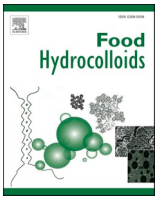
Food Hydrocolloids 135 (2023) 108143

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd



Food matrix impact on rheological and digestive properties of protein-enriched and texture-modified mushroom creams

Marta Gallego, Susana Ribes, Raúl Grau, Pau Talens *

Departamento Tecnología de Alimentos, Universitat Politècnica de València, Camino de Vera s/n, 46022, Valencia, Spain

ARTICLE INFO

Keywords:
Protein enrichment
Thickener
Rheology
In vitro digestion
Proteolysis

ABSTRACT

This study aimed to investigate the food matrix impact of adding two different proteins, whey protein (WP), bovine gelatin protein (BG) or their respective hydrolysates (WPH or BGH), and two hydrocolloids, neutral modified waxy-maize starch (MS) or charged carboxymethyl cellulose (CMC), on flow and viscoelastic properties, oral processing, and *in vitro* gastrointestinal digestion of protein-enriched and texture-modified mushroom creams. All the samples exhibited a weak gel behaviour; however, BG-MS, WP-CMC, WPH-CMC, and BGH-CMC mushroom creams would be considered safer to swallow by dysphagic and elderly people due to their good elasticity degree and resistance to deformation. The addition of saliva during oral processing produced remarkable changes in consistency, adhesiveness, and viscosity of samples containing MS as thickener, which could prevent a safe swallowing. Samples with hydrolysates as protein source and the anionic CMC as thickener could form a compact structure that led to high viscosity and consistency values up to the gastric phase, but they could reduce protein digestibility at the end of the simulated gastrointestinal digestion. This study evidences how protein-hydrocolloid interactions and the food matrix determine the rheological and digestibility properties of samples, which must be considered in the design of foods to meet the specific needs of certain population groups such as dysphagic or elderly people.

1. Introduction

Texture-modified foods (TMF) are used for therapeutic treatment of masticatory or swallowing dysfunctions in individuals with dysphagia or the elderly, which is the fastest-growing segment of the world's population. TMF need to be soft and moist and easily swallowed to achieve a safe and efficient food intake, but they must also cover specific nutritional requirements such as an increased protein intake to avoid the decline in skeletal muscle mass (sarcopenia), common in many older adults (Gallego, Barat, Grau, & Talens, 2022; Lutz, Petzold, & Albala, 2019).

Starch-based hydrocolloids and carboxymethyl cellulose are two widely used thickeners in the food industry. Modified starches are neutral or charged polysaccharides that show higher thermomechanical resistance and stability than native starch. They improve the food system texture by increasing the viscosity of the aqueous phase, but neutral starches do not undergo pH-dependent interactions with proteins (Nguyen, Kravchuk, Bhandari, & Prakash, 2017). Carboxymethyl cellulose is an anionic water-soluble hydrocolloid that can form strong networks or complexes due to interactions between its negatively charged carboxyl group and the cationic domains of the proteins (Yu, Sabato, D'Aprano, & Lacroix, 2004). On the other hand, protein-enriched foods can be obtained by adding intact proteins or their hydrolysates. Despite protein hydrolysates could facilitate subsequent protein digestion and absorption (Koopman et al., 2009; Nguyen, Bhandari, Cichero, & Prakash, 2016), there is little information on differences in physical/mechanical and digestibility characteristics depending on whether proteins are added intact or hydrolysed, which will determine their interactions with the food matrix.

Proteins and polysaccharides can interact through strong interactions (covalent bonds) or weak interactions (electrostatic, van der Waals, hydrophobic or hydrogen bonds) according to their structure, concentration, ratio, ionisation, and charge density, as well as environmental conditions such as the pH, ionic strength, temperature, shearing rate, time, and pressure. Moreover, these complexes can establish competitive interactions with other components of the food matrix (water, sugars, lipids, metal ions, etc.), determining the relationship between the structure and properties of the food system

* Corresponding author.
E-mail address: pautalens@tal.upv.es (P. Talens).

https://doi.org/10.1016/j.foodhyd.2022.108143
Received 10 May 2022; Received in revised form 20 August 2022; Accepted 12 September 2022
Available online 21 September 2022

(Mouécoucou, Villaume, Sanchez, & Méjean, 2004; Ye, 2008), and the food behaviour during oral processing and gastrointestinal digestion (Borreani, Llorca, Larrea, & Hernando, 2016; David, Magram Klaiman, Shpigelman, & Lesmes, 2020).

The aim of this study was to evaluate the food matrix effect of adding two different proteins (whey protein or gelatin protein, both in intact or hydrolysate form) and two hydrocolloids (a neutral modified waxy-maize starch or a charged carboxymethyl cellulose) when formulating protein-enriched and texture-modified mushroom creams. To this end, samples were characterised in terms of flow and viscoelastic properties and evaluated for their behaviour during oral processing and *in vitro* gastrointestinal digestion.

2. Materials and methods

2.1. Materials and reagents

Ingredients used to prepare the mushroom creams were purchased from a local supermarket (Valencia, Spain). Protein enrichment was carried out using two different proteins: whey protein concentrate (WP), which was kindly provided by Brenntag Química S.A.U. (Sevilla, Spain), gelatin from bovine skin (BG) (type B, 225 g bloom gel strength), which was purchased from Sigma Aldrich (St. Louis, MO, USA), and their hydrolysates that were obtained by using Protamex® enzyme (Novozymes A/S (Bagsværd, Denmark). To modify the texture of the mushroom creams two different thickeners were employed: neutral modified waxy-maize starch (MS) in the form of acetylated distarch adipate (E−1422; composed of 3% amylose with an average degree of polymerisation (DP) of 800 and of 97% amylopectin with an average DP of 2000000), which was kindly supplied by Cargill, S.L.U. (Martorell, Spain), and anionic carboxymethyl cellulose (CMC) in the form of sodium salt (E−466; with a molecular weight of 170000 and a degree of substitution of 0.65–0.85), which was acquired from EPSA Aditivos Alimentarios, S.A. (Valencia, Spain).

The gastrointestinal enzymes α-amylase type VI-B, pancreatin from porcine pancreas, and pepsin from porcine gastric mucosa, as well as porcine bile extract and mucin type II from porcine stomach, were purchased from Sigma-Aldrich, Co. (St. Louis, MO, USA).

2.2. Preparation of protein hydrolysates

Hydrolysates from WP and BG proteins named as WPH and BGH, respectively, were obtained following the methodology described by Jancikova, Jamróz, Kulawik, Tkaczewska, and Dordevic (2019) with some modifications. To this end, each protein was dissolved (2.5%, w/v) in Tris buffer (50 mM), the pH was adjusted to 7.5 with NaOH (1 M), and Protamex® enzyme was added in the amount of 5% (w/w) of the protein content. The reaction was performed for 3 h at 50 °C and then stopped by heat shock (98 °C, 5 min) followed by cooling in an ice bath. Samples were centrifuged (8000 *g*, 15 min), and the resultant supernatants were lyophilised (LyoQuest-55, Telstar, Terrassa, Spain).

2.3. Preparation of protein-enriched and texture-modified mushroom creams

Mushrooms (*Agaricus bisporus*) (40% w/w), full-fat bovine milk (40% w/w), tap water (20% w/w), and salt (<0.1%, w/w) were used to prepare the base mushroom cream. All the ingredients were cooked at 90 °C for 10 min in a food processor (Mycook One, Taurus, Spain) and, subsequently, blended until a homogeneous mixture was obtained. Protein-enriched samples were obtained by adding each of the different protein sources (WP, BG, WPH, and BGH) to the mushroom creams previously tempered at 40 °C in a water bath. The addition of proteins or hydrolysates was carried out to obtain 10% of total protein content in each mushroom cream, which was confirmed by the Kjeldahl method (AOAC, 1990). The texture of the protein-enriched samples was modified by the addition of MS or CMC to the mushroom creams, which were previously tempered at 70 °C, up to reach apparent viscosity (η) values of about 2 Pa·s. The viscosity of the samples was measured on a rotational controlled stress Kinexus Pro + Rheometer (Malvern Instruments Ltd., MA, USA), provided with a Peltier cartridge for temperature guidance and rSpace for Kinexus software. A parallel-plate geometry (PLC61/PU40) with a 1-mm gap was employed and a shear rate ramp from 0.1 to 100 s^{-1} at 37 °C was run. The η values at a shear rate of 50 s^{-1} (approx. 2 Pa·s) were used to establish the concentration of each thickener, allowing the comparison among samples. Note that the shear rate of 50 s^{-1} is suggested by the National Dysphagia Diet guidelines as a standard for evaluating apparent viscosity to ensure safe swallowing (Newman, Vilardell, Clavé, & Speyer, 2016; Vieira et al., 2020). Concentrations (%, w/w) of the proteins and thickeners used to prepare the samples are shown in Table 1. Then, all the samples were homogenised until a uniform dispersion was reached and stored for 24 h at 4 °C until analysed. Two sets of every mushroom cream were prepared.

2.4. Characterisation of flow and viscoelastic properties of the mushroom creams

The flow and viscoelastic properties of the samples were characterised by using the rheometer and methodology previously described in section 2.3. Before being analysed, the samples were situated in the measuring system and allowed to stand for 180 s to favour structure recuperation and temperature stability. To prevent the desiccation of the samples, silicone oil was added on the external part of the geometry that, in turn, was also covered with the accessory provided by the supplier. Measurements were made in duplicate.

2.4.1. Flow rheological tests

After eliminating the flow time dependence by applying a previous 300 s shearing time at 100 s^{-1}, the steady-state flow behaviour of the samples was characterised by analysing their flow curves, in which an increasing shear rate from 0.1 to 100 s^{-1} for 180 s was applied. The Herschel-Bulkley model accurately described the flow behaviour of the samples, and yield stress (σ_0), consistency coefficient (K), and flow behaviour index (n) were determined as described by Ribes, Gallego, Barat, Grau, and Talens (2022). The η values of each mushroom cream at 10 s^{-1} and 50 s^{-1} were also calculated. For confirming the proper fit of the Herschel-Bulkley model, the coefficient of determination (R^2) was reported.

2.4.2. Viscoelastic tests

The viscoelastic properties of the samples were evaluated by linear and non-linear viscoelastic tests. To determine the boundary of the linear viscoelastic region (LVR) of each sample, a stress sweep test was run by applying a shear stress comprised between 0.1 and 100 Pa at 1 Hz. The viscoelastic properties of each mushroom cream in the LVR were determined by means of performing a frequency sweep test, in which a frequency range from 0.1 to 10 Hz at a stress value of 1 Pa was applied to the samples. Furthermore, the effect of temperature on the structural changes of the samples was evaluated by running a temperature sweep test from 15 °C to 80 °C at a heating rate of 10 °C min^{-1}. Assays were carried out in the LVR (1 Pa) and at 1 Hz.

The software of the rheometer was utilised to establish the following viscoelastic parameters: complex modulus (G*), complex viscosity (η*), elastic modulus (G′), viscous modulus (G″), and loss tangent (Tan δ).

2.5. Combined squeezing flow and shear force test

This test was carried out following the method described by Chung, Degner, and McClements (2012) to mimic oral processing. To this end, samples were subjected to 10 cycles of compression, constant shear rate (10 s^{-1}), and decompression to simulate the tongue motion when compressing a fluid bolus against the palate. The shear rate of 10 s^{-1} and

Table 1
Concentration (%, w/w) of proteins and thickeners employed to prepare the different mushroom creams and their apparent viscosities (η, Pa·s) measured at 37 °C with a shear rate of 50 s^{-1}.

Sample	Protein	Protein concentration (%, w/w)[a]	Thickener	Thickener concentration (%, w/w)	η (Pa·s)
Base mushroom cream	–	–	–	–	0.16 ± 0.02
WP-MS	WP	15	MS	6.85	2.02 ± 0.11
WP-CMC			CMC	1.15	1.89 ± 0.15
WPH-MS	WPH	17	MS	10.75	1.94 ± 0.17
WPH-CMC			CMC	1.30	1.99 ± 0.11
BG-MS	BG	10	MS	7.20	1.89 ± 0.22
BG-CMC			CMC	0.90	2.15 ± 0.13
BGH-MS	BGH	13	MS	8.50	2.01 ± 0.20
BGH-CMC			CMC	1.30	2.03 ± 0.10

Results are the average of two independent experiments.
WP: whey protein; WPH: whey protein hydrolysate; BG: bovine gelatin; BGH: bovine gelatin hydrolysate. MS: modified waxy-maize starch; CMC: carboxymethyl cellulose.
[a] Each protein concentration indicated in this table was used to obtain 10% of total protein content in each mushroom cream, which was confirmed by the Kjeldahl method (AOAC, 1990).

temperature of 37 °C were established to reproduce physiological conditions (Herranz, Criado, Pozo-Bayón, & Álvarez, 2021). The test was performed in the absence and presence of artificial saliva, which was composed of simulated salivary fluid, α-amylase (75 U/mL), and mucin (3 mg/mL) according to a slightly modified INFOGEST protocol (Minekus et al., 2014). The samples mixed with saliva (1:1, w/v) were incubated for 2 min at 37 °C before conducting the test. On the other hand, the samples were also mixed with water (1:1, w/v) to evaluate the effect of sample dilution resulting from the addition of saliva.

2.6. Simulated gastrointestinal digestion of the mushroom creams

In vitro gastrointestinal digestion (GID) was carried out following the standardised INFOGEST protocol (Minekus et al., 2014). The GID was done in duplicate. To this end, a rotary mixer (Intell-Mixer™ RM-2, ELMI Ltd., Riga, Latvia) at 40 rpm and an incubator chamber (JP Selecta, S.A., Barcelona, Spain) at 37 °C were used. Simulated salivary fluid and α-amylase (75 U/mL) were mixed with each sample (1:1, v/w) for 2 min to mimic the oral phase (pH 7). In the gastric step, after adjusting the pH to 3 with HCl (1 M), simulated gastric fluid and pepsin (2000 U/mL) were mixed with the oral bolus (1:1, v/v) for 2 h. The pH was then adjusted to 7 with NaOH (1 M) in the intestinal phase, in which simulated intestinal fluid containing pancreatin (100 U/mL trypsin activity) and bile salts (10 mM) were mixed with the gastric chyme (1:1, v/v) for 2 h. Enzymatic reactions were stopped by heat shock (98 °C, 5 min) followed by cooling in an ice bath. Individual tubes were used for each sampling time point (after oral, gastric, and intestinal phases) and blank samples containing enzymes and bile (without sample) were subjected to the same conditions. Undigested samples were obtained by mixing the sample with water (1:1, w/v). A part of each sample was centrifuged (8000 g, 4 °C, 10 min) and stored at −20 °C for subsequent analysis, whereas the remaining sample volume was immediately used for flow behaviour analysis. In such a case, additional samples diluted in distilled water, considering the volume of digestive fluids at each phase, were subjected to the same GID conditions (time, temperature, and shaking) and analysed.

2.7. Flow rheological behaviour of digested mushroom creams

The flow rheological properties of the digested and diluted samples were evaluated as previously described in Section 2.4.1 but employing a system of coaxial cylinders (C25/PC25). Experimental data were fitted by the power-law model, and the parameters η, n, K, and R^2 were determined. The η values were measured at 10 s^{-1} as this shear rate was close to that physiologically reported (Hardacre, Yap, Lentle, & Monro, 2015).

2.8. Determination of protein digestibility of the mushroom creams

The protein digestibility of the samples obtained before and during GID was evaluated as described by Gallego, Arnal, Barat, and Talens (2021). To this end, the content of total soluble proteins (determined by the Bradford method), the fraction of peptides soluble in 5% TCA that includes free amino acids and peptides lesser than 10 amino acid residues, as well as the content of free amino groups as proteolysis index (evaluated by the TNBS method) were determined. All the assays were done in triplicate, and results were expressed as mg/g of sample.

2.9. Statistical analysis

Results were statistically processed by the Statgraphics Centurion XVII software (Statgraphics Technologies, Inc., The Plains, VA, USA). The data were subjected to one-way analysis of variance (ANOVA) and the means were compared using the Tukey–Kramer HSD test. The results marked with different letters are significantly different at $p < 0.05$.

3. Results and discussion

3.1. Characterisation of flow and viscoelastic properties of the mushroom creams

3.1.1. Flow behaviour of samples

The flow behaviour of the mushroom creams was described by the Herschel-Bulkley model. The rheological parameters of the samples from the steady flow behaviour test are shown in Table 2. Regarding the apparent viscosity (η) of the mushroom creams, the results are presented at 10 s^{-1} and 50 s^{-1} given that a transient increase in the η of the bolus brings a reduction in the shear rate related to the safe-swallowing procedure, which could be compromised during ageing (Herranz et al., 2021). In this sense, the samples exhibited a marked decrease in their η values as the shear rate increased probably due to the structural breakdown of the particles generated by hydrodynamic forces and the higher alignment of the hydrocolloids' particles (Izidoro, Scheer, Sierakowski, & Haminiuk, 2008). Noteworthy that this outcome was also noticed by Herranz et al. (2021) in commercial instant purees formulated for people with swallowing disorders.

The yield stress (σ_0) represents the minimum shear stress required to initiate the sample's flow and is related to the breakdown of the internal structure of the sample (Augusto, Cristianini, & Ibarz, 2012). Non-significant ($p > 0.05$) differences were observed among the thickened mushroom creams, suggesting similar critical stresses to initiate their flow. The σ_0 observed could be ascribed to the close-packing of the different systems formulated owing to the ability of hydrocolloids to improve the cohesion amongst food particles (Tashiro, Hasegawa,

Table 2
Flow rheological parameters from the steady flow behaviour assay of different mushroom creams at 37 °C.

Sample	η at 10 s^{-1} (Pa·s)	η at 50 s^{-1} (Pa·s)	σ_0 (Pa)	K (Pa·s^n)	n	R^2
WP-MS	6.79 ± 0.99[a]	2.02 ± 0.11[ab]	47.21 ± 15.52[a]	5.35 ± 2.10[a]	0.60 ± 0.06[a]	0.99
WP-CMC	5.62 ± 1.43[a]	1.89 ± 0.15[a]	36.29 ± 15.63[a]	4.99 ± 0.25[a]	0.61 ± 0.05[a]	0.97
WPH-MS	5.85 ± 1.04[a]	1.94 ± 0.17[ab]	35.32 ± 11.21[a]	5.69 ± 0.09[a]	0.61 ± 0.01[a]	0.99
WPH-CMC	6.16 ± 0.95[a]	1.99 ± 0.11[ab]	38.27 ± 11.58[a]	5.88 ± 0.42[a]	0.60 ± 0.01[a]	0.98
BG-MS	5.75 ± 0.83[a]	1.89 ± 0.22[ab]	36.99 ± 9.03[a]	5.23 ± 0.01[a]	0.60 ± 0.02[a]	0.99
BG-CMC	5.71 ± 0.78[a]	2.15 ± 0.13[b]	29.94 ± 7.30[a]	6.04 ± 0.35[a]	0.65 ± 0.02[a]	0.97
BGH-MS	6.33 ± 1.46[a]	2.01 ± 0.20[ab]	39.83 ± 15.73[a]	6.01 ± 0.13[a]	0.61 ± 0.01[a]	0.99
BGH-CMC	6.23 ± 0.62[a]	2.03 ± 0.10[ab]	37.28 ± 8.13[a]	6.27 ± 0.60[a]	0.60 ± 0.01[a]	0.98

Rheological parameters represent: η, apparent viscosity; σ_0, yield stress; K, consistency coefficient; n, flow behaviour index; R^2, coefficient of determination.
Results are the average of two independent experiments. Lowercase letters indicate significant differences among samples ($p < 0.05$).
WP: whey protein; WPH: whey protein hydrolysate; BG: bovine gelatin; BGH: bovine gelatin hydrolysate. MS: modified waxy-maize starch; CMC: carboxymethyl cellulose.

Kohyama, Kumagai, & Kumagai, 2010). In this sense, it is well-known that starch molecules act as filler reinforcement agents that filled into the protein network structure (Yu, Ren, Zhao, Cui, & Liu, 2020), meanwhile, the CMC can create robust networks due to interactions between its negatively charged carboxyl group and the cationic domains of the proteins (Yu et al., 2004). Moreover, it is important to mention that the molecular structural parameters and chemical structure of the cellulose derivative determine its rheological and viscoelastic properties and thus, the structure–property relationships of the thickener (Clasen & Kulicke, 2001).

Concerning the consistency coefficient (K), non-significant differences ($p > 0.05$) were observed among the different mushroom creams. The K values of the samples were between 4.99 ± 0.25 and 6.27 ± 0.60 Pa·s^n, being similar to those reported by Herranz et al. (2021) in commercial instant purees. Furthermore, the flow behaviour index (n) values of all the tested mushroom creams were lower than 1, indicating their shear-thinning flow behaviour once the σ_0 was overtaken. These results were in accordance with those observed by Vieira et al. (2021) in different thickeners' solutions designed for people with swallowing problems. It is worth mentioning that foods with n values lower than 1 are favourable for a swallowing process with a reduced risk of aspiration, giving the neuromuscular structure a greater reaction time for closing the epiglottis (Nakauma, Ishihara, Funami, & Nishinari, 2011) and being, therefore, appropriate for the elderly and for people with swallowing disorders.

3.1.2. *Viscoelastic properties of the samples*

Fig. 1 shows the changes in G′, G″, and Tan δ values of the different mushroom creams at 37 °C, as a function of the frequency stress applied. As can be observed, all the samples exhibited higher G′ than G″ values over the frequency range evaluated, which indicates their weak gel character (Sharma, Kristo, Corredig, & Duizer, 2017). Additionally, Tan δ provides residual information on the product's viscoelastic modulus by considering the contribution of G′ and G'' (Tan δ = G''/G′) (Talens, Castells, Verdú, Barat, & Grau, 2021). Tan δ values over 1 denote diluted solutions, whereas values between 0.1 and 1 connote weak gels (Irani, Razavi, Abdel-Aal, Hucl, & Patterson, 2019). All the samples presented Tan δ values between 0.14 and 0.70 over the entire frequency range studied (Fig. 1), corroborating their weak gel character (Sharma et al., 2017). Similar results were reported by Ribes, Estarriaga, Grau, and Talens (2021) in texture-modified sauces designed for people with swallowing disorders.

To better understand the dependency of G′ and G″ values on frequency and to study the gel characteristics, ln G′ and ln G″ slopes (n' and n'', respectively) *vs.* ln frequency plot of the different mushroom creams

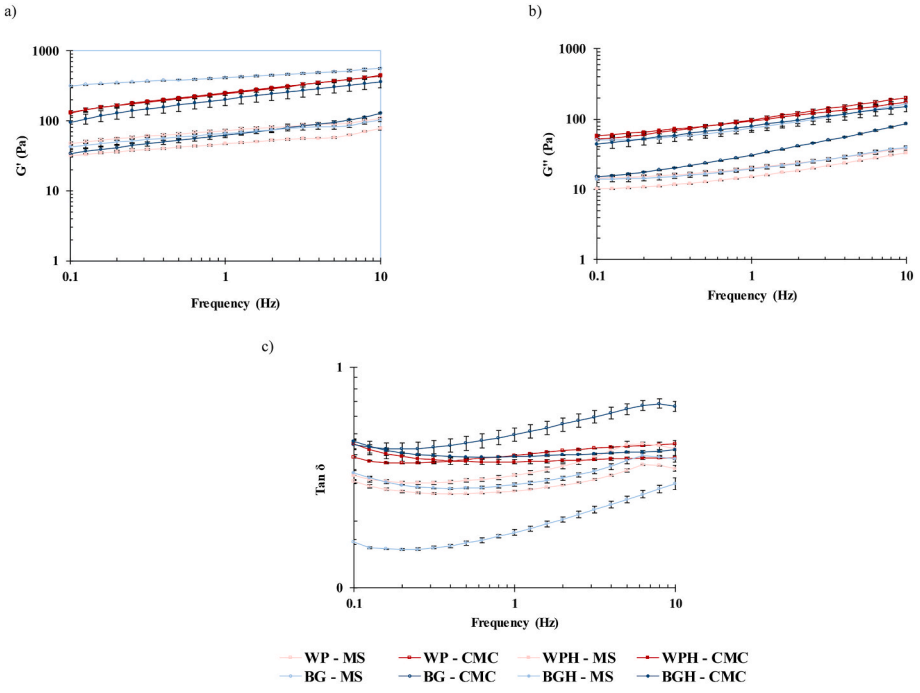


Fig. 1. Changes in a) elastic modulus (G′), b) viscous modulus (G″), and c) loss tangent (Tan δ) values of the different mushroom creams at 37 °C as a function of the applied frequency stress. Curves are representative runs. (WP: whey protein; WPH: whey protein hydrolysate; BG: bovine gelatin; BGH: bovine gelatin hydrolysate. MS: modified waxy-maize starch; CMC: carboxymethyl cellulose).

were evaluated (Alvarez, Fuentes, Guerrero, & Canet, 2017). Furthermore, G'_0-G''_0 values, at a frequency of 1 Hz, were employed as a measure of the gel strength. The gel properties parameters of the different samples obtained from the frequency sweep test performed at 37 °C are summarised in Table 3. As can be observed, all samples presented n' and n'' values between 0 and 1, which could be attributed to the behaviour of weak gels (Irani et al., 2019). Indeed, samples exhibited generally higher n'' than n' values, suggesting that G″ was more frequency-dependent than G'. This behaviour was also described by Alvarez and Canet (2013) in vegetable purees with sea bass, vegetables with fish, and vegetables with chicken. Regarding G'_0-G''_0 values, it is worth mentioning that the BG-MS sample showed the highest value, which could be explained because the gelatin would increase the number of molecular associations and thus the reorganisation of the biopolymer's chains, increasing the gel strength (Simionescu Ibanescu, Danu, Rotaru, & Ibanescu, 2013).

Table 3 also presents the linear viscoelastic parameters of the different mushroom creams obtained from the frequency sweep test performed at 37 °C. For a better comparison of these results, the viscoelastic parameters were reported at a frequency of 1 Hz. All the mushroom creams exhibited a predominant elastic behaviour (G' > G″) that is frequently observed in weak viscoelastic systems (Sharma et al., 2017), as previously mentioned. This fact was also reported by Herranz et al. (2021) and Ribes et al. (2021) in thickened purees and sauces, respectively.

Moreover, the complex modulus (G*) is connected to the rigidity and stiffness of the samples, whereas the complex viscosity (η*) evaluates the whole resistance of the products to flow based on the angular frequency (Talens et al., 2021). The BG-MS sample showed the greatest G* and η* values, probably due to the molecular associations induced by gelatin and the rearrangements of the biopolymer's chains (Simionescu, Ibanescu, Danu, Rotaru, & Ibanescu, 2013), as above-mentioned. On the contrary, the lowest G* and η* values were observed in the case of the WPH-MS sample. Interactions between the swollen starch granules could be weakened by the presence of WPH aggregates in the continuous phase that hampers the packing of the system since swollen starch granules do not interact with each other (Vu Dang, Loisel, Desrumaux, & Doublier, 2009), reducing the rigidity and stiffness of the samples. The WPH aggregates could be generated by the interaction of larger amounts of peptides produced during hydrolysis (Tarhan, Spotti, Schaffer, Corvalan, & Campanella, 2016). It is also important to mention that amongst the samples enriched with WP and WPH, the use of CMC as thickener conferred greater G* and η* values to the samples. Moreover, the same trend was observed in the case of the BGH-CMC mushroom cream. It could be explained by the formation of strong networks between the anionic domains of the hydrocolloid (negatively charged carboxyl group) and the cationic domains of the proteins (amino acids), thereby strengthening the product structure (Yu et al., 2004). In addition, all the mushroom creams showed Tan δ values < 1, suggesting a clear dominance of the elastic properties. It is important to highlight that the Tan δ can be utilised as a rheological parameter of easy-swallowing boluses. In this sense, Nyström, Qazi, Bülow, Ekberg, and Stading (2015) pointed out that dysphagic people noticed easier to swallow thinning fluids with high elasticity. Similarly, Ishihara, Nakauma, Funami, Odake, and Nishinari (2011) suggested that Tan δ values between 0.1 and 1 can be used as rheological criteria to identify easy-swallowable boluses. Thus, taking into consideration that food products with high resistance to deformation (high G* values) and good elasticity degree (low Tan δ values; Tan δ = G''/G′) would be safer to swallow (Herranz et al., 2021), it could be stated that BG-MS, WP-CMC, WPH-CMC, and BGH-CMC mushroom creams would be more suitable for people with swallowing disorders.

On the other hand, to determine the influence of temperature on the structural changes of the samples, a temperature sweep assay was performed from 15 °C to 80 °C as shown in Fig. 2. Generally, G′ and G″ values decreased as the temperature increased. Nevertheless, it is important to highlight the behaviour observed in the case of the samples enriched with BG. As can be seen, when the temperature increased, G′ and G″ values decreased since more thermal energy was available to overcome the energy of the inter- and intra-molecular bonds between the strands inside of the helices (Netter, Goudoulas, & Germann, 2020), but by increasing the temperature above 37 °C, the G′ and G″ values did not show a higher decrease due to the product became liquid. Conversely, when lowering the temperature (<37 °C), the BG samples started to show greater elastic behaviour (G' > G″) because of the formation of the triple helices (Netter et al., 2020). It is worth mentioning that G′ values increased rapidly during cooling compared to G″ values, suggesting that ionic interactions, hydrogen bonding, van der Waals forces, hydrophobic association, and self-assembly of the gelatin chains to triple helices were responsible for the gelling process during cooling (Huang et al., 2017). Lastly, Tan δ values of BG samples also indicated the phase transition by means of the formation of junction zones of the protein chains in the three-dimensional network followed by the creation of a strong gel system during the cooling procedure, and the opposite during the heating process (Sinthusamran, Benjakul, & Kishimura, 2014). Contrarily, the Tan δ values of WP samples and all the hydrolysates remained quite stable during the whole range of temperatures evaluated. It is important to highlight that despite the changes noticed in the Tan δ values of the samples enriched with BG, they were comprised between 0.1 and 1 at 37 °C. Thus, regarding the good elasticity degree of samples (low Tan δ values; Tan δ = G''/G′) they could be considered safe-swallowing for dysphagic and elder people at the temperature evaluated, which corresponds to that of the oral cavity (Herranz et al., 2021; Ishihara et al., 2011; Ribes et al., 2021).

Table 3
Gel properties parameters and viscoelastic parameters of the different mushroom creams obtained from the frequency sweep test performed at 37 °C.

Sample	Gel properties parameters			Viscoelastic parameters				
	n'	n''	G_0'-G_0'' (Pa)	G' (Pa)	G'' (Pa)	G* (Pa)	η* (Pa·s)	Tan δ
WP-MS	0.16 ± 0.01^{b}	0.22 ± 0.01^{d}	52.53 ± 3.08^{c}	72.44 ± 4.21^{a}	19.91 ± 1.13^{a}	75.13 ± 4.36^{a}	11.96 ± 0.69^{a}	0.27 ± 0.00^{b}
WP-CMC	0.26 ± 0.01^{a}	0.30 ± 0.01^{b}	146.75 ± 8.99^{b}	244.30 ± 13.72^{b}	97.56 ± 4.73^{c}	263.10 ± 14.57^{b}	41.88 ± 2.31^{b}	0.40 ± 0.00^{d}
WPH-MS	0.17 ± 0.01^{b}	0.25 ± 0.01^{c}	31.64 ± 0.96^{c}	46.84 ± 1.03^{a}	15.20 ± 0.07^{a}	49.24 ± 0.99^{a}	7.84 ± 0.16^{a}	0.32 ± 0.01^{bc}
WPH-CMC	0.26 ± 0.02^{a}	0.24 ± 0.01^{cd}	158.88 ± 2.84^{b}	253.40 ± 0.99^{b}	94.53 ± 1.85^{bc}	270.45 ± 0.21^{b}	43.05 ± 0.04^{b}	0.37 ± 0.01^{cd}
BG-MS	0.12 ± 0.01^{c}	0.27 ± 0.02^{bc}	341.55 ± 7.65^{a}	415.45 ± 12.09^{c}	73.90 ± 4.44^{b}	422.00 ± 12.73^{c}	67.17 ± 2.02^{c}	0.18 ± 0.01^{b}
BG-CMC	0.27 ± 0.01^{a}	0.35 ± 0.01^{a}	31.39 ± 4.36^{c}	62.17 ± 4.22^{a}	30.78 ± 0.14^{a}	69.38 ± 3.72^{a}	11.04 ± 0.59^{a}	0.50 ± 0.04^{e}
BGH-MS	0.18 ± 0.01^{b}	0.24 ± 0.01^{cd}	46.83 ± 3.82^{c}	66.22 ± 4.94^{a}	19.39 ± 1.12^{a}	69.00 ± 5.05^{a}	10.98 ± 0.81^{a}	0.29 ± 0.00^{a}
BGH-CMC	0.28 ± 0.01^{a}	0.27 ± 0.01^{bc}	123.37 ± 23.44^{b}	203.45 ± 38.68^{b}	80.09 ± 15.24^{bc}	218.60 ± 41.58^{b}	34.80 ± 6.62^{b}	0.39 ± 0.00^{d}

Gel properties parameters represent: n', ln G′ slope *vs.* ln frequency; n'', ln G″ slope *vs.* ln frequency; G_0'-G_0'', gel strength.
Viscoelastic parameters represent: G′, elastic modulus at 1 Hz; G″, viscous modulus at 1 Hz; G*, complex modulus at 1 Hz; η*, complex viscosity at 1 Hz; Tan δ, loss tangent at 1 Hz.
Results are the average of two independent experiments. Lowercase letters indicate significant differences among samples ($p < 0.05$).
WP: whey protein; WPH: whey protein hydrolysate; BG: bovine gelatin; BGH: bovine gelatin hydrolysate. MS: modified waxy-maize starch; CMC: carboxymethyl cellulose.

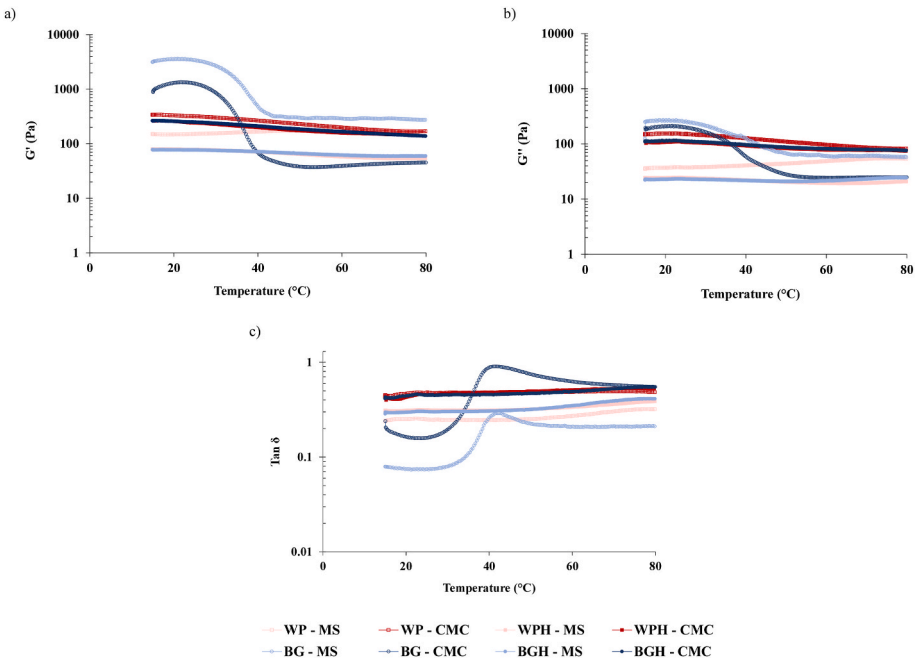


Fig. 2. Temperature sweeps of the mushroom creams from 15 °C to 80 °C. Curves are representative runs. (WP: whey protein; WPH: whey protein hydrolysate; BG: bovine gelatin; BGH: bovine gelatin hydrolysate. MS: modified waxy-maize starch; CMC: carboxymethyl cellulose).

3.2. Combined squeezing flow and shear force test

The movement of the tongue and palate during oral processing was simulated by subjecting mushroom creams to 10 compression/decompression cycles in the presence and absence of artificial saliva and in diluted samples. The values of maximum positive and negative forces, which indicate consistency and adhesiveness of the samples, respectively, as well as the apparent viscosity, which is related to the sliding tongue – palate (Chung et al., 2012), are shown in Fig. 3. Generally, no marked decrease was observed in samples without saliva for any of the parameters evaluated as the number of cycles increased (Fig. 3-a,b), since the grinding process used to obtain the mushroom creams would break the food structure more than the oral processing. However, these values were significantly ($p < 0.05$) reduced when compared to samples treated with saliva and to the diluted samples (Fig. 3-c-f), and the lowest consistency, adhesiveness, and η values were generally observed in the presence of saliva. These results evidence the effect of oral conditions on the loss of the food structure together with the dilution effect resulting from the addition of saliva. In the presence of saliva, samples with CMC showed the highest values of consistency and adhesiveness (Fig. 3-c), whereas WP-CMC samples presented a remarkable greater η, followed by samples with protein hydrolysates and CMC (Fig. 3-d). If compared with diluted samples (Fig. 3-e,f), it is noteworthy the reduction of the consistency and adhesiveness of WP-MS and BG-MS samples, as well as of the η values of samples with MS, when saliva is added. These results suggest that, in the presence of saliva, the action of the α-amylase

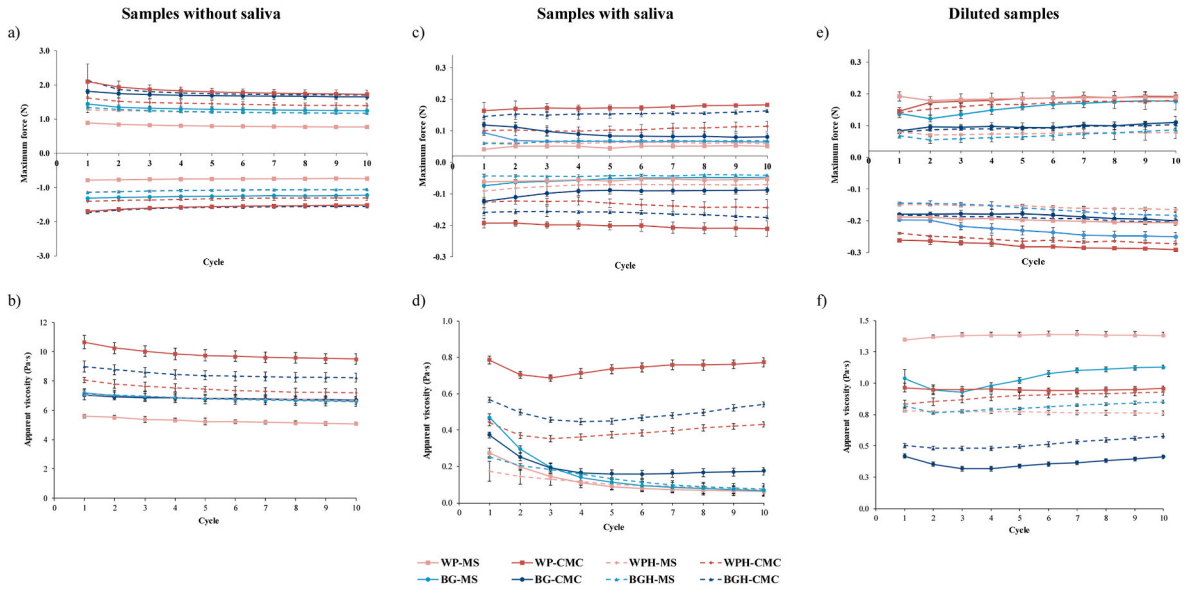


Fig. 3. Combined squeezing flow and shear force test of the mushroom creams showing maximum force *vs.* cycle in the a) absence of saliva, c) presence of saliva, and e) diluted samples, as well as the apparent viscosity (η) at 10 s^{-1} *vs.* cycle in the b) absence of saliva, d) presence of saliva, and f) diluted samples. (WP: whey protein; WPH: whey protein hydrolysate; BG: bovine gelatin; BGH: bovine gelatin hydrolysate. MS: modified waxy-maize starch; CMC: carboxymethyl cellulose).

enzyme by breaking the chains of amylose and amylopectin that are components of the starch would reduce the consistency, adhesiveness, and η of samples prepared with MS as thickener. Thus, in general, samples with CMC maintained the highest consistency, adhesiveness, and η in the presence of saliva, being more miscible with saliva and resulting in a homogenous bolus that could be easier to swallow (Talens et al., 2021).

3.3. Flow rheological characterisation of digested mushroom creams

The action of gastrointestinal enzymes and the changes in ionic strength, pH, and volume at each digestion phase affect samples' conformation and their rheological behaviour during GID (Chen et al., 2020). Moreover, the different composition and physicochemical properties of samples, resulting from the complex food matrix, as well as the nature, size, and structure of the added protein (WP *vs.* BG samples, intact *vs.* hydrolysate) and charge of the thickener (neutral MS *vs.* anionic CMC), can greatly influence their flow characteristics. The calculated parameters η (at 10 s^{-1}), n, K, and R^2 from the rheological characterisation of the digested samples and the diluted samples during *in vitro* GID are shown in Table 4. All the samples presented a non-Newtonian shear-thinning flow behaviour ($n < 1$), without requiring minimum shear stress to initiate the flow of samples. In all cases, the η decreased throughout the GID, but the values in the diluted samples were generally higher than those of the digested samples, especially in the oral phase. This fact would be largely attributed to the effect of the gastrointestinal conditions on the loss of the structure and destabilisation of the food matrix, rather than the effect of sample dilution resulting from the addition of digestive fluids (Malinauskytė et al., 2018). Indeed, the pH and ionic strength values control the degree of ionisation of the charged residues and the range of the electrostatic forces between the protein and the hydrocolloid, thus dominating the behaviour of the protein–hydrocolloid complex (Syrbe, Bauer, & Klostermeyer, 1998). Regarding the digested samples, the highest η and K values after the oral phase were found for BG-MS (0.42 Pa·s and 2.07 $Pa{\cdot}s^n$, respectively), followed by the samples with protein hydrolysates and CMC, indicating that these samples would present the structure less affected by the oral α-amylase enzyme. However, these parameters were intensely reduced in the BG-MS sample after the gastric phase due to an intense action of the pepsin enzyme in the hydrolysis of the gelatin protein, thus reducing its viscosity and consistency (Chen et al., 2020). BGH-CMC and WPH-CMC samples showed the highest η and K values after the gastric phase, suggesting that protein hydrolysates and CMC could form a relatively compact network structure. In this regard, interactions between negatively-charged polysaccharides and positively-charged or hydrophilic amino acids of proteins can give place to large aggregates or complexes, limiting pepsin accessibility and making the sample less susceptible to rupture (Borreani et al., 2016; David et al., 2020). When comparing digested and diluted samples, WP-MS showed the greatest difference in the parameters η and K after the oral and gastric phases; thus, it would be the most hydrolysed sample due to the action of the α-amylase and pepsin enzymes. After the intestinal phase, the digested samples did not present significant differences ($p > 0.05$) in the η and K values. Therefore, although diluted samples showed slight differences between them, the dilution effect of the samples would be the main factor in reducing viscosity and consistency at the end of the GID, as previously observed in texture-modified chicken and vegetables purees (Ribes et al., 2022).

3.4. Protein digestibility of the mushroom creams

The addition of proteins and thickeners to the food matrix can impact on its flow characteristics, limiting or modifying the action of gastrointestinal enzymes and, in turn, nutritional properties (Mao & Miao, 2015; Turgeon & Rioux, 2011). In this context, the digestibility of proteins of the different mushroom creams was evaluated during *in vitro*

Table 4
Flow rheological behaviour of the digested samples and diluted samples during *in vitro* gastrointestinal digestion.

Digestion phase	Parameter	DIGESTED SAMPLES							
		WP-MS	WP-CMC	WPH-MS	WPH-CMC	BG-MS	BG-CMC	BGH-MS	BGH-CMC
Oral	**η (Pa·s)**	0.03 ± 0.01^{d}	0.09 ± 0.01^{c}	0.03 ± 0.01^{d}	0.17 ± 0.01^{b}	0.42 ± 0.01^{a}	0.08 ± 0.01^{c}	0.04 ± 0.01^{d}	0.20 ± 0.01^{b}
	n	0.41 ± 0.03^{bcd}	0.48 ± 0.03^{abc}	0.39 ± 0.04^{cd}	0.62 ± 0.05^{a}	0.30 ± 0.02^{d}	0.54 ± 0.03^{ab}	0.37 ± 0.03^{a}	0.58 ± 0.04^{a}
	K ($Pa{\cdot}s^n$)	0.13 ± 0.02^{d}	0.31 ± 0.04^{bcd}	0.11 ± 0.02^{d}	0.42 ± 0.08^{bc}	2.07 ± 0.12^{a}	0.23 ± 0.04^{cd}	0.18 ± 0.03^{b}	0.53 ± 0.09^{b}
	R^2	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
Gastric	**η (Pa·s)**	0.01 ± 0.01^{cd}	0.03 ± 0.01^{bc}	0.01 ± 0.01^{d}	0.05 ± 0.01^{ab}	0.02 ± 0.01^{cd}	0.02 ± 0.01^{cd}	0.01 ± 0.01^{cd}	0.07 ± 0.01^{a}
	n	0.60 ± 0.22^{a}	0.56 ± 0.08^{a}	0.69 ± 0.29^{a}	0.53 ± 0.06^{a}	0.47 ± 0.08^{a}	0.63 ± 0.11^{a}	0.61 ± 0.27^{a}	0.55 ± 0.05^{a}
	K ($Pa{\cdot}s^n$)	0.02 ± 0.02^{c}	0.09 ± 0.04^{abc}	0.01 ± 0.01^{c}	0.16 ± 0.06^{ab}	0.05 ± 0.02^{bc}	0.06 ± 0.03^{bc}	0.03 ± 0.02^{c}	0.19 ± 0.04^{a}
	R^2	0.95	0.98	0.96	0.98	0.99	0.99	0.97	0.99
Intestinal	**η (Pa·s)**	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
	n	0.77 ± 0.35^{a}	0.85 ± 0.34^{a}	0.80 ± 0.37^{a}	0.86 ± 0.29^{a}	0.80 ± 0.34^{a}	0.83 ± 0.34^{a}	0.76 ± 0.33^{a}	0.89 ± 0.37^{a}
	K ($Pa{\cdot}s^n$)	0.01 ± 0.01^{a}	0.02 ± 0.01^{a}	0.01 ± 0.01^{a}	0.02 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.02 ± 0.02^{a}
	R^2	0.89	0.97	0.83	0.98	0.79	0.98	0.70	0.92
Digestion phase	**Parameter**	**DILUTED SAMPLES**							
		WP-MS	**WP-CMC**	**WPH-MS**	**WPH-CMC**	**BG-MS**	**BG-CMC**	**BGH-MS**	**BGH-CMC**
Oral	**η (Pa·s)**	1.18 ± 0.01^{a}	0.49 ± 0.03^{c}	0.05 ± 0.01^{e}	0.40 ± 0.02^{d}	1.10 ± 0.02^{a}	0.57 ± 0.03^{bc}	0.61 ± 0.01^{b}	0.51 ± 0.03^{c}
	n	0.33 ± 0.02^{b}	0.51 ± 0.04^{a}	0.53 ± 0.06^{a}	0.55 ± 0.04^{a}	0.30 ± 0.01^{b}	0.55 ± 0.04^{a}	0.42 ± 0.02^{ab}	0.51 ± 0.03^{a}
	K ($Pa{\cdot}s^n$)	5.49 ± 0.18^{a}	1.52 ± 0.24^{c}	0.16 ± 0.04^{d}	1.11 ± 0.16^{c}	5.52 ± 0.07^{a}	1.59 ± 0.22^{c}	2.31 ± 0.14^{b}	1.59 ± 0.21^{c}
	R^2	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
Gastric	**η (Pa·s)**	0.17 ± 0.01^{a}	0.04 ± 0.01^{d}	0.01 ± 0.01^{e}	0.05 ± 0.01^{d}	0.13 ± 0.01^{b}	0.05 ± 0.01^{d}	0.08 ± 0.01^{c}	0.06 ± 0.01^{d}
	n	0.34 ± 0.01^{a}	0.67 ± 0.08^{a}	0.69 ± 0.21^{a}	0.64 ± 0.07^{a}	$0.35 \pm 0.02a$	0.69 ± 0.08^{a}	0.45 ± 0.05^{a}	0.63 ± 0.07^{a}
	K ($Pa{\cdot}s^n$)	0.75 ± 0.01^{a}	0.09 ± 0.03^{d}	0.03 ± 0.02^{d}	0.12 ± 0.04^{d}	0.59 ± 0.03^{b}	0.10 ± 0.03^{d}	0.30 ± 0.05^{c}	0.14 ± 0.04^{d}
	R^2	0.99	0.99	0.99	0.99	0.99	0.99	0.97	0.99
Intestinal	**η (Pa·s)**	0.02 ± 0.01^{a}	0.01 ± 0.01^{ab}	0.01 ± 0.01^{b}	0.01 ± 0.01^{ab}	0.02 ± 0.01^{ab}	0.01 ± 0.01^{ab}	0.01 ± 0.01^{ab}	0.01 ± 0.01^{ab}
	n	0.43 ± 0.05^{a}	0.91 ± 0.29^{a}	0.79 ± 0.37^{a}	0.80 ± 0.19^{a}	0.48 ± 0.09^{a}	0.89 ± 0.30^{a}	0.57 ± 0.19^{a}	0.82 ± 0.22^{a}
	K ($Pa{\cdot}s^n$)	0.09 ± 0.02^{a}	0.02 ± 0.02^{ab}	0.01 ± 0.01^{b}	0.02 ± 0.02^{ab}	0.06 ± 0.02^{ab}	0.01 ± 0.01^{ab}	0.04 ± 0.03^{ab}	0.02 ± 0.02^{ab}
	R^2	0.99	0.97	0.90	0.99	0.99	0.98	0.97	0.99

Rheological parameters represent: η, apparent viscosity at 10 s^{-1}; n, flow behaviour index; K, consistency coefficient; R^2, coefficient of determination.
Results are the average of two independent experiments. Lowercase letters indicate significant differences ($p < 0.05$) among samples within the same digestion phase.
WP: whey protein; WPH: whey protein hydrolysate; BG: bovine gelatin; BGH: bovine gelatin hydrolysate. MS: modified waxy-maize starch; CMC: carboxymethyl cellulose.

GID (Fig. 4). Before digestion, the samples with WP presented the highest contents of soluble proteins, whereas the samples with BGH showed the lowest values (<3 mg/g). In such cases, the samples with MS presented around 1.5 times more soluble proteins than those with CMC (Fig. 4a). The reduction in the content of soluble proteins after the oral phase, particularly notable for the sample WP-MS, could be attributed to the precipitation or aggregation of proteins due to interactions with α-amylase enzyme since protein hydrolysis was not expected at this stage (Crosara et al., 2018). At the end of the GID, non-significant differences ($p > 0.05$) were observed between the samples containing the same type of added protein but different thickener except for BGH samples, in which case BGH-MS presented twice as many soluble proteins as BGH-CMC (Fig. 4a). In addition, the content of soluble peptides (<10 amino acid residues) and free amino groups increased in all the samples as de GID advanced (Fig. 4b and c). After the gastric phase, the content of soluble peptides was higher in the samples with whey protein than those with gelatin, whatever the nature of the added protein and hydrocolloid. However, WP and BG samples showed 1.5–2.5 times higher values than WPH and BGH at the end of the GID (Fig. 4b), evidencing the key action of intestinal enzymes (mainly trypsin and chymotrypsin) in the hydrolysis of added intact proteins and the generation of small peptides and free amino acids. After the intestinal stage, the added hydrocolloid within the same type of sample only showed significant differences ($p < 0.05$) for BGH samples, being BGH-CMC that with the lowest content of soluble peptides (24 mg/g). Regarding the content of free amino groups (Fig. 4c), the samples containing protein hydrolysates presented the highest values up to the gastric phase, but significant differences ($p < 0.05$) were generally found after the intestinal phase depending on the type of added hydrocolloid. Values were around 1.5 times higher in the samples containing MS than those with CMC, except for WP samples that did not present differences ($p > 0.05$). Thus, the use of hydrolysates as protein source in combination with the anionic CMC as thickener could reduce protein digestibility. Sample BGH-CMC presented the lowest value of free amino groups (30 mg/g), agreeing with previous determinations of soluble proteins and peptides. These results indicate that, although all the samples had the same initial protein content (10%), the protein digestibility would be affected by the composition, structural organisation, and physicochemical properties of the samples, which resulted from the added proteins and their interactions with the hydrocolloids and the food matrix (Ogawa et al., 2018). Protein-hydrocolloid interactions are dependent on the structure and composition of the compounds, pH value, ionic strength, ionisation degree, charge density, among others (Mouécoucou et al., 2004), and can influence food digestion by increasing or decreasing the action of digestive enzymes. For instance, anionic polysaccharides such as carrageenan or xanthan gum were reported to reduce the breakdown of milk, egg, or soy proteins during GID (David et al., 2020; Fahoum et al., 2017), whereas alginate decreased the proteolysis in the gastric phase (Borreani et al., 2016) but not at the small intestinal phase (Chater, Wilcox, Brownlee, & Pearson, 2015). Electrostatic interactions between the anionic domains of the alginate and cationic domains of proteins would limit the accessibility of the pepsin enzyme, although neutral polysaccharides such as konjac glucomannan could also reduce protein digestion due to its increased viscosity when mixed with water (Borreani et al., 2016).

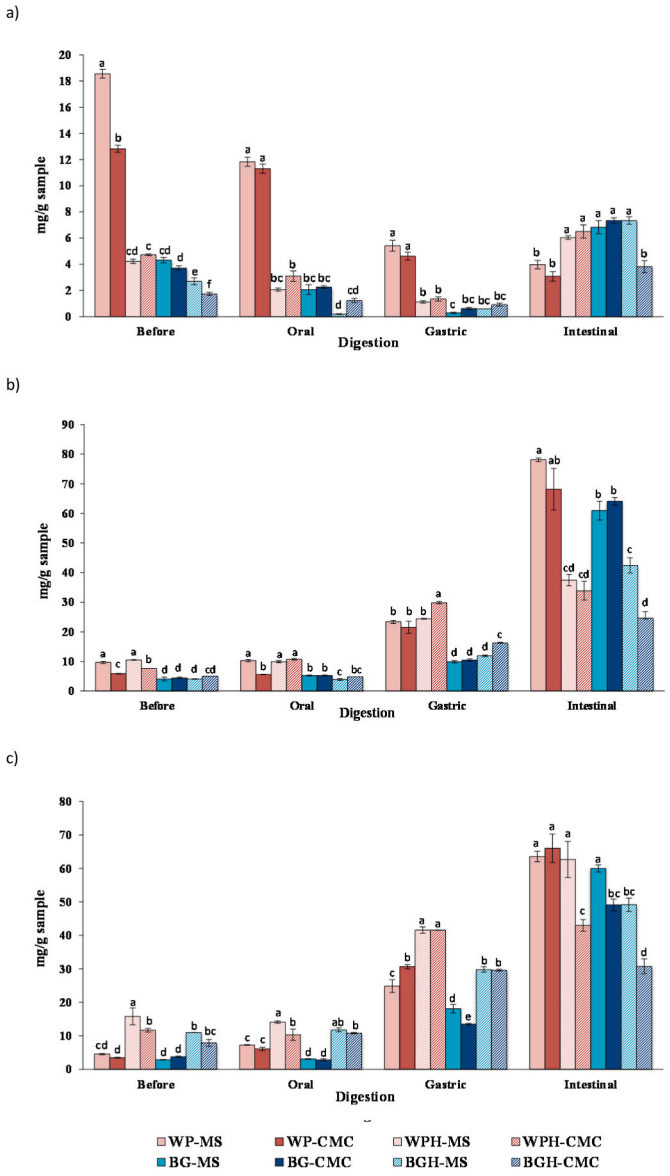


Fig. 4. Protein digestibility of mushroom creams before and during *in vitro* gastrointestinal digestion evaluated as the contents of a) soluble proteins, b) soluble peptides, and c) free amino groups. (WP: whey protein; WPH: whey protein hydrolysate; BG: bovine gelatin; BGH: bovine gelatin hydrolysate. MS: modified waxy-maize starch; CMC: carboxymethyl cellulose).

4. Conclusions

Protein-enriched and texture-modified mushroom creams exhibited similar flow behaviour properties. Nonetheless, the viscoelastic characterisation of the mushroom creams showed that BG-MS sample, as well as WP, WPH, and BGH samples thickened with CMC could be considered more appropriate for dysphagic and elder people owing to their high resistance to deformation and good elasticity degree. The action of the α-amylase enzyme contained in saliva provoked the degradation of the samples containing MS as thickener. Given the fact that the quick depletion in the oral viscosity is critical in dysphagia handling owing to the aspiration's risk, the samples thickened with MS would be considered less suitable for people with swallowing problems than those prepared with CMC. During simulated gastrointestinal digestion, samples with protein hydrolysates and the anionic CMC could form a compact structure that led to high viscosity and consistency values up to the gastric phase, but they could reduce protein digestibility at the end of the simulated gastrointestinal digestion, mainly in the case of the sample BGH-CMC. This work confirms that the interactions between added proteins and hydrocolloids and the food matrix determine the rheological and digestibility properties of samples, being those prepared with WP as protein source and CMC as thickener a good option when designing food products suitable for dysphagics or elderly people.

Author contributions

Conceptualisation, M.G., S.R., and P.T.; Methodology, M.G., S.R.,

and P.T.; Formal Analysis, M.G. and S.R.; Investigation, M.G. and S.R.; Writing – Original Draft Preparation, M.G. and S.R.; Writing – Review & Editing, M.G., S.R., and P.T.; Visualisation, M.G. and S.R.; Supervision, M.G., S.R., and P.T.; Project Administration, R.G. and P.T.; Funding Acquisition, R.G and P.T.

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

Data will be made available on request.

Acknowledgements

Grant RTI 2018-098842-B-I00 funded by MCIN/AEI/10.13039/501100011033 and by "ERDF A way of making Europe" is acknowledged. M.G. gratefully thanks the Postdoctoral grant (PAID-10-19) from the *Universitat Politècnica de València* and S.R. acknowledges the *Generalitat Valenciana* for her Postdoctoral Fellowship (APOSTD/2020/264).

References

Alvarez, M. D., & Canet, W. (2013). Dynamic viscoelastic behavior of vegetable-based Infant purees. *Journal of Texture Studies, 44*(3), 205–224.
Alvarez, M. D., Fuentes, R., Guerrero, G., & Canet, W. (2017). Characterization of commercial Spanish hummus formulation: Nutritional composition, rheology, and structure. *International Journal of Food Properties, 20*(4), 845–863.
AOAC. (1990). *Association of official analytical chemist. Official methods of analysis* (15th ed.) Washington D.C, USA.
Augusto, P. E. D., Cristianini, M., & Ibarz, A. (2012). Effect of temperature on dynamic and steady-state shear rheological properties of siriguela (*Spondias purpurea* L.) pulp. *Journal of Food Engineering, 108*(2), 283–289.
Borreani, J., Llorca, E., Larrea, V., & Hernando, I. (2016). Adding neutral or anionic hydrocolloids to dairy proteins under *in vitro* gastric digestion conditions. *Food Hydrocolloids, 57*, 169–177.
Chater, P. I., Wilcox, M. D., Brownlee, I. A., & Pearson, J. P. (2015). Alginate as a protease inhibitor *in vitro* and in a model gut system; selective inhibition of pepsin but not trypsin. *Carbohydrate Polymers, 131*, 142–151.
Chen, M., Guo, L., Nsor-Atindana, J., Goff, H. D., Zhang, W., Mao, J., et al. (2020). The effect of viscous soluble dietary fiber on nutrient digestion and metabolic responses: *In vitro* digestion process. *Food Hydrocolloids, 107*, Article 105971.
Chung, C., Degner, B., & McClements, D. J. (2012). Instrumental mastication assay for texture assessment of semi-solid foods: Combined cyclic squeezing flow and shear viscometry. *Food Research International, 49*(1), 161–169.
Clasen, C., & Kulicke, W. M. (2001). Determination of viscoelastic and rheo-optical material functions of water-soluble cellulose derivatives. *Progress in Polymer Science, 26*(9), 1839–1919.
Crosara, K. T. B., Zuanazzi, D., Moffa, E. B., Xiao, Y., Machado, M. A., & Siqueira, W. L. (2018). Revealing the amylase interactome in whole saliva using proteomic approaches. *BioMed Research International, 2018*, 1–15.
David, S., Magram Klaiman, M., Shpigelman, A., & Lesmes, U. (2020). Addition of anionic polysaccharide stabilisers modulates *in vitro* digestive proteolysis of a chocolate milk drink in adults and children. *Foods, 9*(9), 1253.
Fahoum, L., Moscovici, A., David, S., Shaoul, R., Rozen, G., Meyron-Holtz, E. G., et al. (2017). Digestive fate of dietary carrageenan: Evidence of interference with digestive proteolysis and disruption of gut epithelial function. *Molecular Nutrition & Food Research, 61*(3), Article 1600545.
Gallego, M., Arnal, M., Barat, J. M., & Talens, P. (2021). Effect of cooking on protein digestion and antioxidant activity of different legume pastes. *Foods, 10*(1), 47.
Gallego, M., Barat, J. M., Grau, R., & Talens, P. (2022). Compositional, structural design and nutritional aspects of texture-modified foods for the elderly. *Trends in Food Science & Technology, 119*, 152–163.
Hardacre, A. K., Yap, S. Y., Lentle, R. G., & Monro, J. A. (2015). The effect of fibre and gelatinised starch type on amylolysis and apparent viscosity during *in vitro* digestion at a physiological shear rate. *Carbohydrate Polymers, 123*, 80–88.
Herranz, B., Criado, C., Pozo-Bayón, M. A., & Álvarez, M. D. (2021). Effect of addition of human saliva on steady and viscoelastic rheological properties of some commercial dysphagia-oriented products. *Food Hydrocolloids, 111*, Article 106403.
Huang, T., Tu, Z. C., Wang, H., Liu, W., Zhang, L., Zhang, Y., et al. (2017). Comparison of rheological behaviors and nanostructure of bighead carp scales gelatin modified by different modification methods. *Journal of Food Science & Technology, 54*(5), 1256–1265.
Irani, M., Razavi, S. M. A., Abdel-Aal, E. S. M., Hucl, P., & Patterson, C. A. (2019). Viscoelastic and textural properties of canary seed starch gels in comparison with wheat starch gel. *International Journal of Biological Macromolecules, 124*, 270–281.
Ishihara, S., Nakauma, M., Funami, T., Odake, S., & Nishinari, K. (2011). Swallowing profiles of food polysaccharide gels in relation to bolus rheology. *Food Hydrocolloids, 25*(5), 1016–1024.
Izidoro, D. R., Scheer, A. P., Sierakowski, M. R., & Haminiuk, C. W. I. (2008). Influence of green banana pulp on the rheological behaviour and chemical characteristics of emulsions (mayonnaises). *LWT - Food Science and Technology, 41*, 1018–1028.
Jancikova, S., Jamróz, E., Kulawik, P., Tkaczewska, J., & Dordevic, D. (2019). Furcellaran/gelatin hydrolysate/rosemary extract composite films as active and intelligent packaging materials. *International Journal of Biological Macromolecules, 131*, 19–28.
Koopman, R., Crombach, N., Gijsen, A. P., Walrand, S., Fauquant, J., Kies, A. K., … van Loon, L. J. (2009). Ingestion of a protein hydrolysate is accompanied by an accelerated *in vivo* digestion and absorption rate when compared with its intact protein. *The American Journal of Clinical Nutrition, 90*(1), 106–115.
Lutz, M., Petzold, G., & Albala, C. (2019). Considerations for the development of innovative foods to improve nutrition in older adults. *Nutrients, 11*(6), 1275.
Malinauskytė, E., Ramanauskaitė, J., Keršienė, M., Jasutienė, I., Leskauskaitė, D., Devold, T. G., et al. (2018). Impact of interfacial composition on emulsion digestion using *in vitro* and *in vivo* models. *Journal of Food Science, 83*(11), 2850–2857.
Mao, L., & Miao, S. (2015). Structuring food emulsions to improve nutrient delivery during digestion. *Food Engineering Reviews, 7*(4), 439–451.
Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., et al. (2014). A standardised static *in vitro* digestion method suitable for food – an international consensus. *Food & Function, 5*(6), 1113–1124.
Mouécoucou, J., Villaume, C., Sanchez, C., & Méjean, L. (2004). β-Lactoglobulin/polysaccharide interactions during *in vitro* gastric and pancreatic hydrolysis assessed in dialysis bags of different molecular weight cut-offs. *Biochimica et Biophysica Acta (BBA) - General Subjects, 1670*(2), 105–112.
Nakauma, M., Ishihara, S., Funami, T., & Nishinari, K. (2011). Swallowing profiles of food polysaccharide solutions with different flow behaviors. *Food Hydrocolloids, 25*(5), 1165–1173.
Netter, A. B., Goudoulas, T. B., & Germann, N. (2020). Effects of Bloom number on phase transition of gelatin determined by means of rheological characterization. *LWT - Food Science and Technology, 132*, Article 109813.
Newman, R., Vilardell, N., Clavé, P., & Speyer, R. (2016). Effect of bolus viscosity on the safety and efficacy of swallowing and the kinematics of the swallow response in patients with oropharyngeal dysphagia: White paper by the European Society for Swallowing Disorders (ESSD). *Dysphagia, 31*, 232–249.
Nguyen, T. T., Bhandari, B., Cichero, J., & Prakash, S. (2016). *In vitro* digestion of infant formulations with hydrolysed and non-hydrolysed proteins from dairy and soybean. *Food & Function, 7*(12), 4908–4919.
Nguyen, P. T., Kravchuk, O., Bhandari, B., & Prakash, S. (2017). Effect of different hydrocolloids on texture, rheology, tribology and sensory perception of texture and mouthfeel of low-fat pot-set yoghurt. *Food Hydrocolloids, 72*, 90–104.
Nyström, M., Qazi, W. M., Bülow, M., Ekberg, O., & Stading, M. (2015). Effects of rheological factors on perceived ease of swallowing. *Applied Rheology, 25*, Article 63876.
Ogawa, Y., Donlao, N., Thuengtung, S., Tian, J., Cai, Y., Reginio, F. C., Jr., … Tamura, M. (2018). Impact of food structure and cell matrix on digestibility of plant-based food. *Current Opinion in Food Science, 19*, 36–41.
Ribes, S., Estarriaga, R., Grau, R., & Talens, P. (2021). Physical, sensory, and simulated mastication properties of texture-modified Spanish sauce using different texturing agents. *Food & Function, 12*(17), 8181–8195.
Ribes, S., Gallego, M., Barat, J. M., Grau, R., & Talens, P. (2022). Impact of chia seed mucilage on technological, sensory, and *in vitro* digestibility properties of a texture-modified puree. *Journal of Functional Foods, 89*, Article 104943.
Sharma, M., Kristo, E., Corredig, M., & Duizer, L. (2017). Effect of hydrocolloid type on texture of pureed carrots: Rheological and sensory measures. *Food Hydrocolloids, 63*, 478–487.
Simionescu, B., Ibanescu, S. A., Danu, M., Rotaru, I., & Ibanescu, C. (2013). Rheology of gelatin – starch systems. Influence of system composition. *Revista de Chimie, 64*(8), 909–913.
Sinthusamran, S., Benjakul, S., & Kishimura, H. (2014). Characteristics and gel properties of gelatin from skin of seabass (*Lates calcarifer*) as influenced by extraction conditions. *Food Chemistry, 152*, 276–284.
Syrbe, A., Bauer, W. J., & Klostermeyer, H. J. I. D. J. (1998). Polymer science concepts in dairy systems—an overview of milk protein and food hydrocolloid interaction. *International Dairy Journal, 8*(3), 179–193.
Talens, P., Castells, M. L., Verdú, S., Barat, J. M., & Grau, R. (2021). Flow, viscoelastic and masticatory properties of tailor made thickened pea cream for people with swallowing problems. *Journal of Food Engineering, 292*, Article 110265.
Tarhan, O., Spotti, M. J., Schaffer, S., Corvalan, C. M., & Campanella, O. H. (2016). Rheological and structural characterization of whey protein gelation induced by enzymatic hydrolysis. *Food Hydrocolloids, 61*, 211–220.
Tashiro, A., Hasegawa, A., Kohyama, K., Kumagai, H., & Kumagai, H. (2010). Relationship between the rheological properties of thickener solutions and their velocity through the pharynx as measured by the ultrasonic pulse Doppler method. *Bioscience, Biotechnology, and Biochemistry, 74*, 1598–1605, 2010.
Turgeon, S. L., & Rioux, L. E. (2011). Food matrix impact on macronutrients nutritional properties. *Food Hydrocolloids, 25*(8), 1915–1924.
Vieira, J. M., Andrade, C. C. P., Santos, T. P., Okuro, P. K., Garcia, S. T., Rodrigues, M. I., et al. (2021). Flaxseed gum-biopolymers interactions driving rheological behaviour

of oropharyngeal dysphagia-oriented products. *Food Hydrocolloids, 111*, Article 106257.
Vieira, J. M., Oliveira, F. D., Jr., Salvaro, D. B., Maffezzolli, G. P., de Mello, J. B., Vicente, A. A., et al. (2020). Rheology and soft tribology of thickened dispersions aiming the development of oropharyngeal dysphagia-oriented products. *Current Research in Food Science, 3*, 19–29.
Vu Dang, H., Loisel, C., Desrumaux, A., & Doublier, J. (2009). Rheology and microstructure of cross-linked waxy maize starch/whey protein suspensions. *Food Hydrocolloids, 23*(7), 1678–1686.
Ye, A. (2008). Complexation between milk proteins and polysaccharides via electrostatic interaction: Principles and applications–a review. *International Journal of Food Science and Technology, 43*(3), 406–415.
Yu, B., Ren, F., Zhao, H., Cui, B., & Liu, P. (2020). Effects of native starch and modified starches on the textural, rheological and microstructural characteristics of soybean protein gel. *International Journal of Biological Macromolecules, 142*, 237–243.
Yu, H., Sabato, S. F., D'Aprano, G., & Lacroix, M. (2004). Effect of the addition of CMC on the aggregation behaviour of proteins. *Radiation Physics and Chemistry, 71*(1–2), 131–135.