Food Research International 173 (2023) 113297

Contents lists available at ScienceDirect

Food Research International

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

Influence of food oral processing, bolus characteristics, and digestive conditions on the protein digestibility of turkey cold meat and fresh cheese

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ARTICLE INFO

Keywords:
Food oral processing
Deficient mastication
Bolus properties
In vitro gastrointestinal digestion
Protein digestibility

ABSTRACT

During mastication, foods are progressively transformed to achieve swallowable boluses and their characteristics are crucial for the subsequent digestion events. The main goal of this work was to evaluate the impact of food oral processing, bolus properties, and different digestive conditions on the protein digestibility of turkey cold meat and fresh cheese. *In vivo* normal and deficient masticated food boluses were prepared by a young volunteer. Besides, three digestion models were used to simulate the different physiological conditions frequently observed in adults and the elderly, presenting good or poor oral health: i) Normal Masticated-Normal Digested model; ii) Deficient Masticated-Normal Digested model; and iii) Deficient Masticated-Elderly Digested model. The oral processing behaviour (number of chews, chewing time, chewing rate, and saliva uptake), bolus particle size, textural and viscoelastic properties of boluses, and protein digestibility of samples were determined. Results showed that deficient masticated boluses exhibited lower amounts of saliva uptake and greater particle sizes, hardness, stiffness, and rigidity, notably in deficient masticated turkey cold meat boluses. Moreover, the worst digestive scenario (Deficient Masticated-Elderly Digested model) negatively impacted on the proteolysis extend of samples, especially for total soluble proteins and soluble peptides contents. The current study demonstrates that the oral processing behaviour and degree of food fragmentation impacted on the granulometric, texture, and viscoelastic properties of both food boluses, whereas the worst digestive scenario commonly observed in the elderly reduced the proteolysis extend of the products evaluated.

1. Introduction

Nowadays, the worldwide population is three times larger than it was in the 20th century and could grow from 8.5 billion in 2030 to 9.7 billion in 2050. However, a continuous demographic evolution has been observed in the last decade due to the increasing life expectancy and decreasing mortality. In this sense, the number of people over 65 years-old is estimated to rise from 727 million in 2020 to 1.5 billion in 2050 (United Nations, 2020).

Ageing is a natural process in which several physiological changes take place, including masticatory deficiencies, loss of muscle mass, and gastrointestinal alterations (Assad-Bustillos et al., 2020; Peyron et al., 2018) compared to healthy adults. One of the most important factors employed to achieve a healthy ageing is the enhancement of elderly dietary patterns, among which meat and dairy proteins are essential to prevent sarcopenia (Hernández-Olivas et al., 2022; Lorieau et al., 2018). In this context, the European Society for Clinical Nutrition and Metabolism (ESPEN) recommends a daily protein intake of 1.0 – 1.2 g protein/kg body weight for people over 65 years-old (Melchior et al., 2023), whereas the recommended daily protein intake for healthy adults is 0.8 g protein/kg body weight (Peyron et al., 2021). Nevertheless, nutrient deficits are frequently observed in seniors due to changes in the oral and gastrointestinal process. During oral processing, products are submitted to several transformations in the oral cavity and progressively modified to achieve a swallowable bolus. Its formation begins with a decrease in food hardness along with an increase in bolus hydration because of mastication and lubrication, and it finalises with an increase in bolus cohesion and stickiness, which could be crucial for safe-swallowing (Loret et al., 2011; Panouillé et al., 2014). The characteristics of these boluses are based on the oral capabilities of each individual and type of food. In this sense, seniors without any important oral disorder provoke a slight impact on masticatory performance as they are capable to generate suitable food boluses for swallowing with minor adaptations to compensate physiological changes (Rémond et al.,

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https://doi.org/10.1016/j.foodres.2023.113297
Received 20 May 2023; Received in revised form 12 July 2023; Accepted 20 July 2023
Available online 22 July 2023
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2015). On the contrary, when presenting poor oral health, food boluses produced by the elderly are generally characterised by low levels of food breakdown, which favours the presence of large particles that rise bolus hardness and negatively impact on the nutrient bioaccessibility of the elderly (Blanquet-Diot et al., 2021; Peyron et al., 2018; Sugimoto et al., 2020). An impaired oral health often leads the elderly to modify their diet for adjusting it to their limited oral functional capabilities (Rémond et al., 2015). Furthermore, gastrointestinal declines have also been reported to diminish the nutrient bioaccessibility in seniors in comparison with healthy adults (Hernández-Olivas et al., 2020; Hernández-Olivas et al., 2022; Menard et al., 2023).

In the last years, an increasing demand of texture-modified products, rich in protein content, to cover the oral and digestive capabilities of specific populations has been observed (Assad-Bustillos et al., 2020; Gallego et al., 2023; Lorieau et al., 2018; Ribes et al., 2022). The characteristics of food boluses are decisive for understanding the oral processing and digestion in adults and the elderly. For instance, the rheological and viscoelastic properties of food boluses are linked to food texture, its breakdown, and capacity to be swallowed, finally impacting on the gastrointestinal digestion and nutrient bioaccessibility of foods. Some studies investigated the granulometric, textural, and rheological properties of boluses (Gibouin et al., 2022; Panouillé et al., 2014; Peyron et al., 2018), as well as the consequences of the elderly digestion on the nutrient bioaccessibility of a huge variety of products (Blanquet-Diot et al., 2021; Denis et al., 2016; Peyron et al., 2021; Shani-Levi et al., 2017). Nonetheless, no works have investigated at the same time the impact of food oral processing, mechanical properties of boluses, and different digestive conditions on the protein bioaccessibility of meat and dairy products.

Thus, this study aimed to evaluate the impact of food oral processing, bolus characteristics, and digestive conditions on the protein digestibility of turkey cold meat and fresh cheese. To this end, the oral processing behaviour of samples and the granulometric, textural, and viscoelastic properties of normal and deficient masticated food boluses were determined, as well as their proteolysis extend after simulating the oral and gastrointestinal alternations commonly observed in adults and the elderly, having good or poor oral health.

2. Materials and methods

2.1. Materials

Turkey cold meat (T) and fresh cheese (C) employed in this study were bought from a local supermarket (Valencia, Spain). The nutritional composition of T sample was 77.8% water, 1.0% fat, 6.0% carbohydrates, 13.0% proteins, and 2.2% salt; whereas C sample had 82.3% water, 0.2% fats, 4.4% carbohydrates, 12.3% proteins, and 0.8% salt.

Gastrointestinal enzymes, such as pepsin from gastric porcine mucosa (P7012), pancreatin from porcine pancreas (P7545), and porcine bile extract (B8631), were provided by Sigma-Aldrich Co. (St. Louis, MO, USA). Bovine serum albumin (BSA), L-Tirosine, L-Leucine, and trinitrobenzenesulfonic acid (TNBS) employed in analytical determinations were also supplied by Sigma-Aldrich Co. (St. Louis, MO, USA). Sodium bicarbonate, sodium chloride, potassium chloride, potassium dihydrogen phosphate, magnesium chloride hexahydrate, ammonium carbonate, calcium chloride dehydrate, phosphoric acid, ethanol, sodium hydroxide, and hydrochloric acid were purchased from Scharlab, S. L. (Barcelona, Spain).

2.2. Food bolus formation and oral processing behaviour

A young volunteer (female, 35 years-old) with good oral health formed all the boluses employed in this study. To prepare normal masticated (NM) boluses, the volunteer was invited to chew 5.0 ± 0.1 g of sample as usually and to expectorate the bolus when feeling the desire of swallowing. Deficient masticated (DM) boluses, commonly observed in seniors with poor oral health and characterised by large particle sizes, were formed by reducing to 50% the mean number of chews performed when preparing turkey cold meat and fresh cheese NM boluses (Hernández-Olivas et al., 2022). Noteworthy that this percentage was selected to produce boluses presenting higher particle sizes commonly observed in elderly people with poor oral health. To analyse the food oral processing response, the number of chews, the chewing time at the end of mastication, and the chewing rate (number of chews divided by chewing time) were recorded. The amount of saliva uptake was also calculated by removing from each expectorated bolus the weight of the corresponding sample served to the volunteer (Álvarez et al., 2020). Five boluses per condition were prepared. All *in vivo* boluses were produced and collected before conducting each analysis.

2.3. Characterisation of food boluses

2.3.1. Granulometric analysis of food boluses

The granulometric analyses of each NM and DM bolus were performed by manual dry sieving according to Peyron et al. (2018), with minor changes. Briefly, boluses were poured onto a nylon cloth of 0.2 mm, washed with tap water to attain a great particle dispersion and to eliminate saliva, and dried for 40 min at 37 °C in an oven. Dried particles were poured onto a pile of 10 sieves with orifices of 10.0, 8.0, 5.0, 4.0, 3.2, 1.5, 1.25, 1.0, 0.71, and 0.25 mm (Mecánica Científica, S.A., Madrid, Spain), and manually sieved with the aid of a brush. Particles held on each sieve were weighted and data were expressed as cumulative curves by employing the weight of the particles dropping through each specific sieve. The median particle size (d_{50}) from each curve, which is defined as the aperture of a theoretical sieve throughout which the 50% of the particles weight could pass, was also reported. The assays were run in quintuplicate.

2.3.2. Texture properties of food boluses

Textural properties of NM and DM boluses were measured by performing a Textural Profile Analysis (TPA), using a double compression cycle test, in a TA-TX2 texture analyser (Stable Micro Systems, Surrey, UK) equipped with a 25 kg load cell. Immediately after its production, each bolus was placed in a measuring container (28 mm diameter, 50 mm high) and held in a water bath at 37 °C for 5 min. A 20 mm cylindrical compression probe was employed to compress the boluses. Test settings were fixed at 70% compression strain, pre- and post-test speed of 5 mm/s, and test speed of 3 mm/s. Measurements were performed in quintuplicate and the TPA parameters, including hardness, adhesiveness, and cohesiveness, were reported. Hardness is defined as the maximum force achieved during the first compression, adhesiveness is described as the negative area obtained after the first compression, and cohesiveness is defined as the ratio of the second compression area to the first compression area (Wee et al., 2019).

2.3.3. Viscoelastic properties of food boluses

Viscoelastic properties of NM and DM food boluses were determined by non-linear and linear viscoelastic assays in a rotational stress-controlled Kinexus Pro + Rheometer (Malvern Instruments Ltd., MA, USA), equipped with a Peltier cartridge for temperature regulation and rSpace for Kinexus software. Measurements were performed at 37 °C using a PLC61/PU40 parallel-plate circular geometry with a 3-mm gap. Immediately after its preparation, each bolus was loaded in the measuring system, covered with the accessory supplied by the provider to minimise the water evaporation during tests, and allowed to stand for 300 s for structure relaxation and temperature equilibration.

For establishing the boundary of the linear viscoelastic region (LVR) and determining the non-linear viscoelastic characteristics of food boluses, a large amplitude oscillatory shear (LAOS) test was performed. Strain sweeps tests were run at 1 Hz in a strain varying from 0.01% to 40%. Stress sweeps assays were also conducted at 1 Hz within a stress comprised between 0.01 and 100 Pa to set the flow point (G' = G''). For

characterising the linear viscoelastic properties of food boluses, a small amplitude oscillatory shear (SAOS) test ranging from 0.1 up to 10 Hz at 0.5% strain was performed. From all these assays, changes in elastic (G') and viscous modulus (G''), complex modulus (G*), complex viscosity (η*), loss tangent (Tan δ), G' value at LVR (G'_{LVR}), stress and strain values at LVR, and flow point were reported. The boundary of the LVR was determined as the strain/stress at which the G' value was reduced from 100% to 90% of G' plateau value (Ribes et al., 2021; Sharma et al., 2017). Assays were done in triplicate.

2.4. In vitro digestion tests

Samples were digested by following different oral and gastrointestinal conditions (Fig. 1). To this end, the standardised INFOGEST protocol defined by Minekus et al. (2014) and Brodkorb et al. (2019) was slightly modified to mimic the digestion of healthy adults (Normal Masticated-Normal Digested model, NM-ND). NM boluses were prepared as described in section 2.2 and immediately placed on ice until use. On the contrary, to evaluate the contribution of different oral and gastrointestinal alterations, two digestion models were employed: i) Deficient Masticated-Normal Digested model (DM-ND), in which DM boluses were produced as defined in section 2.2 and rapidly placed in an ice bath, and gastric and intestinal phases were run as described in the INFOGEST protocol; and, ii) Deficient Masticated-Elderly Digested model (DM-ED), in which DM boluses were produced as above-mentioned but, in the gastric phase, pepsin activity decreased (1500 U/mL) and pH increased (pH 6). Besides, in the intestinal phase, the pancreatin activity (50 U/mL) and bile salts content (5 mM) decreased, whereas the duration of this phase increased (4 h) compared to the INFOGEST protocol (Denis et al., 2016; Hernández-Olivas et al., 2022; Shani-Levi et al., 2017).

All tests were performed in duplicate, using two different boluses per condition, in a rotary mixer (Intell-Mixer™ RM-2, ELMI Ltd., Riga, Latvia) programmed at 40 rpm and placed in an incubator chamber (JP Selecta, S.A., Barcelona, Spain) at 37 °C. Individual test tubes were used to conduct digestion tests. Moreover, an aliquot of 1 mL was taken out from each reaction tube at the end of the gastric phase, which was previously treated with NaOH (1 M) to stop the enzymatic reactions by increasing the pH to 7. These reactions were also stopped by heat shock (98 °C, 5 min) at the end of the intestinal phase, and samples were subsequently cooled in an ice bath. Blank samples (without food but containing enzymes and bile) were also prepared and subjected to the same conditions. Finally, aliquots of 3 mL were withdrawn and centrifuged (8000 g, 4 °C, 10 min), and supernatants were kept at − 20 °C for further analysis.

2.4.1. Protein digestibility of samples

The protein digestibility of samples was evaluated by determining the content of total soluble proteins, TCA-soluble peptides, and free amino groups formed during digestion.

The total soluble protein content was assessed as described by Bradford (1976). To this end, 40 μL of each sample was blended with 2 mL of Bradford reagent and incubated at room temperature for 5 min. The absorbance was measured at 595 nm and data were expressed as mg bovine serum albumin (BSA) protein/g sample.

TCA-soluble peptides content was analysed according to Ketnawa and Ogawa (2019). Briefly, 50 μL of each sample was added to 450 μL of TCA (5%, w/v), mixed, and stored at 4 °C for 1 h. The mixture was then centrifuged (8000 g, 4 °C, 10 min) and the absorbance of the supernatant was measured at 280 nm. Results were expressed as mg tyrosine equivalents/g sample.

Finally, TNBS method was used to determine the content of free amino groups (Adler-Nissen, 1979). For that, 40 μL of each sample was mixed with 320 μL of TNBS and 320 μL of sodium phosphate buffer (0.2 M, pH 8.2), vortexed, and incubated at 50 °C for 1 h. Subsequently, 640 μL of HCl (0.1 N) was added and the mixture was incubated at room

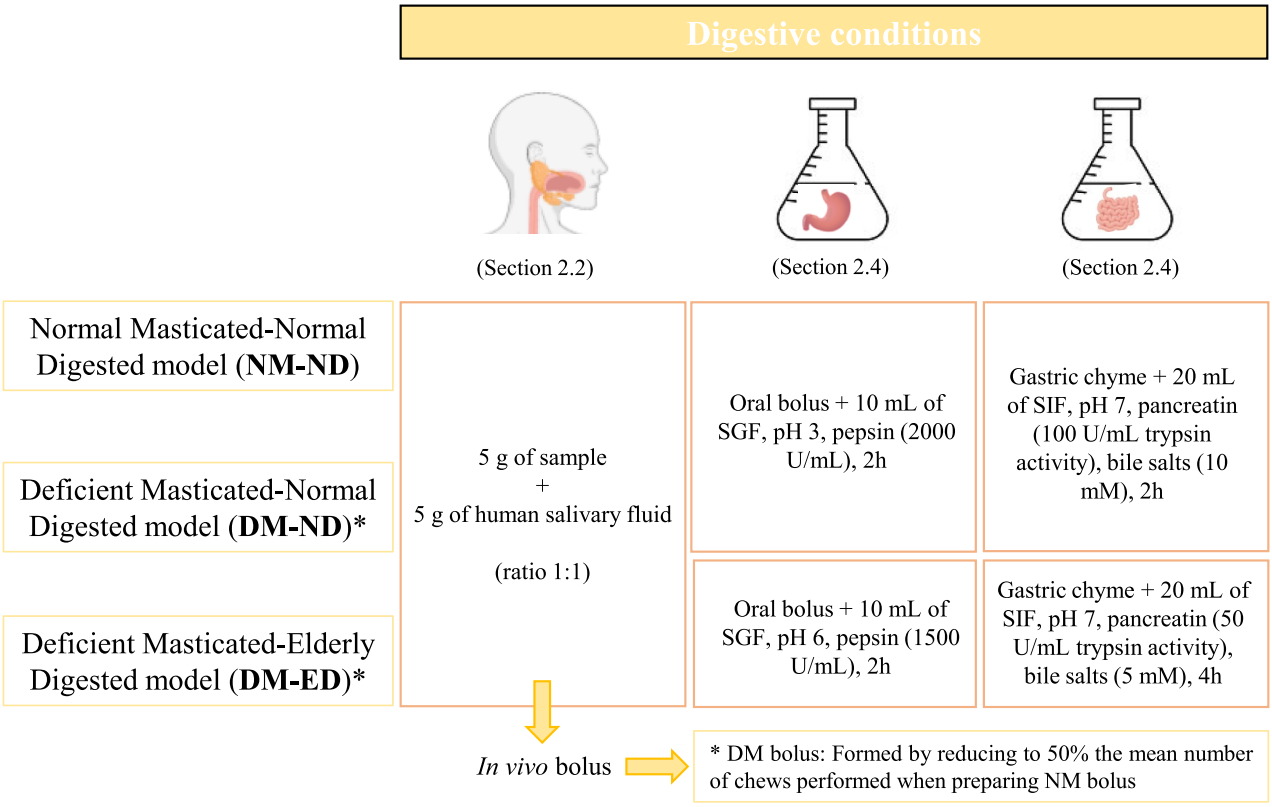

Fig. 1. Oral and gastrointestinal parameters of the different models employed to simulate several digestive alterations. SGF: Simulated Gastric Fluid; SIF: Simulated Intestinal Fluid.

temperature for 30 min. The absorbance was measured at 340 nm and data were expressed as mg L-leucine equivalents/g sample.

All determinations were made in triplicate by measuring the absorbance with an UV–Visible spectrophotometer (Helios Zeta, Thermo Scientific, UK).

2.5. Statistical analysis

Statistical analyses were performed with XLSTAT 2020.3.1 software (XLSTAT statistical and data analysis solution, Addinsoft, New York, USA). One-way repeated measures ANOVA test, followed by Tukey-Kramer *post-hoc* test for mean comparisons, was used to study the differences between the particle size distribution of T-NM and T-DM boluses and C-NM and C-DM boluses. One-way ANOVA test, followed by Tukey-Kramer *post-hoc* test for mean comparisons, was conducted to determine the differences between the d_{50} of NM and DM boluses, texture and viscoelastic properties of NM and DM boluses, and protein digestibility of T and C samples under different digestion models. The statistical significance level was fixed at $p < 0.05$.

3. Results and discussion

3.1. Oral processing behaviour

Mastication is the key aspect of food oral processing, and its main goals are the breakdown of foods into smaller particles and the formation of safe-swallowable boluses with saliva incorporation (Hollis, 2018).

Fig. 2 summarises the results of the oral processing behaviour of samples during mastication. Significant ($p < 0.05$) differences were observed among samples in relation to the number of chews and chewing time. In this sense, T-NM required significantly ($p < 0.05$) higher number of chews than C-NM, whereas in DM products the number of chews was reduced to 50% to simulate the oral declines observed in the elderly (section 2.2). Furthermore, T-NM exhibited the highest chewing time as a consequence of the highest number of chews, while the C-DM showed the lowest chewing time (Fig. 2 B). Previous research studies have shown positive correlations between the instrumental properties of food products and their oral processing behaviour. Foster et al. (2006) demonstrated that the number of chewing cycles, the duration of mastication, and the muscle activities significantly increased with food hardness. Similarly, Bolhuis and Forde (2020) pointed out that harder foods require more chewing cycles and longer time for modifying their texture, as well as for disrupting the innate structures and fibres.

Regarding the chewing rate, significant ($p < 0.05$) differences were noted among samples. T-NM showed the highest chewing rate, whereas C-NM presented the lowest chewing rate (Fig. 2 C). This fact could also be attributed to the instrumental properties of foods. Çakir et al. (2012) observed that an increase in products' adhesiveness resulted in a slowing down of the chewing rate. Lastly, significant ($p < 0.05$) differences were observed among samples in relation to saliva uptake. Greater saliva content was incorporated in T-NM boluses than in T-DM boluses, and the same trend was noted in C-NM and C-DM boluses. Besides, T-NM showed the highest saliva uptake, meanwhile C-DM showed the lowest saliva uptake (Fig. 2 D). The rate of saliva uptake is highly influenced by the available surface area, moisture content, and absorption properties of food boluses (Bolhuis & Forde, 2020; Mosca & Chen, 2017). Those samples requiring high lubrications will increase the number of chews to extend the particle surface area and moisten the bolus completely (Bolhuis & Forde, 2020).

3.2. Granulometric analysis of food boluses

The granulometric properties of food boluses are commonly determined to provide insights about the in-mouth comminution and agglomeration mechanisms. Different techniques have been employed depending on food products and ranges of particle size, including sieving methods, image analysis, and laser diffraction (Panouillé et al., 2016). In this study, the granulometric properties of all *in vivo* boluses collected after performing the NM and DM were determined by dry

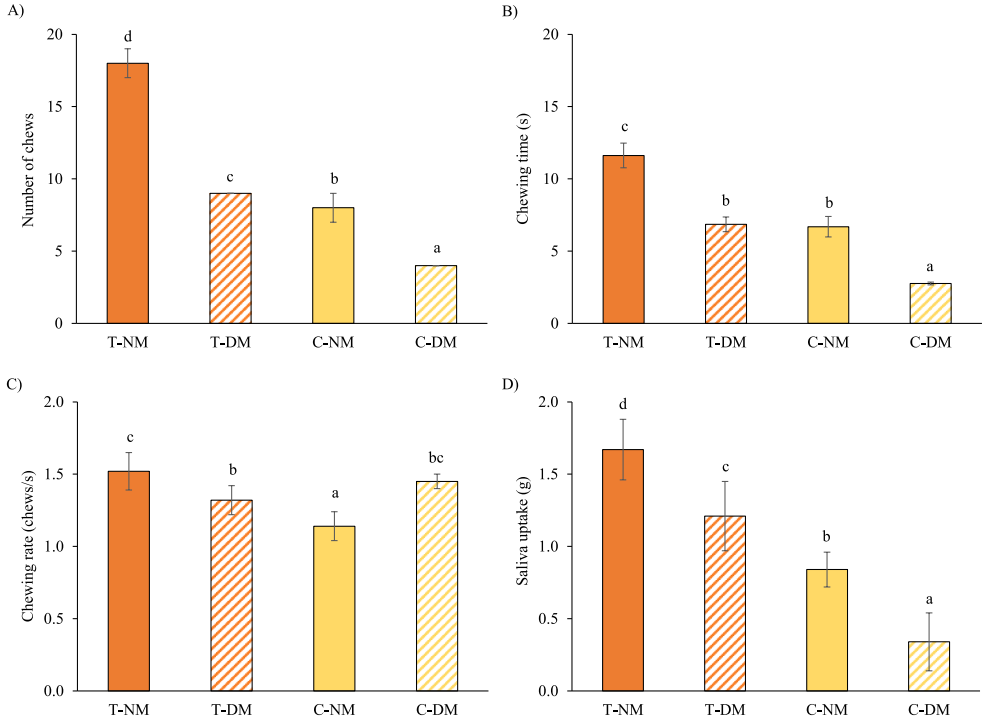

Fig. 2. Oral processing behaviour of turkey cold meat and fresh cheese after *in vivo* normal and deficient mastication: number of chews (A), chewing time (s) (B), chewing rate (chews/s) (C), and saliva uptake (g) (D). Mean values (n = 5) ± SD. T-NM: Turkey-Normal Masticated; T-DM: Turkey-Deficient Masticated; C-NM: Cheese-Normal Masticated; C-DM: Cheese-Deficient Masticated. Different superscripts indicate significant differences among boluses ($p < 0.05$).

sieving.

Fig. 3 presents the particle size distribution and the median particle size (d_{50}) values of T and C boluses. When DM boluses were produced, their particle size distribution were significantly ($p < 0.05$) different from those of NM boluses (Fig. 3 A). As can be seen, greater proportions of large particles were obtained when preparing DM boluses and their cumulative weight did not arrive at 100% owing to the existence of particles bigger than the higher sieve orifice. Moreover, the d_{50} values of both DM boluses were significantly ($p < 0.05$) higher than those observed in the case of NM boluses, indicating the formation of poorly fragmented boluses (Fig. 3 B). Food boluses produced by seniors presenting different oral deficiencies have been largely reported to possess greater proportion of large particles than boluses produced in a normal mastication. Assad-Bustillos et al. (2020), who performed a masticatory study involving 20 seniors with poor and satisfactory dental status, reported that seniors with satisfactory dental status produced food boluses with smaller particle sizes. Likewise, Woda et al. (2006) reported that the particles size of peanut and carrot boluses produced by seniors with denture wearers were larger than those produced by dentate subjects.

3.3. Texture properties of food boluses

During the food oral processing, the mechanical effect of mastication and saliva impregnation lead to significant changes in the texture properties of boluses (Pu et al., 2021). Table 1 shows the texture parameters of all *in vivo* NM and DM boluses. Significant ($p < 0.05$) differences were observed among boluses in relation to hardness parameter. T-DM was the hardest bolus, followed by T-NM, C-DM, and C-NM. Furthermore, both DM boluses presented higher hardness values than NM boluses, which could be attributed to the lower number of chews and saliva uptake. Similar results were reported by Peyron et al. (2018) when evaluating the hardness of meatball boluses. These authors indicated that whatever the degree of the oral deficiency, DM boluses were harder than NM boluses.

Concerning the adhesiveness of food boluses, it is worth mentioning that C boluses presented significantly ($p < 0.05$) higher values than T boluses. In addition, C-NM boluses showed higher adhesiveness values than C-DM, which could be ascribed to the mastication procedure, α-amylase activity, and mucin impregnation (Pu et al., 2021). This outcome was also observed by Blanquet-Diot et al. (2021) when evaluating the textural properties of NM and DM wholegrain pasta boluses.

Table 1
Texture parameters of turkey cold meat and fresh cheese bolus after *in vivo* normal and deficient mastication. Mean values (n = 5) ± SD.

Sample	Hardness (N)	Adhesiveness (N·s)	Cohesiveness (%)
T-NM	7.08 ± 1.37^{a}	− 0.08 ± 0.06^{c}	41.44 ± 1.87^{a}
T-DM	10.73 ± 0.82^{b}	− 0.05 ± 0.01^{c}	46.11 ± 9.10^{a}
C-NM	6.40 ± 1.03^{a}	− 0.51 ± 0.11^{a}	40.32 ± 5.64^{a}
C-DM	6.57 ± 0.46^{a}	− 0.35 ± 0.12^{b}	37.23 ± 2.45^{a}

T-NM: Turkey-Normal Masticated; T-DM: Turkey-Deficient Masticated; C-NM: Cheese-Normal Masticated; C-DM: Cheese-Deficient Masticated.
Texture parameters: hardness (N), adhesiveness (N·s), and cohesiveness (%).
Different superscripts indicate significant differences among boluses ($p < 0.05$).

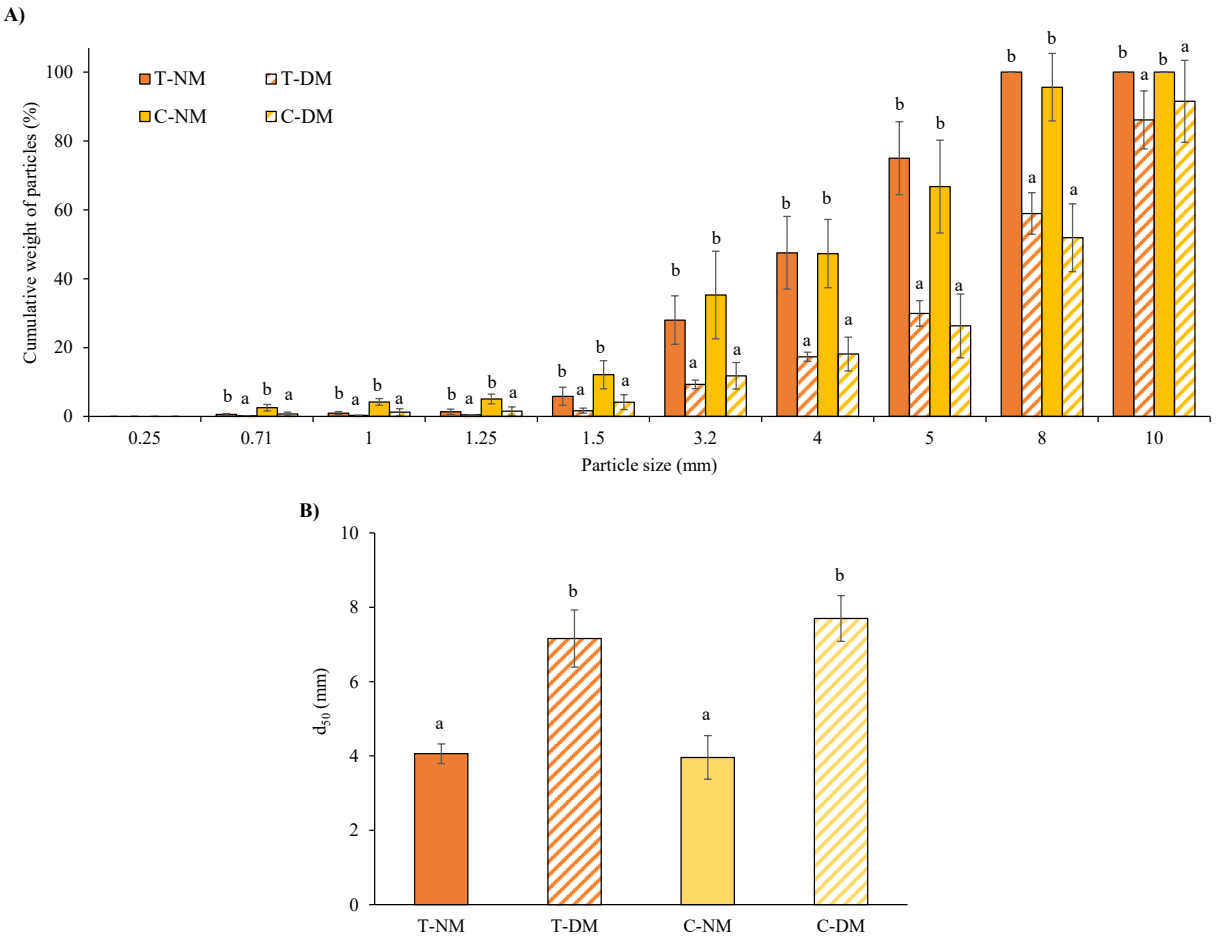

Fig. 3. Granulometric analysis: Particle size distribution of turkey cold meat and fresh cheese bolus collected after performing *in vivo* normal and deficient mastication (A). Median particle size value, expressed as d_{50} in mm, of turkey and fresh cheese bolus after *in vivo* normal and deficient mastication (B). Mean values (n = 5) ± SD. NM: Normal Masticated; DM: Deficient Masticated. Different superscripts indicate significant differences among boluses ($p < 0.05$).

Finally, despite non-significant ($p > 0.05$) differences were noted among boluses regarding their cohesiveness, it is important to mention that C-DM exhibited the lowest value. This could be linked with the lower saliva uptake achieved during the oral processing given that a good saliva impregnation enhances bolus cohesiveness, which plays an important role in safe-swallowing (Mishellany et al., 2006).

3.4. Viscoelastic properties of food boluses

LAOS tests were performed to establish the boundary of the LVR and flow point (G' = G''). Fig. 4 shows the changes in the elastic (G') and viscous modulus (G'') of all the boluses analysed at 37 °C, according to the strain applied. Higher G' than G'' values were recorded over the LVR, which is distinctive of weak viscoelastic systems (Ribes et al., 2022). Beyond the LVR, G' values of all the boluses decreased as the strain increased. Conversely, it is worth mentioning the slight increase observed in the G'' values of all boluses, which lowered again when higher strain levels were applied to them. A similar trend was observed in the study performed by Gibouin et al. (2022) when evaluating the rheological properties of cereal boluses. According to Hyun et al. (2002), these boluses would present weak strain-overshoot behaviour at the onset of the non-LVR, probably due to the use of greater quantities of energy during the deformation procedure. Microfissures in the bolus structure can be originated, and the layers' friction at the fissure sites provokes energy losses as heat.

Table 2 presents the viscoelastic parameters of the different boluses recorded from the LAOS test at 37 °C (strain sweep and stress sweep tests). G'_{LVR} is related to the stiffness of the product, whereas $Stress_{LVR}$ and $Strain_{LVR}$ values may be employed as indexes of the product stability and extensibility (Campo-Deaño et al., 2009; Ribes et al., 2022). In this work, the stability of the bolus should be interpreted as less degraded or chewed bolus. As expected, DM boluses presented significantly ($p <$

Table 2
Viscoelastic parameters of turkey cold meat and fresh cheese bolus obtained from the LAOS test at 37 °C. Mean values (n = 3) ± SD.

Sample	G'_{LVR} (Pa)	$Stress_{LVR}$ (Pa)	$Strain_{LVR}$ (%)	Flow point (Pa)
T-NM	3954 ± 28^{b}	40.52 ± 0.32^{a}	1.00 ± 0.00^{a}	12.29 ± 0.35^{b}
T-DM	7214 ± 797^{c}	116.80 ± 12.90^{c}	1.59 ± 0.00^{a}	20.98 ± 1.25^{c}
C-NM	1530 ± 135^{a}	40.54 ± 3.55^{a}	2.52 ± 0.00^{b}	9.37 ± 0.27^{a}
C-DM	1839 ± 416^{a}	66.15 ± 13.52^{b}	3.44 ± 0.47^{c}	12.64 ± 0.12^{b}

Viscoelastic parameters from the LAOS test: elastic modulus value at LVR, G'_{LVR}; stress value at LVR, $Stress_{LVR}$; strain value at LVR, $Strain_{LVR}$: strain value at LVR; and flow point.
T-NM: Turkey-Normal Masticated; T-DM: Turkey-Deficient Masticated; C-NM: Cheese-Normal Masticated; C-DM: Cheese-Deficient Masticated.
Different superscripts indicate significant differences among boluses ($p < 0.05$).

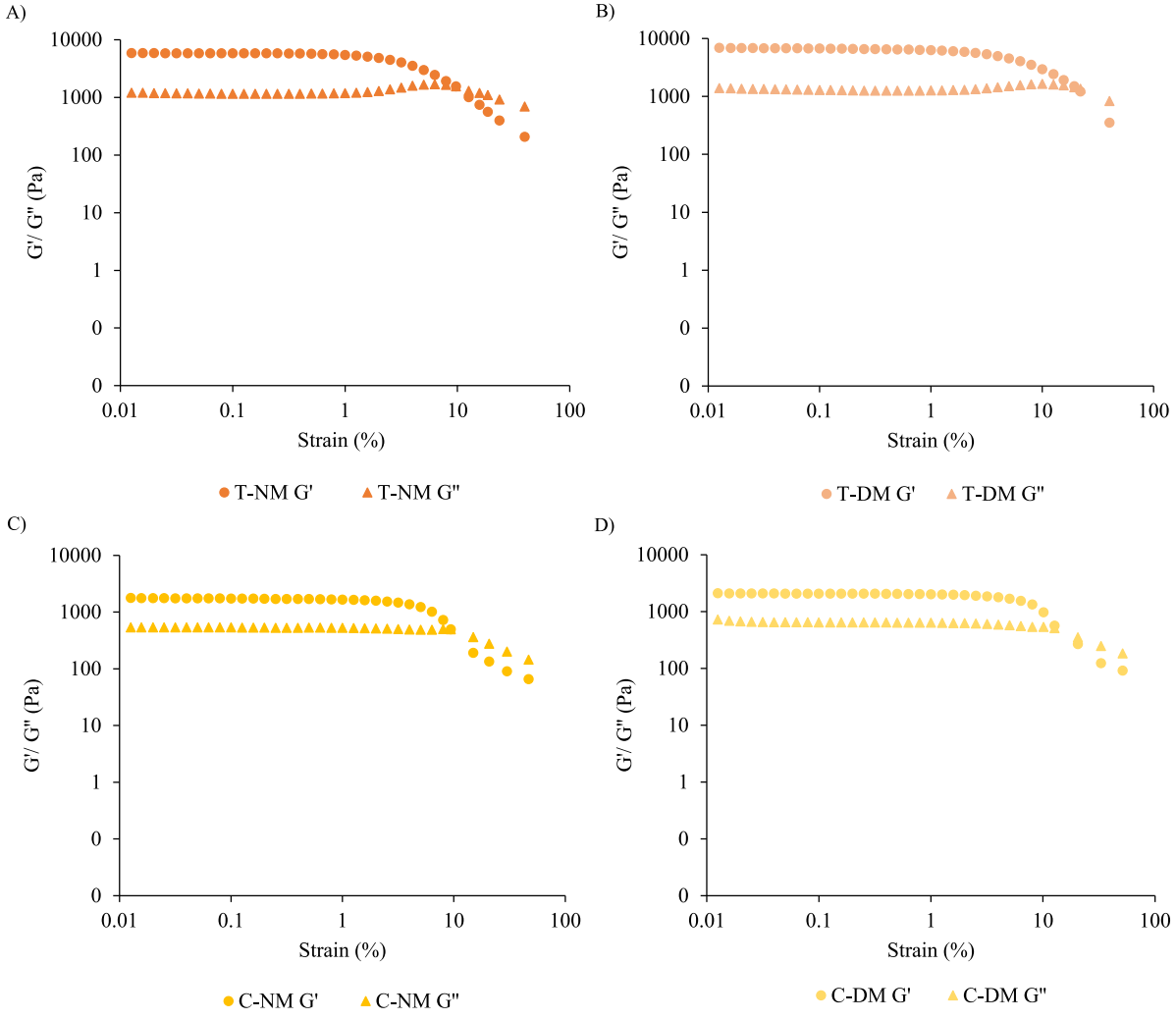

Fig. 4. Changes in elastic modulus, G', and viscous modulus, G'', of normal and deficient masticated turkey cold meat (A-B) and fresh cheese (C-D) bolus according to the strain applied. Curves are representative runs. T-NM: Turkey-Normal Masticated; T-DM: Turkey-Deficient Masticated; C-NM: Cheese-Normal Masticated; C-DM: Cheese-Deficient Masticated.

0.05) greater G'_{LVR} values than NM boluses probably due to the lower number of chews applied during mastication and saliva impregnation. Moreover, it is important to highlight that T-DM boluses exhibited the greatest stiff structure whereas C-NM boluses showed the lowest stiff structure, which is in accordance with the data observed in section 3.3 for bolus hardness. Concerning the $Stress_{LVR}$ and $Strain_{LVR}$ values, significant ($p < 0.05$) differences were noted among samples. Higher $Stress_{LVR}$ and $Strain_{LVR}$ values were observed in DM boluses compared to NM boluses, suggesting the formation of less chewed/degraded and extensible boluses. C-NM and C-DM boluses were also perceived as more extensible than T-NM and T-DM boluses, which could be linked with the emulsion formed with saliva during cheese mastication. Nonetheless, it is important to consider that the highest G'_{LVR} and the low $Strain_{LVR}$ of T-DM bolus could be translated in an increased brittleness, which could favour the preparation of structurally inhomogeneous bolus at the swallowing point (Sharma et al., 2017). A scattered bolus might fail to pass as a cohesive mass trough the pharynx and could led to highly irregular flow rates during swallowing, subsequently rising the aspiration risk (Ishihara et al., 2011). Furthermore, the flow point provides information about the disruption of the gel-like structure, which make difficult the formation of a gel-like network between food particles and saliva (Loret et al., 2011). Non-significant ($p > 0.05$) differences were observed between T-NM and C-DM boluses. Conversely, T-DM bolus showed the highest flow point, whereas the lowest value was reported for C-NM bolus. These results could be explained by the water content of samples. Gibouin et al. (2022) demonstrated that the stress needed to make the bolus flowable (G' = G'') was mainly influenced by water in extruded cereals foods.

Table 3 summarises the viscoelastic parameters of the different boluses obtained from the SAOS test at 37 °C. The viscoelastic parameters were reported at 1 Hz to make a better comparison of the data. All *in vivo* boluses displayed a dominant elastic behaviour (G' > G'') that is distinctive of weak viscoelastic systems (Ribes et al., 2022), as above-mentioned. This behaviour was also reported by Tobin et al. (2020) when investigating the bolus rheology and easy-swallowing of particulated foods. Besides, the complex modulus (G*) could be employed as a measure of the rigidity and stiffness of the samples, while the complex viscosity (η*) determines the resistance of samples to flow, relative to the angular frequency (Mezger, 2006; Ribes et al., 2021). Significant ($p < 0.05$) differences were observed among boluses in relation to G* and η* values. T-DM bolus exhibited the highest G* and η* values, whereas the lowest values were recorded in the case of C-NM bolus. It is also important to indicate that both DM boluses showed higher G* and η* values than NM boluses, which could be ascribed to the lower number of chews, saliva uptake, and hardness values reported (section 3.1 and 3.3). Differences in bolus rheology may come from variations in the mechanical properties of food particles and their capacity to absorb saliva, which potentially undergo hydration, swelling, and/or dissolution (Witt & Stokes, 2015). Lastly, all *in vivo* boluses showed Tan δ values < 1, denoting the prevalence of the elastic properties. This parameter can be employed as a rheological indicator of easy-swallowable boluses. Ishihara et al. (2011) reported that Tan δ values between 0.1 and 1 can be utilised as rheological factor to detect easy-swallowable boluses but other parameters, such as G'_{LVR} and $Strain_{LVR}$, must be taken into consideration, as previously indicated.

Table 3
Viscoelastic parameters of turkey cold meat and fresh cheese bolus from SAOS test at 1 Hz and 37 °C. Mean values (n = 3) ± SD.

Sample	G' (Pa)	G'' (Pa)	G* (Pa)	η* (Pa·s)	Tan δ
T-NM	4228 ± 11^{b}	856 ± 16^{b}	4314 ± 14^{b}	686 ± 2^{b}	0.200 ± 0.005^{a}
T-DM	8036 ± 852^{c}	1526 ± 150^{c}	8179 ± 865^{c}	1282 ± 142^{c}	0.190 ± 0.002^{a}
C-NM	1731 ± 167^{a}	533 ± 49^{a}	1811 ± 174^{a}	288 ± 28^{a}	0.308 ± 0.004^{b}
C-DM	2078 ± 434^{a}	647 ± 117^{ab}	2176 ± 449^{a}	346 ± 71^{a}	0.312 ± 0.010^{b}

Viscoelastic parameters form the SAOS test: elastic modulus (G'), viscous modulus (G''), complex modulus (G*), complex viscosity (η*), and loss tangent (Tan δ).
T-NM: Turkey-Normal Masticated; T-DM: Turkey-Deficient Masticated; C-NM: Cheese-Normal Masticated; C-DM: Cheese-Deficient Masticated.
Different superscripts indicate significant differences among boluses ($p < 0.05$).

3.5. Protein digestibility of samples

Protein is an essential nutrient in adults' and elders' diet and its digestion involves mechanical breakdown of solid matrices, pH variations, pH-dependent protease-catalysed hydrolysis, transit across the gastrointestinal tract, and absorption (Baugreet et al., 2019; Rivera del Rio et al., 2022). Proteolysis starts in the stomach due to the pepsin and HCl action. The acidic environment provokes proteins to unfold and uncoil as a result of hydrogen and electrostatic bonds, which enables the pepsin activity for breaking down proteins in peptides and amino acids (Gropper & Smith, 2013).

In this work, the protein digestibility of T and C samples was studied by determining the total soluble proteins content, TCA-soluble peptides, and free amino groups after the different digestion phases (Fig. 5). For T sample, a greater total soluble proteins content was reported as digestion advanced. Moreover, significant ($p < 0.05$) differences were observed among digestion models when mimicking the alterations frequently noted in the elderly (Fig. 5 A). After the oral phase, the total soluble proteins content was lower in NM-ND model than in DM-ND and DM-ED models. However, mastication did not exert a significant ($p < 0.05$) impact on the total soluble proteins content of NM-ND and DM-ND models during digestion, which could be explained by the particle size reduction suffered by food boluses as digestion progressed. In this sense, Zou et al. (2018) observed similar particle size distribution curves after *in vitro* gastric and intestinal digestion of boluses presenting different initial particle sizes. It is also important to mention that protein hydrolysis was not expected at this stage, suggesting the aggregation or precipitation of proteins owing to interaction with salivary α-amylase (Crosara et al., 2018). After the gastric phase, significantly ($p < 0.05$) lower amounts of total soluble proteins were noted in the DM-ED model, which could be explained by pH changes from 3 to 6 along with the lower pepsin activity employed (1500 U/mL). Upon completion of the intestinal phase, DM-ED model exhibited the lowest total soluble proteins content (Fig. 5 A), probably due to the reduction of the pancreatin activity (50 U/mL), and concentration of bile salts (5 mM) employed. Noteworthy that a reduction in the pancreatic activity has been linked to poor digestion processes and, in turn, to protein malabsorption that can lead to nutritional deficiencies (Rémond et al., 2015).

In the case of C sample, the total soluble proteins content increased at the end of the gastric phase but lowered again after the intestinal phase (Fig. 5 B). This behaviour could be attributed to the greater water content and lower compact structure of C sample compared to T sample, which could favour the enzyme diffusion in the gastric compartment (Gunasekaran & Ak, 2003). A similar trend was reported by Asensio-Grau et al. (2019) while investigating the *in vitro* digestion of different types of cheese. When simulating the digestive alterations typically noted in the elderly, non-significant ($p > 0.05$) differences were recorded among digestion models after the oral stage (Fig. 5 B). Conversely, at the end of gastric and intestinal phases, DM-ED model exhibited a significantly ($p < 0.05$) lower total soluble proteins content. This can also be explained by pH modifications (pH 6 instead of pH 3), as well as the low pepsin activity (1500 U/mL), pancreatin activity (50 U/mL), and concentration of bile salts (5 mM) used.

Concerning the TCA-soluble peptides, higher values were detected as digestion of samples progressed (Fig. 5 C and D). For T samples, the greatest content of soluble peptides was observed at the end of digestion, given that the fraction of proteins soluble in TCA 5% would be composed

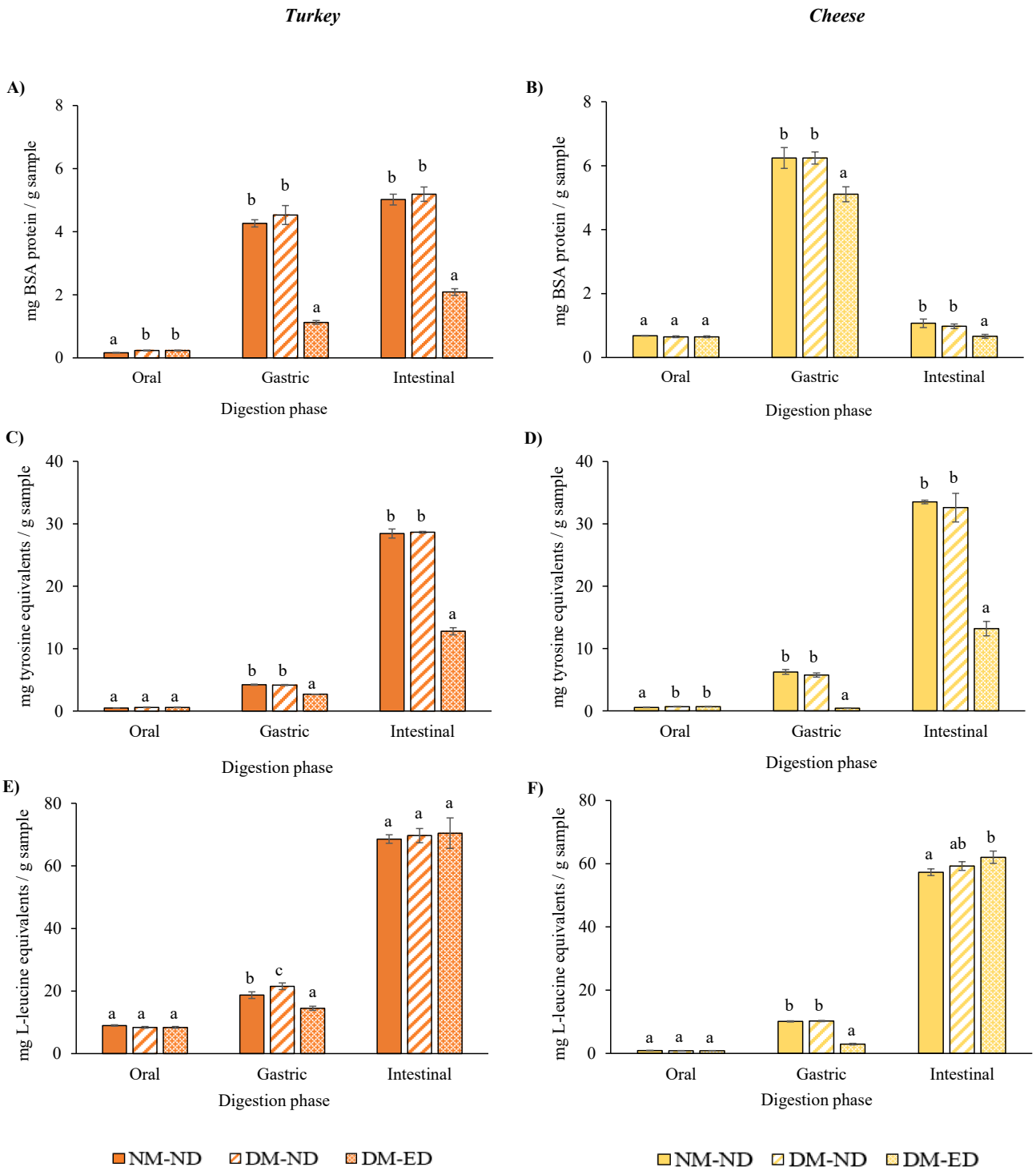

Fig. 5. Protein digestibility of turkey cold meat (orange colour) and fresh cheese (yellow colour) during oral and gastrointestinal digestion tests evaluated as the contents of soluble proteins (A-B), soluble peptides (C-D), and free amino groups (E-F). Mean values (n = 3) ± SD. NM-ND: Normal Masticated-Normal Digested; DM-ND: Deficient Masticated-Normal Digested; DM-ED: Deficient Masticated-Elderly Digested. Different superscripts indicate significant differences among digestion models ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of small peptides (<10 amino acid residues) and free amino acids (Chen et al., 2010). At the end of the oral phase, non-significant ($p < 0.05$) differences were observed among digestion models (Fig. 5 C). Conversely, the content of soluble peptides was drastically reduced at the end of the gastric and intestinal phases when using the DM-ED model. As previously indicated, this outcome could be explained by the lower acidification of the simulated fluid, enzymes activity (pepsin and pancreatin), and concentration of bile salts employed. Similar results on the proteolysis extent of meat products were reported by Hernández-Olivas et al. (2022) when mimicking the elderly digestive conditions tested in the present work.

Following with C sample, it is important to mention that slightly higher amounts of soluble peptides were recorded as digestion advanced in comparison with T sample (Fig. 5 D), probably due to its lower

compact/rigid structure and high susceptibility of caseins toward pancreatic proteases (Egger et al., 2021). Besides, significant ($p < 0.05$) differences were noted among digestion models when simulating the main oral and gastrointestinal alterations of seniors. After the oral phase, NM-ND model presented the lowest content of soluble peptides; but mastication did not significantly ($p > 0.05$) impact on the soluble peptides content of NM-ND and DM-ND models during digestion. As above-mentioned, it could be attributed to the particle size reduction suffered by food boluses as digestion advanced. After the gastric and intestinal phases, significantly ($p < 0.05$) lower amounts of soluble peptides were reported when mimicking the DM-ED model owing to the reduced acidification, enzymes activity, and concentration of bile salts used (Fig. 5 D). These results fall in line with those observed by Hernández-Olivas et al. (2020) when studying the consequences of the elderly digestive conditions on the protein digestibility of different dairy products.

In relation to the free amino groups' content, greater values were reported in T and C samples through digestion (Fig. 5 E and F). For T sample, non-significant ($p > 0.05$) differences were noted among digestion models at the end of the oral phase. Conversely, after the gastric phase, the DM-ED model showed the lowest content of free amino groups because of the low acidity of the simulated fluid used, as well as the reduced pepsin activity (1500 U/mL) (Hernández-Olivas et al., 2022). Non-significant ($p > 0.05$) differences were observed among models upon completion of digestion. Despite the drastic digestive conditions employed in DM-ED model, similar amounts of free amino groups were reported after the intestinal phase (Fig. 5 E). It can be ascribed to the duration of this phase (4 h instead of 2 h), which could favour the action of the intestinal enzymes for breaking down small peptides in free amino acids.

Similarly, mastication did not directly impact on the free amino groups' content of C sample, but significant ($p < 0.05$) differences were observed among models after the gastric and intestinal phases (Fig. 5 F). The lowest amount of free amino groups reported in DM-ED model at the end of the gastric phase could be explained by the lower enzymatic activity and acidity employed, as previously indicated. Finally, after the intestinal phase, the slightly greater content of free amino groups observed while simulating the DM-ED model could be attributed to the extension of the intestinal phase (4 h). The greatest duration of this phase and the complete disintegration of C sample at the end of digestion would facilitate the enzyme diffusion (Žolnere et al., 2019), releasing higher contents of free amino groups. In this sense, Egger et al. (2021) pointed out a rapid and important increase in the content of free amino acids and free R-NH_2 of cheese up to 3 h of intestinal dynamic digestion.

4. Conclusions

The results of this work evidenced that the oral processing behaviour and level of food breakdown attained during mastication played an important role in the granulometric, texture, and viscoelastic properties of turkey cold meat and fresh cheese boluses. Additionally, gastrointestinal alterations appearing with ageing negatively impacted on the content of total soluble proteins and TCA-soluble peptides of both samples. The present study broadens knowledge of food oral processing behaviour, bolus properties, and protein digestibility by simulating different digestive conditions, which is crucial to develop new products with improved functionalities that cover the specific necessities of several populations, such as healthy adults or the elderly. However, further studies investigating the impact of mastication, boluses features, and several digestive conditions on the nutrient bioaccessibility of a huge variety of foods are needed.

Declaration of Competing Interest

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

Data availability

The authors do not have permission to share data.

Acknowledgements

Susana Ribes gratefully acknowledges the postdoctoral grant (APOSTD/2020/264) from *Generalitat Valenciana* (Spain). Milagros Arnal gratefully thanks the Predoctoral contract (FPU19/02401) from the *Spanish Ministry of Science, Innovation, and Universities*. Funding for open access charge: CRUE-Universitat Politècnica de València.

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