nutritional quality of food products while keeping attractive prices (Green et al., 2022). In particular, yellow field pea (Pisum sativum L.) has received much attention to produce plant-based protein ingredients because of its low allergenicity, high nutritional value and good functionalities (McClements, Lu, et Grossmann 2022; Siddique et al., 2012). Yet, for liquid products, pea protein isolate (PPI) has been reported to be astringent in sensory analysis (Cosson, Delarue, et al., 2020; García Arteaga et al., 2021). Such astringency severely limits its use also considering that there are reports on beany and bitter notes (Canon et al., 2021).

Astringency is defined as the "complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alumns or tannins'' by the American Society for

Testing and Materials (ASTM) (ASTM, 2004). Even if astringency is one of the sensations having the strongest negative impact on consumers acceptability in plant-based products, its mechanisms in plant proteins remain principally unexplored even if the mechanisms for polyphenol astringency is well-researched in literature. Astringency caused by polyphenols is generally considered to be a tactile sensation, detected by mechanoreceptors, and the most well-known astringent compounds such as tannins bind and precipitate salivary proteins (Canon et al., 2013). However, astringency was also perceived in dairy protein beverages at low pH, and studies showed that it was associated with interactions between cationic whey proteins and negatively-charged mucins (Carter et al., 2020). It is thought that by precipitating salivary proteins, astringent compounds alter the structure of salivary mucosal pellicle and consequently increase frictional forces in the oral cavity leading to the activation of mechanoreceptors (Canon et al., 2021). In a similar way to astringent polyphenolic compounds, one can hypothesize that astringency perception of plant proteins is a result of interactions

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between plant proteins and salivary proteins (Assad-Bustillos et al., 2023). In a recent systematic review, Brown et al. (2021) pointed out a clear gap in research about interactions between plant and saliva proteins and the subsequent impact on mouthfeel perception.

Mouthfeel perceptions such as creaminess, smoothness and astringency are governed by the interactions between food components and saliva and the salivary pellicle coating oral surfaces during mastication (Sarkar & Krop, 2019; Stokes et al., 2013). Saliva primarily provides lubrication in the mouth - thanks to the formation of a salivary film coating oral surfaces. The lubrication properties of the food bolus and the friction forces associated with interacting oral surfaces can be measured by oral tribology. Determination of friction coefficients have been successfully used to understand the complex dimensions of mouthfeel perception including astringency in red wines (Brossard et al., 2016), tea (Rossetti et al., 2009), plant-based and dairy proteins (Vlădescu et al., 2023). In these studies, friction coefficients measured over a wide range of lubrication speeds tend to correlate with surface related sensory properties (astringency, creaminess, …) (Sarkar et al., 2021; Shewan et al., 2019).

In addition to tribological analysis, quartz crystal microbalance with dissipation monitoring (QCM-D) enables real-time measurements of protein adsorption on surfaces with varying degree of surface hydrophobicities. QCM-D and oral tribology are highly complementary as the film properties can be related to the frictional behavior of the salivary proteins (Xu et al., 2019) as well as plant proteins (Kew et al., 2021).

Pea proteins are a complex mixture of proteins primarily comprising salt-soluble globulin proteins (55–65 %) while water-soluble albumin proteins represent the second major class of proteins (18–25%) (Lam et al., 2018). Legumin (11S, 300 kDa) and vicilin (7S, 150 kDa) are the two main storage proteins belonging to the globulin family. The wet extraction and separation of pea proteins to produce protein ingredients is based on their different isoelectric points and solubilities (Rubio et al., 2014). Pea protein ingredients such as protein concentrates and isolates are rich in globulins, and exhibit interesting gelling and emulsifying functionalities (Shanthakumar et al., 2022). Kornet et al. (2022) and Yang et al. (2022) also highlighted the good emulsifying and foaming properties of albumin proteins from pea.

Given the complexity of astringency perception, it is likely that multiple mechanisms coexist and participate to different extents in astringent sensation during the consumption of plant-based products. In this context, it remains a challenge to link the different levels of astringency perception obtained by sensory analysis to instrumental results obtained to gain an overall understanding of the phenomenon.

The aim of this study was to gain mechanistic understanding on the impact of pea protein-salivary protein interactions on astringency perception. We asked three questions 1) do different fractions of pea proteins have different astringency perception, in other words are globulin fractions more astringent than the albumin fraction? 2) is there a correlation between sensory astringency and oral friction or adsorption? 3) do the salivary protein-plant protein complexes vary depending upon the protein type? To do so, we investigated the astringency levels of the three main pea protein fractions (legumin-rich, vicilin-rich and albumin-rich fractions). A complimentary suite of experimental methods from *in vivo* trained panel-based sensory analysis, to the *in vitro* impact of the pea protein fractions on salivary lubrication (saliva collected *ex vivo*) and protein adsorption on *ex vivo* salivary conditioning film-coated surfaces, to the identification of the proteins *via* proteomics that precipitated when interacting with salivary proteins.

2. Materials and methods

2.1. Materials

Commercial pea flour F200X with 24.5% protein was kindly gifted by Vestkorn (Vestkorn, Norway). Sodium chloride (NaCl), hydrochloric acid (HCl) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

(HEPES) buffer were purchased from Thermo Fisher Scientific (Loughborough, UK). All the chemicals for sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) including NuPAGE™ 4–12%, Bis-Tris, 1.0 mm, Mini Protein Gel, MES™ Running Buffer (20X), NuPAGE™ LDS sample buffer (4X), NuPAGE™ Sample Reducing Agent (10X), PageRuler™ Unstained Broad Range Protein Ladder, Imperial™ Protein Stain, were purchased from Thermo Fisher Scientific (Loughborough, UK). Protein solutions were prepared with ultrapure water (water purified by a Milli-Q apparatus, Millipore Corp., USA) with a resistivity of 18.2 MΩ at 25 °C. Polydimethylsiloxane (PDMS) (base fluid and cross-linker (10:1 w/w)) was bought from Clearco (Sylgard 184, Dow Corning, Midland, MI, USA), and was used for creating PDMS-coated QCM-D sensors. PDMS balls and disc were purchased from PCS Instruments (London, UK). To prepare the 3D biomimetic tongue like surface, Ecoflex 00–30 kit was purchased from Smooth-on Inc. (Pennsylvania, US) and the surfactant Span 80 was purchased from Sigma-Aldrich (Dorset, UK). All solutions were prepared from analytical grade chemicals unless otherwise mentioned.

2.2. Fractionation of pea proteins

The procedure for protein extraction from the pea flour (24.5% protein) was as from Rubio et al. (2014) with some modifications. The extraction protocol was based on the difference of isoelectric points between legumin and vicilin proteins (4.6 and 5.5 respectively) and solubility (water for albumin proteins and salt solutions for globulin proteins). The pea flour was diluted in buffer solution 20 mM, pH 8 with 0.5 M NaCl for 2 h under magnetic stirring at room temperature, and centrifuged (15 min, 5000 rpm, 4 °C). The supernatant was kept, and the insoluble sediments were re-extracted in the conditions described previously and centrifuged. The extract was combined with the first supernatant and adjusted to pH 4.5 with HCl, stirred for 1 h, and centrifuged (15 min, 5000 rpm, 4 °C). The sediment corresponded to the first protein fraction called the legumin-rich fraction. It was re-dissolved in buffer, dialyzed extensively against ultrapure water and freeze-dried (Alpha 3–4 LSCbasic, Christ, Germany). The supernatant was also dialyzed extensively against ultrapure water and centrifuged (15 min, 5000 rpm, 4 °C). The new sediment was freeze-dried and corresponded to the second protein fraction of the study called the vicilin-rich fraction. The new supernatant was treated with ammonium sulphate $(NH_4)_2SO_4$ (600 g/L), stirred for 2 h at room temperature and centrifuged (15 min, 5000 rpm, 4 °C). The sediment was extensively dialyzed against ultrapure water and freeze-dried. The sediment obtained corresponded to the third protein fraction investigated in this study called albumin-rich fraction.

2.3. Collection of human saliva

Fresh unstimulated human saliva was collected before each experiment. Participants were informed about the study and asked to sign a consent form (Faculty Research Ethics Committee of the University of Leeds (MEEC-16-046)). Participants (n = 8) were asked to refrain from eating and drinking (except water) for 2 h before collection to reduce the influence from the intake of foods and beverages. They were also instructed to rinse their mouths with water. During saliva collection, participants were asked to passively accumulate saliva in the mouth and then spit it into a clean tube kept on ice during the whole collection time (15 min). Immediately after collection, saliva samples from all the participants were pooled and centrifuged (10 min at 15 000 rpm). The supernatant was collected and stored at 4 °C before use. Saliva was diluted in HEPES buffer (20 mM, pH 7) in a 1:1 w/w ratio to match the high volume of saliva needed for tribological measurements. HEPES is widely used in biological research because of its optimal buffer capacity within the physiological range (pH 6.8–8.2) and because it does not form complexes with metal ions and may prevent the damage of certain proteins (Ferreira et al., 2015; Good et Izawa 1972).

2.4. Preparation of protein suspensions

Aqueous solutions of the three pea protein fractions *i.e.*, legumin-rich, vicilin-rich and albumin-rich fractions (100 mg/ml w/v protein) were prepared by dispersing and mixing the protein powders in ultra-pure water (pH 5.6) for 2 h to ensure optimum dissolution. The protein suspensions were centrifuged (5 min at 15 000 rpm) to get rid of the insoluble fractions. The supernatants were collected and diluted to reach the same protein concentration of 10 mg/mL for all three pea protein fractions. The protein concentration was determined colorimetrically with the BiCinchoninic acid Assay *(*BCA) (Pierce BCA assay kit, Thermo Fisher Scientific, UK).

2.5. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis

SDS-PAGE analysis was carried out to monitor the electrophoretic pattern of the different protein fractions extracted. Protein suspensions at 1 mg/mL of the three pea protein fractions (legumin-rich, vicilin-rich and albumin-rich fractions) were mixed in NuPAGE™ Sample Reducing Agent (10X) and NuPAGE™ LDS sample buffer (4X). The protein suspensions were denatured (heated 10 min at 70 °C) and 15 µl of the mix was loaded on the precast gels. The gels were immersed in an Invitrogen™ Mini Gel Tank system (Thermo Fisher Scientific, Loughborough, UK) and submerged in a solution of running buffer: ultrapure water (1:20 v/v) and protein molecular weights (5 µl) were loaded on the first lane of the gels. The gels were run for 45 min at constant voltage 200 mV. Proteins were stained using a Coomassie Blue staining kit and destained overnight in ultrapure water before being scanned using GelDoc Go imaging system (Bio Rad, München, Germany).

2.6. Sensory analysis

Sensory analysis was performed using a trained panel (n = 12) regularly used for evaluation of plant protein beverages. The attributes to evaluate were defined during a pre-tasting session involving 3 sensory experts, therefore no language generation was needed with the panel. Protein solutions (0.5% w/v protein) were evaluated on green pea (smell only), bitterness and astringency on an unstructured scale (0–100). During a first training part, the 12 trained panelists practiced language, scale usage and sensory methodology while evaluating samples. During the data collection part, the samples were presented one by one, in blind, in a complete balanced order. For each descriptor, the panelists rated the perceived intensity on a 100-point scale. To facilitate the task, they were given an indication on the score given on the previous sample, for each attribute. To avoid build-up effect, panelists used palate cleansers (sour cream, warm water, apple slices, unsalted crackers) and 20-min time breaks in between samples. Moreover, the three replicates were performed on three different days.

2.7. Rheology

Shear viscosity was measured using a rotational rheometer Kinexus Ultra+ (Malvern, UK) equipped with a 50 mm diameter parallel plate geometry. The gap was fixed at 1.0 mm and the temperature was kept at 37 °C to mimic the oral conditions. Shear viscosity was measured at a range of shear rates from 0.1 to 1000.0 s^{-1}. Measurements were carried out in triplicates.

2.8. Soft tribology

Friction coefficients were measured using a Mini Traction Machine MTM2 from PCS instruments (UK). The testing set-up was a ball (19.0 mm diameter) on disc contact, with both surfaces made of silicone (PDMS). To emulate oral processing, temperature was fixed at 37 °C (Assad-Bustillos et al., 2023; Ye et al., 2021) and contact normal force were fixed at and 2.0 N (Hertzian contact pressure of 343 kPa). The entrainment speed U represents the relative motion of rolling/sliding surfaces and is defined as $U = (U_B + U_D)/2$, where U_B and U_D are the ball and disc linear speeds respectively. The sliding rolling ratio was set at 50% to mimic oral conditions. In addition, tribology was also measured to understand boundary friction in 3D tongue-like surface that emulated the wettability, contact pressure and topography of human tongue as described in Andablo-Reyes et al. (2020). The two elastomeric components of the Ecoflex 00–30 kit (Smooth-on Inc, Pennsylvania, US) were crosslinked in a 1:1 w/w ratio and the surfactant Span 80 (Sigma-Aldrich, Dorset, UK) was used at 0.5 wt % as to modify the wettability of Ecoflex 00–30 tongue-like surface (Andablo-Reyes et al., 2020).

2.9. Adsorption behavior using QCM-D

The real time adsorption of proteins was measured by QCM-D (E4 system, Q-Sense, Gothenburg, Sweden) by measuring the shifts in frequency and dissipation at different overtones. This measurement provided information on the adsorption kinetics, mass and viscoelastic properties of the adsorbed film (Xu et al., 2019).

2.9.1. Preparation of PDMS – coated QCM-D sensors

PDMS-coated sensors coated with human saliva were used to mimic the oral surfaces (Xu et al., 2019). Prior to measurements, the PDMS surfaces were cleaned by 30 s immersion in toluene, followed by 30 s in isopropanol, then 2 min immersion in ultrapure water, drying with nitrogen gas.

2.9.2. QCM-D measurements

Protein solutions were made at a concentration of 2.0 wt% and were equilibrated in a buffer at 25 °C before measurements. A peristaltic pump was used to control the flow rate (100 µL/min) at 25 °C. The first step was to inject the buffer solution until a stable baseline was obtained. The second step was to inject human saliva diluted in HEPES buffer (1:20 v/v) to allow the formation of a salivary pellicle on the QCM-D sensor. Human saliva was injected into the system and left to adsorb for at least 1 h under the flow conditions until no change in frequency (f) and dissipation (D) was recorded. The surface was then rinsed with HEPES buffer for at least 30 min to remove the non-adsorbed proteins. The third step was to inject the prepared pea protein solutions (2.0 wt%) to study their adsorption on QCM-D sensors coated with human saliva and PDMS. The three pea protein solutions were left to adsorb for at least 1 h under the flow conditions until no change in frequency (f) and dissipation (D) was recorded. Finally, HEPES buffer was injected once more for at least 30 min to remove the non-adsorbed proteins.

Hydrated mass was calculated from the frequency data using viscoelastic Voigt's model (Voigt, 1889), using "Smartfit Model" by Dfind (Q-Sense, Biolin Scientific, Sweden) software. The 3rd, 5th, 7th and 11th overtones were taken into account for data analysis and only 5th overtone is shown in the results. A minimum of three replicates were measured for each protein sample (Kew et al., 2021).

2.10. Identification of proteins by proteomics analysis

2.10.1. In vitro protein precipitation

The rationale of the experiments involving proteomics analysis consisted in allowing the different pea protein fractions to interact with human saliva to form precipitates. The precipitates were then separated and analyzed to identify the proteins involved in interactions. The protein suspensions (described in section 2.4) were mixed in a 1:1 ratio either with unstimulated human saliva or HEPES buffer (20 mM, pH 7) which was used as a negative control for saliva. HEPES has been used in the past by other authors as a control when studying the lubrication properties of pea proteins or the interactions between pea and salivary proteins (Kew et al., 2021). Therefore, the final pea protein concentration was 0.5% w/v after mixing the pea protein suspensions with whole

human saliva. Due to the low pea protein concentration (0.5% w/v), a 1:1 vol ratio of pea proteins and saliva leads to an overall "true" protein ratio of 1.6. This value is close to the 2.0 protein ratio used by Assad--Bustillos et al. (2023), and it is likely to be closer to the remaining concentration of pea proteins in the mouth after swallowing, which is also the phase of consumption in which astringency perception is the strongest (Cosson, Souchon, et al., 2020). The mixtures were left for 15 min at room temperature before further measurements to allow proteins to interact and form stable complexes and subsequently centrifuged (15 min at 15 000 rpm) to separate the pellets containing the precipitated proteins and supernatants.

2.10.2. Proteomics analysis

2.10.2.1. Sample preparation for mass spectrometry.
Mass spectrometry-based proteomics-related experiments were performed by the Proteomics Core Facility at EPFL. Each sample was digested by filter aided sample preparation (FASP) (Wiśniewski et al., 2009) with minor modifications. Proteins (20 µg) were reduced with 10 mM TCEP in 8M Urea, 0.1M Tris-HCl pH 8.0 at 37 °C for 60 min and further alkylated in 40 mM iodoacetamide at 37 °C for 45 min in the dark. Proteins were digested overnight at 37 °C using 1/50 w/w enzyme-to-protein ratio of mass spectrometry grade Trypsin and LysC. Generated peptides were desalted in StageTips using 6 disks from an Empore C18 (3 M) filter based on the standard protocol (Rappsilber, Mann, et Ishihama 2007). Residual SDS and impurities were cleared using Detergent Removal Cartridges (Thermo Scientific HiPPR) as described by the manufacturer.

2.10.2.2. Liquid chromatography tandem mass spectrometry (LC-MS/MS).
LC-MS/MS is particularly suitable for the analysis of complex protein mixtures (van Vliet 2014). Samples were resuspended in 2% acetonitrile (Biosolve), 0.1% FA and nano-flow separations were performed on a Dionex Ultimate 3000 RSLC nano UPLC system (Thermo Fischer Scientific) on-line connected with an Exploris 480 Mass Spectrometer (Thermo Fischer Scientific). A capillary precolumn (Acclaim Pepmap C18, 3 µm-100 Å, 2 cm × 75 µm ID) was used for sample trapping and cleaning. A 50 cm long capillary column (75 µm ID; in-house packed using ReproSil-Pur C18-AQ 1.9 µm silica beads; Dr. Maisch) was then used for analytical separations at 250 nl/min over 90 min biphasic gradients. Acquisitions were performed through Top Speed Data-Dependent acquisition mode using a cycle time of 2 s with scan range between 350 and 1200. First MS scans were acquired with a resolution of 60′000 (at 200 m/z) and the most intense parent ions were selected and fragmented by High energy Collision Dissociation (HCD) with a Normalized Collision Energy (NCE) of 30% using an isolation window of 1.4 m/z. Fragmented ions were acquired with a resolution 15′000 (at 200m/z) and selected ions were then excluded for the following 20 s.

2.10.2.3. Data analysis.
Raw data were processed using MaxQuant 1.6.10.43 (Cox & Mann, 2008) against concatenated database from Uniprot of human and pea (Pisum sativum, 10305 Sequences downloaded June 2021; Homo sapiens 79052 sequences LR2022_01), Carbamidomethylation was set as fixed modification, whereas oxidation (M), phosphorylation (S, T, Y), acetylation (Protein N-term) and glutamine to pyroglutamate were considered as variable modifications. A maximum of two missed cleavages were allowed and "Match between runs" option was enabled. A minimum of 2 peptides was required for protein identification and the false discovery rate (FDR) cutoff was set to 0.01 for both peptides and proteins. Label-free quantification and normalization was performed by Maxquant using the MaxLFQ algorithm, with the standard settings (Cox et al., 2014).

The statistical analysis was performed using Perseus version 1.6.12.0 (Tyanova et al., 2016) from the MaxQuant tool suite. Reverse proteins, potential contaminants, proteins only identified by sites and Pisum proteins were filtered out. Common proteins across all samples were conserved for further quantitative analysis. A two-sample *t*-test with permutation-based FDR statistics (250 permutations, FDR = 0.05, S0 = 0.1) was performed to determine significant differentially abundant candidates.

2.11. Statistical analysis

All results are reported as means and standard deviations on at least three replicates. Statistical analysis on the significance between data sets was calculated using analysis of variance (ANOVA, $p < 0.05$) with Tukey post hoc test.

3. Results and discussion

3.1. Protein composition

The protein composition of the three pea protein fractions was characterized using SDS-PAGE in denaturing and reducing conditions (Fig. 1). As expected, the first fraction obtained in the fractionation protocol showed high intensity electrophoretic bands corresponding to the α-chain (40 kDa) and a β-chain (20 kDa) of globulin 11S – legumin proteins (Dziuba et al., 2014) as the legumin subunits were cleaved under denaturing conditions. 11S – globulins are the predominant proteins in the legumin-rich fraction which has a very similar composition to the one of commercial pea protein isolates with the presence of bands corresponding to 7S – globulin proteins and albumin proteins. The analysis of the electrophoretic pattern gives a rough estimation of the degree of cross-contamination between the different protein fractions. It can be estimated that 14% of the protein bands visible in the legumin-rich fraction corresponded to proteins belonging to the albumin fraction (mainly Albumin-2 at 26 kDa).

The second fraction obtained showed major electrophoretic bands
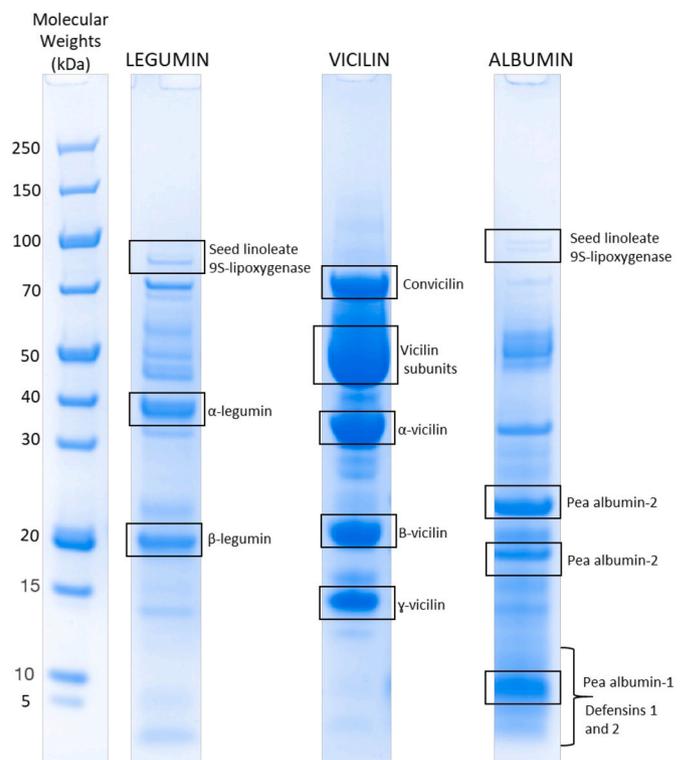
Fig. 1. Sodium dodecyl sulphate polyacrylamide gels electrophoresis (SDS-PAGE) of extracted pea protein fractions in reducing and denaturing conditions. Protein fractions are as follow a) legumin-rich, b) vicilin-rich, c) albumin-rich fractions.

corresponding to vicilin and convicilin polypeptides. The higher molecular weights (70 kDa) corresponded to convicilin and polypeptides with molecular weights of 50 kDa that have been reported to be derived from vicilin precursors by limited post-translational processing (Gatehouse et al., 1981). The vicilin-rich fraction was free of contamination in agreement with the results obtained with the same protocol by Rubio et al. (2014).

In the last fraction recovered, the major electrophoretic bands were albumin-2 with a molecular weight of 26 kDa. This third fraction was also rich in low molecular weight water-soluble proteins involved in the plant metabolism such as albumin-1 (6 kDa), lectin, defensin-1 and defensin-2. Bands corresponding to vicilin subunits are visible on the electrophoretic pattern of the albumin-rich fraction. It could be estimated that these bands (bands at 50 kDa) represent roughly 25% of the overall proteins present in the albumin-rich fraction. The composition of the albumin-rich fraction is in line with previous studies that evidenced that the albumin-rich fraction is made of proteins with a broad range of molecular weights and isoelectric points (Dziuba et al., 2014).

3.2. Sensory analysis

The results of sensory assessments are shown in Fig. 2 and only the results regarding astringency will be discussed in this study. It can be noted that the astringency levels of the three pea protein fractions were higher than 25/100, which highlights the fact that astringency is a sensory characteristic common to all the three pea protein fractions. The astringent properties of pea protein ingredients are well known, and pea protein isolate has been reported to be astringent (García Arteaga et al., 2021). In addition to the basal astringency level, the results of the sensory test evidenced that the albumin-rich fraction had a significantly (See Table S2, supplementary material) higher astringency level (38/100) compared to the one of the vicilin-rich and legumin-rich fractions (28/100 and 26/100 respectively). This result is in line with the conclusions of the study performed by Cosson et al. (2021) on fractions obtained from commercial pea protein isolates. In this study, the authors evidenced that the fractions made of soluble proteins were more astringent than the fractions made of insoluble proteins.
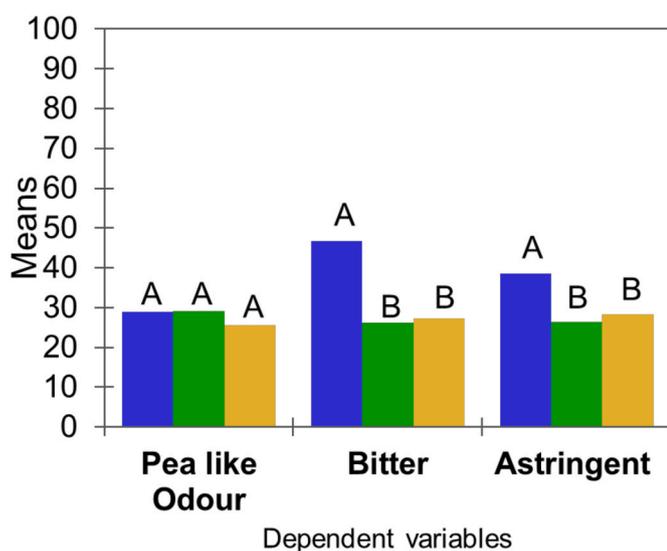

Fig. 2. Plot of the ratings obtained by QDA profiling on the protein solutions. The scores represent the mean of the scores attributed in triplicates by 12 panelists. Bars within one attribute with different letters denote a statistically significant difference (p < 0.05).

3.3. Soft tribology

Using soft PDMS tribo-contact surfaces, the friction coefficient (μ) was measured as a function of entrainment speed for the buffer, fresh unstimulated human saliva, protein suspensions (0.5 mg/mL) and mixtures of saliva and protein suspension. Fig. 3a, b, c showed that for entrainment speeds in the range 0.001–1 m.s-1, the friction coefficients were in the boundary and mixed regimes which have been previously correlated with sensory perception (Dresselhuis, Klok, Stuart, J de Vries, & A van Aken, 2007; Sarkar et al., 2017). The friction coefficient of the buffer was high (μ ≥ 1.0) in these two lubrication regimes, because of the inability of the buffer to adsorb on the hydrophobic PDMS substrate to form a load-bearing film, which is in line with previous results obtained on comparable tribological set ups (Xu et al., 2019). On the contrary, human unstimulated saliva represented the lower bound for μ, with a 20-time reduction of friction coefficient in the boundary regime as compared to the buffer. This is in agreement with previous studies that demonstrated the excellent lubrication properties of saliva by showing one order of magnitude reductions of boundary friction coefficient compared to the buffer (Xu et al., 2019; Vardhanabhuti et al., 2011; Sarkar & Krop, 2019). This decrease of friction is thought to be due to the formation of a complex salivary film involving salivary glycosylated proteins on the PDMS surfaces which also contributes to the reduction in friction coefficient at higher entrainment speeds (>0.01 m.s-1) (Aguirre et al., 1989; Yakubov et al., 2015).

Fig. 4a and b shows that the friction coefficients of the three pea protein fractions were significantly lower compared to the one of the buffer in both the boundary (0.01 m.s-1) and mixed (0.1 m.s-1) regimes. This suggests the surface-active properties and the ability of the three fractions of pea proteins to entrained in the contact region (Sarkar et al., 2019). Kew et al. (2021) also used soft tribology to compare the lubrication properties of different plant and dairy proteins and evidenced that commercial pea protein concentrates and isolates have lubrication properties for protein concentrations between 1 and 5 % (w/v). Fig. 4a highlights that the albumin-rich and the vicilin-rich fractions elicited a significantly stronger decrease of friction in the boundary regime (0.45 and 0.48 respectively) compared to the legumin-rich fraction (0.76). This property could be related to the higher solubility of the albumin-rich and vicilin-rich fractions (see Table S1, supplementary material) which may be an important parameter for lubrication properties in terms of the ability to bind water and form a hydrated layer upon entrainment (Kew et al., 2021).

The mixture of human saliva with the legumin-rich, vicilin-rich and albumin-rich fractions gave information on the impact of the protein fractions on the delubrication properties of saliva. Fig. 3a, b, c evidence that the friction coefficients of the mixtures of pea proteins and saliva were significantly higher compared to saliva alone. It can be noted that only a very low concentration of pea proteins (0.5 mg/mL) was necessary to induce a complete loss of salivary lubrication, and that the lubrication properties of the mixtures of saliva and pea protein fractions were very close to the ones of the pea protein fraction suspensions, suggesting that the surface-active properties of the pea proteins dominated in the mixtures. The loss of salivary lubrication was likely to be due to interactions between the different pea protein fractions and saliva in the mixture prior to the tribological test. Since the lubricating salivary proteins were involved in interactions with pea proteins, they were not available to adhere onto the PDMS surface, which prevented the formation of the lubricating salivary film in the contact zone and resulted in high friction coefficients.

As pea proteins are present in excess in the mixture (pea: saliva proteins ratio of 1.6), it is likely that the pea proteins not involved in interactions with salivary proteins adhered to the PDMS surface, thus dominating the lubrication behavior. It is worth mentioning that rheology could not explain the tribological behavior of the protein fractions. In particular, at high plateau shear rates of 1000 s-1 which is often considered as the shear rate for tribological limits with narrow
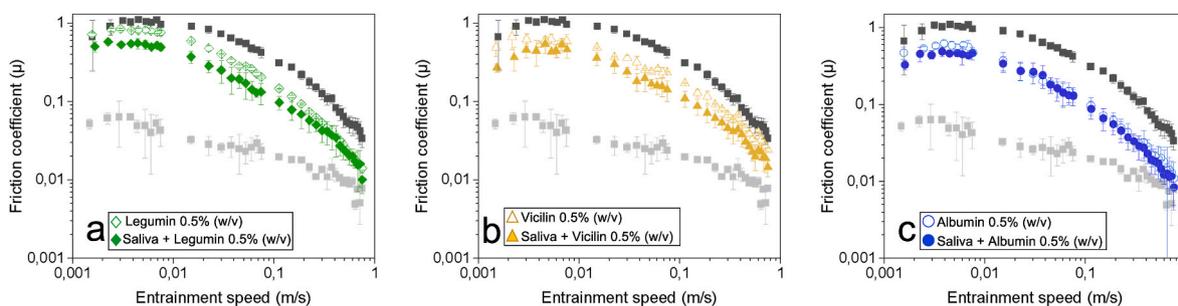
Fig. 3. Plots of mean friction coefficient (μ) as a function of entrainment speed (m.s^{-1}) determined between ball and disc, both surfaces made by PDMS, at 2N load in presence of protein solutions at 0.5% (w/v) for a) legumin-rich b) vicilin-rich c) albumin-rich fractions respectively. The same friction curves of HEPES buffer (■) and whole human saliva (■) are represented in all the graphs.
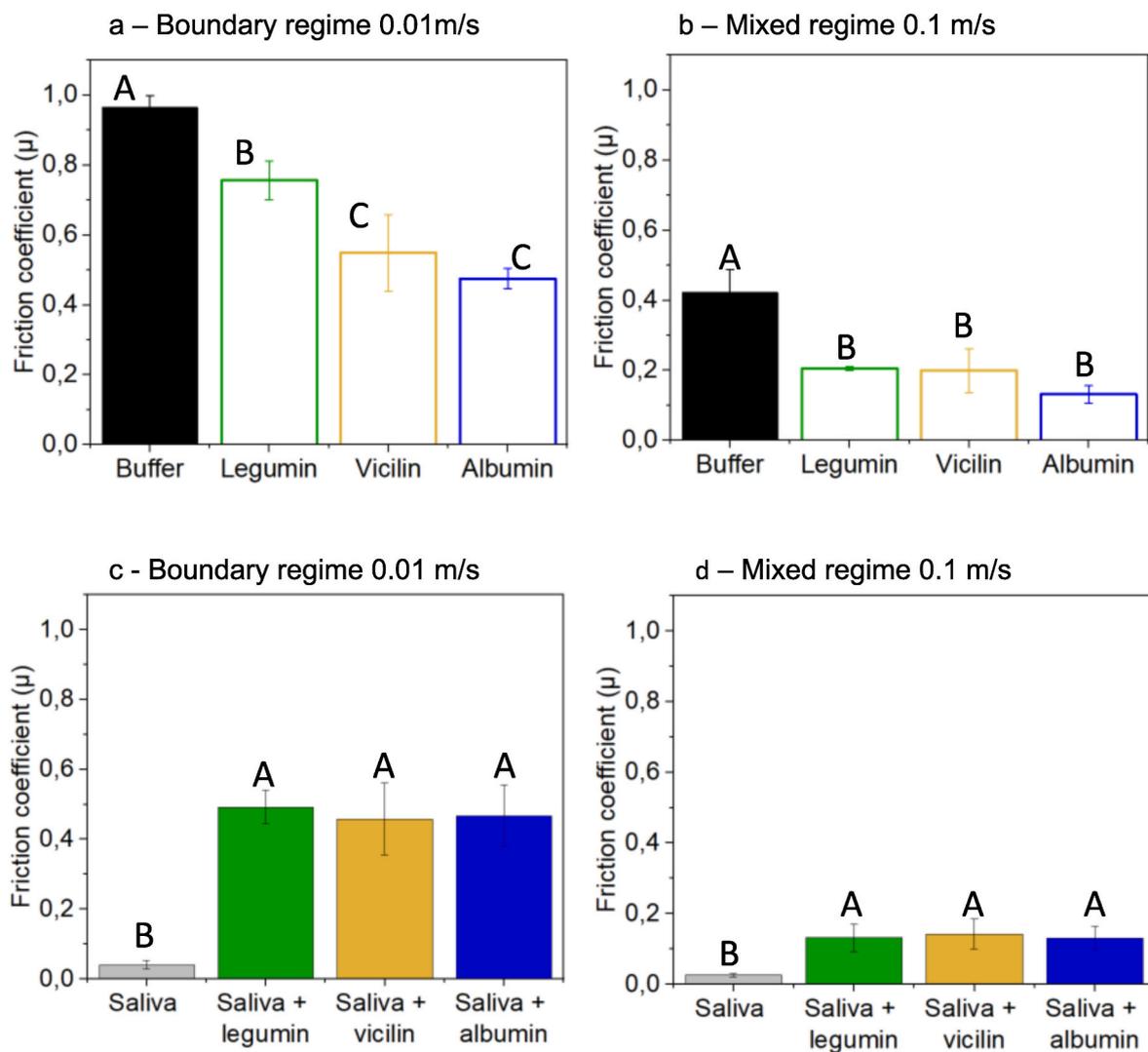

Fig. 4. Plots of mean friction coefficients (μ) obtained in soft tribology for protein solutions (0.5% w/v) in buffer (white bars) a) boundary (0.01 m s^{-1}), b) mixed (0.1 m s^{-1}) regimes and for protein solutions in human saliva (colored bars) c) boundary (0.01 m s^{-1}) and d) mixed (0.1 m s^{-1}) regimes. Different letters in the same graph indicate a significant difference (p < 0.05).

gaps (Kew et al., 2023; Stokes et al., 2011), all the three pea protein fractions had very similar apparent viscosities (see Fig. S1, supplementary). In their study about astringency perception in red wines, Brossard et al. (2016) also investigated the impact of astringent compounds on salivary lubrication and evidenced a loss of salivary lubrication with the addition of astringent compounds (catechin and tannic acid) in saliva,

with a good correlation with sensory results obtained in the boundary regime (sliding speed of 0.075 mm/s). This is also in line with the results obtained by Vlădescu et al. (2023) using different pea protein isolates and *ex-vivo* human saliva. The use of *ex-vivo* human saliva seems to be a key parameter to investigate frictional behavior of proteins. Indeed, Liamas, Connell, et Sarkar (2023) used bovine submaxillary mucin

(BSM) as proxies for salivary pellicles coating surfaces and reveal a different trend with superior lubricating properties due to interactions between plant proteins and mucins.

Fig. 4c and d shows that in the boundary and mixed regimes, the friction coefficients of saliva were increased 10 times by the addition of the pea protein fractions with no significant difference between legumin-rich, vicilin-rich and albumin-rich fractions. The fact that the three pea protein fractions had the exact same impact on the lubricating properties of saliva might be related to the basal level of astringency perception evidenced in the sensory analysis and indicated that there was a commonality between the pea proteins fractions.

Fig. 5 shows the friction coefficients measured using a 3D biomimetic tongue set up, enabling a better emulation of the complex features of a real tongue surface. These measurements were carried out at low linear speeds to focus on the boundary regime (0.01 m.s-1) (full data shown in Fig. S2, supplementary material) which is particularly relevant when looking at correlations between tribology and sensory studies. The results obtained using the 3D biomimetic tongue set up show a similar trend as compared to the ones obtained using the Mini Traction tribometer, further confirming the lubrication behavior of the pea proteins in solution and the lubrication breakdown of human saliva when mixed with all three pea protein fractions.

3.4. Surface adsorption characteristics

The adsorption behavior of the three pea protein fractions was monitored by recording the frequency and dissipation shifts as a function of time by QCM-D measurements. Fig. 6 shows the results for the adsorption of the three protein fractions on hydrophobic PDMS-coated sensors after adsorption of a layer of unstimulated human saliva. The adsorption of salivary conditioning film on the sensor was important to recreate conditions closer to the in-mouth salivary pellicle (Canon et al., 2021). The sharp and instantaneous decrease of frequency suggests that the adsorption of saliva on the PDMS-coated sensor was fast. The mean hydrated mass adsorbed reached 29.20 mg $\pm$ 0.25, which is in line with previous studies (Glumac, Ritzoulis, et Chen 2019; Ash et al., 2014). Some weakly adsorbed salivary proteins were removed by rinsing with water after saliva injection to obtain a stable baseline. The injection of the three protein suspensions (2.0 mg/mL) led to a decrease of frequency shift and an increase in dissipation shift for the three pea protein fractions, but the trends were clearly different depending on the pea protein fraction injected. Injection of the vicilin-rich fraction resulted in the smallest frequency shift (-69 Hz), followed by the legumin-rich fraction (-112 Hz) and the very sharp decrease of frequency measured after the

injection of the albumin-rich fraction (-348 Hz). The injection of the albumin-rich fraction also led to a moderate increase in dissipation (25 ppm), while the increase was significantly higher for the legumin-rich fraction (57 ppm) and the dissipation remained constant after the injection of the vicilin-rich fraction. To the best of our knowledge, no study investigated the adsorption behavior of pea proteins on saliva-coated PDMS sensors until now. Zembyla et al. (2021) investigated the adsorption of pea protein isolates on mucin-coated surfaces and the resulting frequency shift decreased by -15 Hz. It can be hypothesized that the presence of the salivary pellicle on the sensor created a more favorable environment for pea protein adsorption, which is in line with the results obtained by Liamas, Connell, et Sarkar (2023). The protein injection was followed by a rinsing step, highlighting additional differences between the protein films adsorbed. The injection of water induced an increase in frequency for the albumin-rich fraction (+52 Hz) and an increase of dissipation (6 ppm) which suggests that a significant amount of weakly adsorbed proteins was rinsed away with water. Only minor changes in frequency shift and dissipation were observed for the vicilin-rich fraction, indicating very little adsorption of the protein layer. Subsequent rinsing of the legumin-rich fraction induced a moderate rise of frequency shift (+17 Hz) and an unexpected rise of the dissipation (+9 ppm) which might indicate that water was bound in the adsorbed legumin proteins, thus increasing the viscoelasticity of the film. The data about the total hydrated mass of saliva and pea protein fraction shown in Fig. 7 a) is in line with the variations of frequency and dissipation shift. The albumin-rich fraction exhibited a significantly higher adsorption (62.8 ± 7.64 mg m^{-2}) than legumin-rich and vicilin-rich fractions on saliva-coated PDMS surfaces (21.44 ± 4.13 mg m^{-2} and 7.51 ± 1.02 mg m^{-2} respectively). The ratio of $-\Delta D/\Delta f$ gave information on the viscoelastic properties of the film adsorbed on the sensor, with a higher $-\Delta D/\Delta f$ commonly associated with a more viscous/less elastic film and vice versa (Xu et al., 2019). The film formed with the legumin-rich fraction was significantly more viscoelastic than the films formed by the albumin-rich and the vicilin-rich fractions (Fig. 7b). This special property of the legumin film can be related to the rise of dissipation shift after the rinsing of the legumin film with water. It can be concluded from the QCM-D results that the injection of the legumin-rich fraction led the formation of a thin and viscoelastic film, the injection of the vicilin-rich fraction led to the formation of a thin and rigid film, whilst the injection of the albumin-rich fraction led to the formation of a very thick and rigid film with highest adsorbed mass. QCM-D was a successful method to discriminate the properties of the different protein fractions, and evidenced the specific adsorption behavior of the albumin-rich fraction which forms a thick and rigid film on salivary proteins adsorbed on
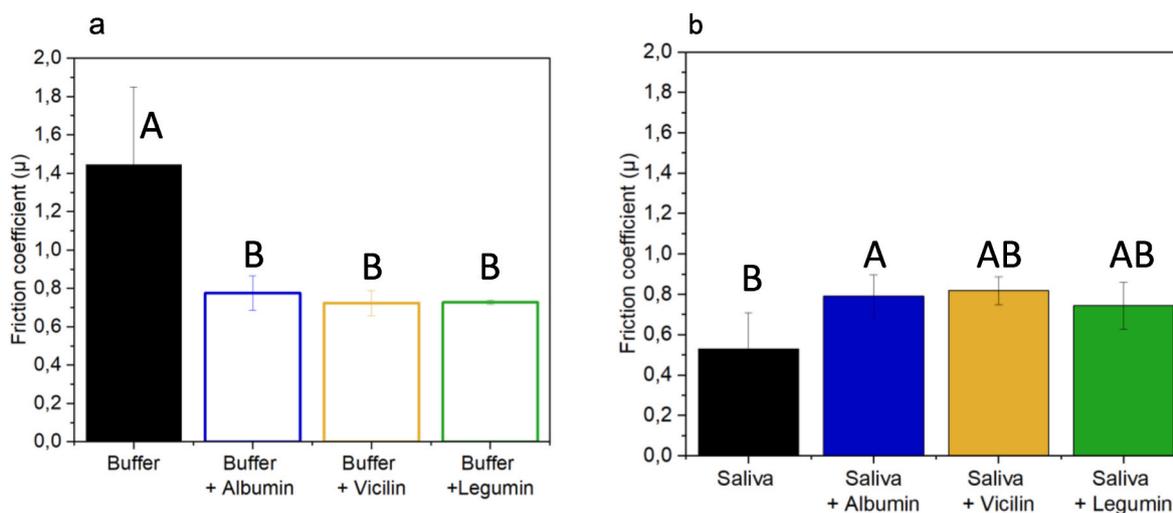

Fig. 5. Plots of mean friction coefficients (μ) obtained in soft tribology using a 3D biomimetic tongue set up for protein solutions (0.5% w/v) a) in buffer (white bars) and b) for protein solutions in human saliva (colored bars) at 0.01 m s^{-1}.
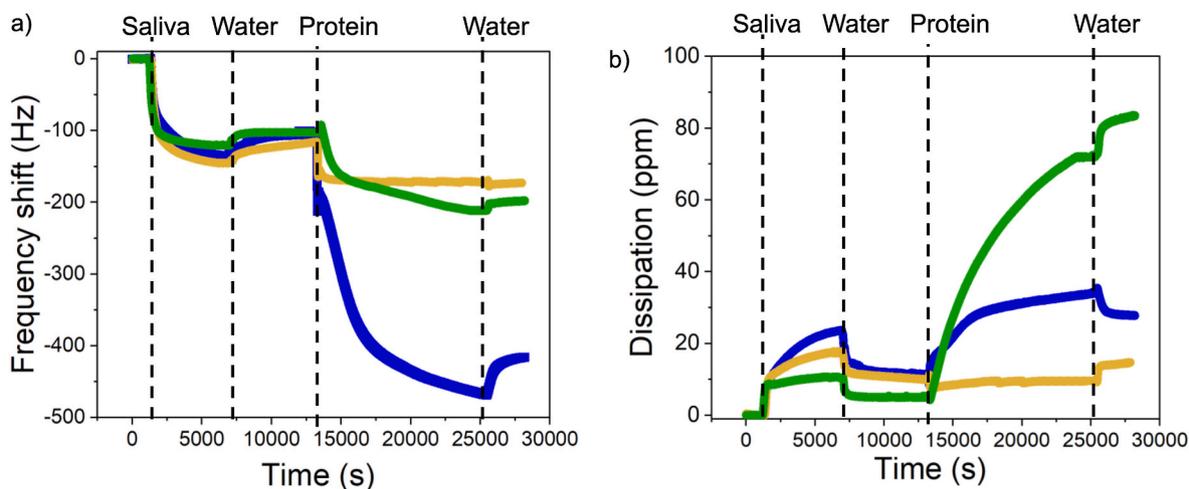
Fig. 6. Plots of a) frequency shift and b) dissipation shift obtained (5th overtone shown) as a function of time for the three pea protein fractions (legumin-rich, vicilin-rich and albumin-rich fractions) at 2.0 mg/mL (w/v) protein on PDMS-coated surface.
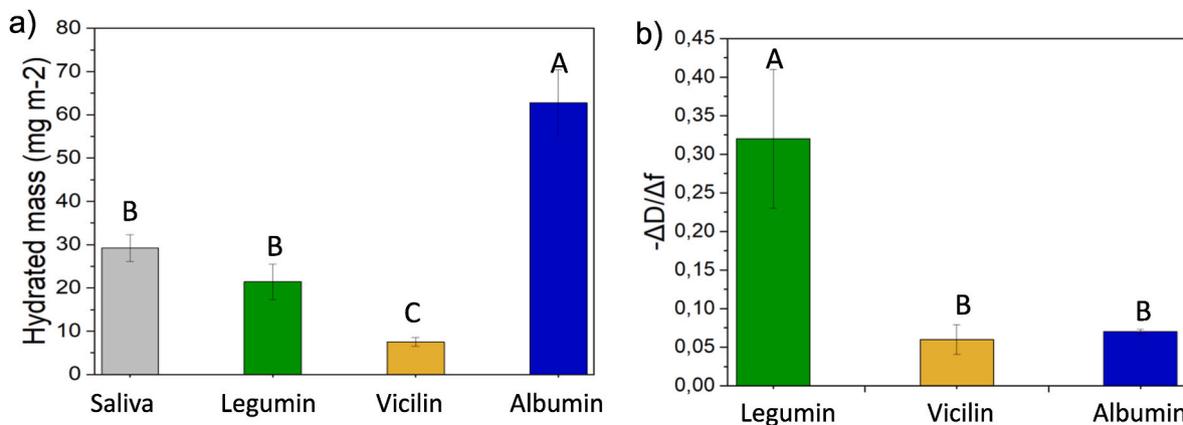

Fig. 7. Plots of a) hydrated mass and b) −ΔD/Δf obtained (5th overtone) for 2.0 mg/mL protein suspensions (legumin-rich, vicilin-rich, albumin-rich fractions).

sensors. Contrary to in-mouth conditions, movement is not considered when measuring protein adsorption in QCM-D, as the flow used to inject the different protein suspensions is very low (100 μl/min). One can hypothesize that the continuous movements *in vivo* would prevent the formation of a thick and rigid film of albumin proteins on the salivary pellicle. However, the strong interaction capacity between the albumin fraction and the saliva film suggests that the latter can be damaged to a greater extent, thus leading to a reduced lubrication and an increase in astringency perception. This specific adsorption behavior of the albumin-rich fraction does not induce a specific lubrication behavior when measured with MTM on PDMS-coated sensors or the 3D bio-mimetic tongue like surface. This might be attributed to the tribology techniques focusing on protein interactions happening with bulk saliva that are continuously depleted and replenished in the contact unlike the static salivary conditioning film adsorbed on a surface in QCM-D that allows probing specific nanoscale interaction.

3.5. Proteomics analysis of proteins involved in the saliva - PPI interactions

For the proteomic analysis, we let pea protein suspensions and human saliva interact together for 15 min to allow for the formation of a precipitate, that in turn was isolated to identify its constitutive proteins. The pellets were then analyzed by the techniques described in section 2.9, with the aim of identifying the main proteins involved in interactions.

Table 1 summarizes the data achieved and shows the number of proteins from pea and saliva identified in the pellets, which gives an overview of the propensity of each pea protein fraction to interact with salivary proteins. The results show that the number of salivary proteins precipitating with the albumin-rich fraction was significantly higher (260 proteins ± 13.04) compared to the salivary proteins precipitating with the vicilin-rich and legumin-rich fractions (185.75 proteins ± 6.17 and 214 proteins ± 18.68, respectively).

To discuss the results of the proteomic analysis, first, we focus on the pea and salivary proteins common to all the pellets. Then, we will discuss pea and salivary proteins specific to the albumin-rich fraction, that was found to be the most astringent one (3.2) and with the highest ability to adsorb on a salivary pellicle (3.4).

Table 1
Number of pea and salivary proteins identified in the pellets obtained after centrifugation of the mixture of saliva and each of the three pea protein fractions. The means and standard deviations were obtained on three replicates. Different letters in the same column indicate significant difference ($p < 0.05$).

	NUMBER of proteins identified in the pellets	
	Salivary proteins	Pea proteins
Legumin	214 ± 18.68[B]	85.67 ± 3.21[A]
Vicilin	185.75 ± 6.17[B]	51.75 ± 2.62[B]
Albumin	260 ± 13.04[A]	52.25 ± 1.5[B]

3.5.1. Salivary proteins common to the pellets obtained with the three pea protein fractions

The Venn diagrams for pea and salivary proteins give a qualitative comparison of the composition of the pellets obtained with the three pea protein fractions (see Fig. 8a). 84 different pea proteins were identified among the three pellets, with 33 of these pea proteins common to all the pellets. These proteins represented 99% of the amount of the pea proteins precipitated in the albumin-rich, vicilin-rich and legumin-rich fractions, and most of them were highly abundant pea storage proteins (vicilin or vicilin subunits and legumin or legumin subunits, present in all the fractions due to cross-contaminations during protein extraction).

Fig. 8b) shows that more than 53% of the salivary proteins identified in the pellets precipitated with all three pea protein fractions (n = 135 salivary proteins out of 254 salivary proteins identified). We focused on the 19 most abundant common salivary proteins (representing 85% of the salivary protein content of the pellets) and used a hierarchical cluster analysis with Euclidean distance as a metric and heat mapping to determine whether their behavior was similar in all fractions or whether differences were present. The results are shown in Fig. 9. Mucin 7 and mucin 5B, abundant negatively charged salivary glycoproteins well-known to be involved in the maintenance of the viscoelastic and lubricating properties of human saliva Yakubov et al. (2015), strongly precipitated with all three pea protein fractions. This suggests that protein aggregates were formed, thus leading to the significant loss of salivary lubrication measured in tribology (section 3.3). Cystatin S, previously connected to interactions with astringent compounds (Ployon et al., 2018; Vingerhoeds et al., 2009), also precipitated in the same way in the three pea protein fractions. Some salivary proteins such as Lysozyme C, zymogen granule protein 16, prolactin inducible protein and immunoglobulin J chain interacted significantly more with the globulin (legumin-rich and vicilin-rich) pea protein fractions than with albumin-rich fraction. 6 out of the 19 most abundant salivary proteins were significantly more abundant in the pellet obtained with the albumin-rich fraction. Among them, α-amylase, BPI fold-containing family A2 (BPI-A2) and carbonic anhydrase are known to precipitate, not only with astringent compounds such as tannins (Gambuti et al., 2006; de Freitas et Mateus 2001), but also with pea proteins (Assad-Bustillos et al., 2023).

Among the other salivary proteins that precipitate with all protein fractions from pea, we were specifically interested in certain protein families that were already reported to be involved in interactions with astringent compounds. It has been previously demonstrated that proline rich proteins (PrPs) have a high affinity for tannins, and for many years the most studied mechanism of astringency was focused on the interaction between astringent compounds and salivary PrPs (Bennick, 2002; Canon et al., 2021; Soares et al., 2018). In our study, the basic PrPs identified in the pellets (basic salivary PrPs 1, 3, 4, and proline rich protein 27) were all significantly more abundant in the pellets obtained with the albumin-rich fraction compared to the pellets obtained with the vicilin-rich and legumin-rich fraction (see Fig. S3, Supplementary material). This was not the case for the acidic PrPs which precipitated in the same way with all three pea protein fractions. The fact that PrPs are intrinsically disordered proteins gives them the ability to efficiently bind to different molecules, including tannins (Canon et al., 2011).

3.5.2. Proteins specific to the pellet obtained with the albumin fraction

The Venn diagrams for pea and salivary proteins Fig. 8 a) and b) highlight the set of pea proteins (n = 13 pea proteins) and salivary proteins (n = 68 salivary proteins) specific to the pellet obtained with the albumin-rich fraction. It can be hypothesized that the 13 pea proteins specific to the albumin-rich fraction (Table 2) triggered interactions between albumin and salivary proteins and were therefore responsible for the high number of precipitated salivary proteins and strong adsorption ability of the albumin-rich fraction. The 13 specific pea proteins of interest were mostly proteins of the plant secondary metabolism with several of them being involved in the plant defense mechanisms (defensin-2, dirigent protein, superoxyde dismutase, …), yet they accounted for only 0.28% of the albumin proteins present in the pellet obtained with the albumin-rich fraction. Given that the albumin-rich fraction behaved differently than the other fraction, it is possible that these 13 pea proteins (or a subset of them) could initiate and facilitate interactions between the proteins present in the albumin-rich fraction and salivary proteins, leading to the precipitation of more salivary proteins. The salivary proteins specific to the albumin pellet were very diverse with a broad range of molecular weights (from 192 to 11 kDa) and were very low abundant salivary proteins, representing less than 1% of the salivary proteins present in the pellet of the albumin-rich fraction (0.88 % ± 0.073) (see Table S3, supplementary material). This fact is also strongly suggestive of a specific and strong interaction between many salivary proteins and the 13 proteins highlighted above. Indeed, we notice that the most abundant salivary proteins known to be involved in astringency perception were not present in this list.
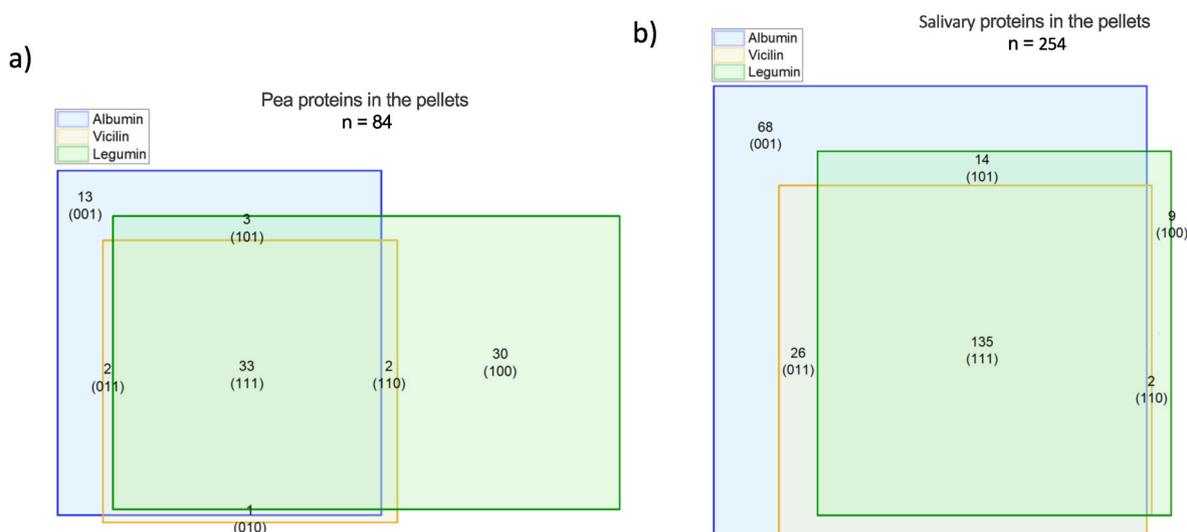
Fig. 8. a) Venn Diagram of the pea proteins b) Venn diagram of the salivary proteins; in both cases these proteins were identified in the proteomic analysis of the pellets obtained after centrifugation of the mixtures of saliva with the different pea protein fractions.
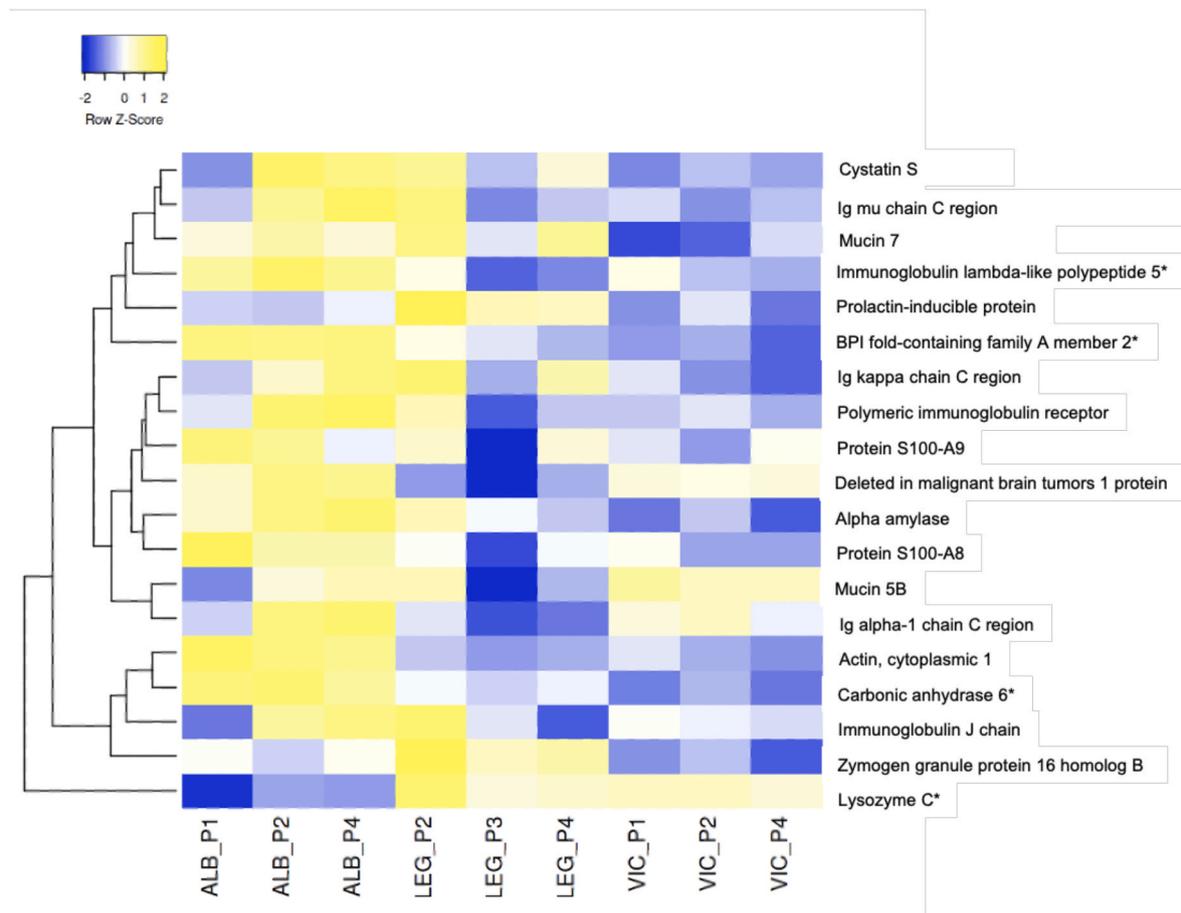
Fig. 9. Hierarchical clustering of the 19 most abundant salivary proteins (accounting for 85% of the common salivary proteins) in precipitates formed upon incubation with the pea protein fractions. Columns of the heat map display three replicates for each pea protein fraction. Each row represents a protein (Uniprot ID on the right). Heat-map color scales from blue to yellow corresponds to z-scores values. Hierarchical clustering of the most abundant salivary proteins is shown on the left. Differences in protein abundance between the three protein fractions are significantly different ($p < 0.05$) for proteins marked with an asterisk (*).

Table 2
13 pea proteins identified only in the pellet obtained after centrifugation of the mixture of saliva and the albumin fraction. The standard deviation is reported for three replicates.
** according to Uniprot or source indicated.

Uniprot accession number	Protein names	Molecular Weight (kDa)	LFQ_Protein/sum LFQ_albumin_ pellet (%)	*stdev*	Functions ** (Uniprot)
P81930	Defensin-2	5.4	0.0575	*0,017*	Antifungal activity sensitive to inorganic cations
Q76KV9	Polygalacturonase inhibiting protein	20.9	0.0514	*0,0049*	Inhibition of the action of polygalacturonase produced by bacterial and fungal pathogens
Q9SYU1	Dirigent protein	20,3	0.0404	*0,006*	Central role in plant secondary metabolism, biosynthesis of lignans
P28641	Dehydrin DHN3	23,9	0.0372	*0,005*	Metal ion binding, response to cold and water deprivation
Q5DWE8	Superoxide dismutase [Cu-Zn]	15,3	0.0255	*0,007*	Destroys radicals which are normally produced within the cells and which are toxic to biological systems
A0A158V755	Non-specific lipid-transfer protein 2	12,1	0,0155	*0,003*	Transfer phospholipids as well as galactolipids across membranes
Q5NJL5	Late embryogenesis abundant protein		0,0157	*0,008*	Protection against dessication
Q92522	Histone H1	22.5	0,0155	*0,007*	Condensation of nucleosome chains into higher-order structures
A1IVX0	Putative glutamate dehydrogenase	21,1	0.0073	*0,001*	Reversible amination of 2-oxoglutarate to form glutamate
O82134	Proliferating cell nuclear antigen	29,5	0.0049	*0,002*	Involved in the control of eukaryotic DNA replication
O82711	Kunitz-type trypsin inhibitor-like 2 protein	23,6	0.0051	*0,0002*	Involved in plant defense responses
Q9SQJ2	Short-chain alcohol dehydrogenase	27,1	0.0036	*0,004*	Enzyme
Q5GQ66	Alpha-dioxygenase PIOX	73,3	0.0027	*0,0005*	Oxidation pathways of fatty acids. Protection of infection

3.6. Discussion and conclusions

Starting from a commercial pea flour we successfully separated pea proteins into three protein fractions corresponding to the main families of storage proteins in pea (legumin-rich, vicilin-rich and albumin-rich fractions). The sensory analysis performed on the three pea protein fractions in solution showed that all three protein solutions were perceived as astringent, but the level of astringency of the solution containing the albumin fraction was significantly higher when compared to the solutions containing the other protein fractions.

We then performed a series of physicochemical analyses of the three fractions. Measurements in soft-tribology showed that all three pea protein fractions induced a comparable loss of salivary lubrication. Proteomics analysis highlighted the existence of a common pool of salivary proteins interacting in a non-specific way with all three pea protein fractions. Salivary glycoproteins (MUC 7 and MUC5B), well-known to be involved in the maintenance of viscoelastic and lubricating salivary properties, were identified in this pool of salivary proteins. Taken all together, the results from the soft-tribological measurements and the proteomics analysis suggests that the basal level of astringency perception in all three pea protein fractions is because there is a common non-specific interaction of salivary proteins with pea proteins that appears to be very similar in all fractions. It is also possible to hypothesize that the basal level of astringency perceived in all pea protein fractions is a lubrication breakdown process due to protein interactions involving abundant storage pea proteins and salivary proteins in bulk saliva.

However, the loss of salivary lubrication measured in soft-tribology did not account for the higher astringency level perceived in the albumin-rich fraction. QCM-D results evidenced that the albumin-rich fraction formed a significantly thicker and more rigid film on a salivary pellicle compared to the vicilin-rich and legumin-rich fractions. The proteomics results showed that the albumin-rich fraction induced the precipitation of a higher number of salivary proteins. By comparing the pea-protein/saliva-protein precipitates for all three pea protein fractions we could identify the subset of proteins unique to the precipitate of the albumin-rich fraction. This subset contained many salivary proteins (n = 63) but only 13 pea proteins, strongly suggesting the existence of specific and strong interactions between the 13 pea proteins (or a subset of these 13 pea proteins) and the salivary proteins (or a subset of these proteins). We find that these 13 proteins are all part of the secondary metabolism of the plant and many of them are involved in defense mechanisms (i.e. antifungal and antibacterial). We postulate that these proteins have the ability to interact with many foreign proteins and thus end up interacting with a number of salivary proteins that otherwise would not be involved in interactions. These extra interactions could be the cause of the perceived increase in astringency for the albumin-rich fraction. It is also possible that the ability of these "defense" proteins to interact with many other foreign proteins is also the cause of the increase of interaction between the albumin-rich fraction and the salivary pellicle layer. Astringency is a complex phenomenon and hence drawing conclusions is always challenging. The extra astringency of the albumin-rich fraction could be solely due to the interactions of this fraction with the salivary pellicle layer and not to the extra aggregation induced in bulk saliva. This interaction with the salivary pellicle could be due to the reason presented above but could also be due to another independent mechanism that we have not identified.

In conclusion, here we present a series of studies aimed at differentiating the astringency of the three main pea protein fractions (legumin-rich, vicilin-rich and albumin-rich fractions). Remarkably albumin-rich fraction although more soluble is significantly more astringent than the other globulin fractions and we present through a series of physicochemical analyses a possible explanation for this observation. We believe that this approach could pave the way for furthermore in-depth studies for pea protein isolates as well as for other legumes protein ingredients in plant-based products.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2023.109489.

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