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Behaviour of texture-modified meats using proteolytic enzymes during gastrointestinal digestion simulating elderly alterations

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ABSTRACT

This study aimed to apply different proteolytic enzymes (bromelain, papain, and flavourzyme) to develop texture-modified meats suitable for people with chewing or swallowing problems. The samples were categorised at level 6 (soft and bite-sized food) of the dysphagia diet, characterised in terms of physicochemical and textural parameters, and evaluated for their behaviour during gastrointestinal digestion simulating elderly alterations. In general, the enzyme-treated samples had lower moisture content, weight, and diameter of the piece of meat, and presented colour differences compared to the control samples. Textural analyses did not show significant differences in terms of hardness and cohesiveness for the texture-modified meats, while flavourzyme-treated samples presented less elasticity. Instrumental mastication assay showed the breakdown of samples' structure mainly during the first mastication cycles, with flavourzyme-treated samples presenting slightly higher consistency. The protein digestibility of the meats greatly increased after simulated gastrointestinal digestion, but a decrease in proteolysis for the control and papain-treated samples in the altered gastric model and an increase for flavourzyme-treated samples in the altered both gastric and intestinal model were shown compared to standard conditions. These results allow integrating knowledge to design foods that better meet the requirements of dysphagics or elderly people.

1. Introduction

Elderly people are a growing segment of the world population due to increased life expectancy and decreased mortality. In fact, it is expected that people over 65 years old will rise from 10% in 2022 to 16% in 2050, reaching 1.6 billion (United Nations, 2022). Consequently, there are global challenges related to the well-being of the elderly in terms of lifestyle and nutritional aspects, since they may experience chewing or swallowing problems due to anatomical and physiological dysfunctions such as dysphagia (difficulty in swallowing safely), dysmasesis (difficulty in masticating), xerostomia (limited salivation), loss of appetite and sensory perception, as well as osteoporosis (loss of bone mass and strength), sarcopenia (decreased skeletal muscle mass), and altered gastrointestinal conditions. In this regard, texture-modified foods (TMF) are developed to achieve a safe and efficient food intake in the elderly, although they should also meet nutritional requirements and provide a pleasant sensory perception for consumer acceptance (Gallego, Barat, Grau, & Talens, 2022; Lutz, Petzold, & Albala, 2019).

Meat is a good source of bioavailable protein and can help to boost muscle protein synthesis and delay sarcopenia, making it a valuable food in the meals of elderly consumers. However, it can present chewing difficulties when eaten as steaks, so developing products that require reduced mastication effort is a particularly interesting strategy (Botinestean, Hossain, Mullen, Kerry, & Hamill, 2021). The application of proteolytic enzymes is commonly used for meat tenderisation, as they have shown effects on meat fibre integrity and structure by hydrolysing myofibrillar proteins and collagen (Gagaoua et al., 2021). Fruit-derived enzymes such as bromelain and papain were applied by permeation method to prepare chicken meat for people with difficulties in mastication, obtaining a softened meat while retaining the fibrous texture of chicken (Takei, Hayashi, Umene, Kobayashi, & Masunaga, 2016). Also, the injection of several commercial enzymes (alcalase, neutrase, flavourzyme, protamex, collupulin, alphalase, and bromelain) was used to soften the texture of chicken and beef, with bromelain and collupulin having the highest effect (Eom, Lee, Chun, Kim, & Park, 2015). The use of enzymatic treatments can also help ensure better digestibility of muscle proteins. In fact, the composition, physicochemical and textural characteristics of food as well as the source, structure, solubility, and

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amino acid composition of proteins affect their digestibility in the gastrointestinal tract and, therefore, their nutritional quality (Gallego et al., 2022; Golding, 2019; Picariello et al., 2023). The alteration and deterioration of certain gastrointestinal parameters in the elderly, such as the secretion of enzymes and digestive fluids, pH, peristaltic movements, and transit times, should be considered as they can lead to maldigestion and malabsorption of nutrients (Hernández-Olivas, Muñoz-Pina, García-Hernández, Andrés, & Heredia, 2022; Shani-Levi et al., 2017). Thus, further understanding of the digestive fate of food in the elderly would facilitate the design of products adapted to their physiological capacities, improving the bioaccessibility and bioavailability of nutrients and helping to combat malnutrition in this population group (Rémond et al., 2015).

Several studies have evaluated the impact of elderly gastrointestinal alterations on protein digestion of different meats such as beef, pork, chicken or turkey (Hernández-Olivas et al., 2022; Wang et al., 2022), but information on the digestibility of texture-modified meat products is scarce. The deterioration of chewing and/or swallowing in the elderly, and the resulting adverse impact on their food consumption decisions, make it essential to evaluate the potential options to develop texture-modified meats that can be consumed by this population segment, considering both oral processing and gastrointestinal digestion for a beneficial impact on their health status. In this context, the aim of this study was the application of different proteolytic enzymes (bromelain, papain, and flavourzyme) to obtain texture-modified meats suitable for people with chewing or swallowing problems such as the elderly. Samples were characterised in terms of physicochemical and textural parameters, and evaluated for their behaviour during gastrointestinal digestion simulating elderly alterations.

2. Materials and methods

2.1. Materials and reagents

Fresh pork loin was purchased from a local supermarket (Valencia, Spain). Proteolytic enzymes used were bromelain 80 and papain 30,000 from Cygyc Biocon, S.L. (Les Franqueses del Vallés, Barcelona, Spain), which were extracted from pineapple and papaya, respectively, as well as Flavourzyme® 1000 L that was the blend of endo- and exopeptidases produced by *Aspergillus oryzae*, which was kindly supplied by Novozymes A/S (Bagsværd, Denmark).

For simulating gastrointestinal digestion, α-amylase type VI-B from porcine stomach (A3176), pepsin from porcine gastric mucosa (P7012), pancreatin from porcine pancreas (P7545), and bile extract porcine (B8631) were purchased from Sigma-Aldrich, Co. (St. Louis, MO, USA).

2.2. Preparation of meat samples

Meat samples were prepared from fresh pork loin pieces (*Longissimus thoracis et lumborum* muscle; 3–5 days post-slaughter) ($n = 6$) by removing the external fat and connective tissue, and then cutting into medallions 1.5 cm thick and 5 cm in diameter. Meat medallions were immersed, individually, in solutions of the commercial enzymes bromelain, papain, and flavourzyme, each of them prepared to reach 6000 U/g to obtain similar hardness values. Samples were incubated at 50 °C in an oven for 24 h to be softened by the action of proteases, and finally heated at 95 °C for 3 min for enzyme inactivation. Control samples were subjected to the same conditions but immersed in distilled water.

2.3. Characterisation of meat samples

Texture-modified meats ($n = 3$) were subjected to the IDDSI tests for their classification in the dysphagia diet framework. Moreover, meat samples ($n = 6$) were analysed for their physicochemical characteristics (moisture content, weight loss, diameter reduction, pH, and colour) as well as for textural parameters.

2.3.1. IDDSI testing methods

Different testing methods were carried out to categorise the texture-modified meat samples within the IDDSI framework (IDDSI, 2019). Spoon tilt test, fork drip test, and fork pressure test were performed for levels 4 and 5, while fork separation test, fork pressure test, and spoon pressure test were employed for levels 6 and 7. When testing the level 6, 15 × 15 mm cubes were cut from the meat medallions.

2.3.2. Physicochemical parameters

The moisture content was determined according to the official AOAC method 950.46 (AOAC, 2005) and calculated by Eq. (1), where W_1 is the weight of the empty crucible, W_2 is the weight of the wet sample in the crucible, and W_3 is the weight of the dry sample in the crucible.

$$\text{Moisture}\ (\%) = (W_2 – W_3)/(W_2 – W_1)\ \text{x}\ 100 \quad (1)$$

The weight loss of each sample was calculated by the difference between the initial weight (raw meat) and the final weight (enzyme-treated meat) according to Eq. (2):

$$\text{Weight loss}\ (\%) = [(\text{initial weight} – \text{final weight})/\text{initial weight}]\ \text{x}\ 100 \quad (2)$$

The diameter reduction of each sample was calculated by the difference between the initial diameter (raw meat) and the final diameter (enzyme-treated meat) according to Eq. (3):

$$\text{Diameter reduction}\ (\%) = [(\text{initial diameter} – \text{final diameter})/\text{initial diameter}]\ \text{x}\ 100 \quad (3)$$

The pH was measured in the meats obtained after incubation and enzyme inactivation using a pH-meter (Basic 20+, Crison Instruments, S.A., Barcelona, Spain), which was calibrated with 4.01 and 7.00 buffers at 22 °C. For that, 1 g of each sample was homogenised with 20 mL of water at 3000 rpm for 5 s using an Ultra-Turrax® T-25 (IKA®-Werke, Staufen, Germany), and then the pH was measured at room temperature.

The colour was determined by using a Minolta CM-700d spectrophotometer (Konica Minolta Sensing, Inc., Osaka, Japan) with an 8-degree viewing angle geometry and 8 mm diameter illumination area as optical system. Samples were placed on a white standard plate, and the standard observer 10 and standard light source D65 were used to obtain colour coordinates L^*, a^*, and b^* in the CIELab space. Chroma (C^*), hue (h^*), and colour differences (ΔE^*) with respect to the control samples were calculated, respectively, by Eqs. (4), (5), and (6).

$$C^* = \left(a^{*2} + b^{*2}\right)^{1/2} \quad (4)$$

$$h^* = \text{arctg}(b^*/a^*) \quad (5)$$

$$\Delta E^* = \left((\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta L^*)^2\right)^{1/2} \quad (6)$$

2.3.3. Textural parameters

The textural properties of the samples were measured with a TA-XT. plus texture analyser (Stable Microsystems Ltd., Godalming, UK). A texture profile analysis (TPA) was performed with a cylindrical probe (75 mm diameter; Stable Microsystems Ltd., Godalming, UK) in 8 meat medallions of each treatment. Each sample was compressed to 80% in a double cycle at a rate of 1 mm/s, and several texture profile parameters, including hardness, adhesiveness, elasticity, cohesiveness, and chewiness, were calculated based on the force/time data collected during the test. In addition, a punch test was performed using a stainless-steel cylindrical probe (5 mm diameter; Stable Microsystems Ltd., Godalming, UK). Each sample was compressed to 80% of its height at a speed of 2 mm/s, taking 5 measurements per sample in 8 meat medallions of each treatment. The average values of hardness and positive area were obtained.

2.4. Instrumental mastication assay

The assay was used to mimic oral processing according to the method described by Chung, Degner, and McClements (2012) with some modifications. For that, a rotational Kinexus Pro+ Rheometer (Malvern Instruments Ltd., MA, USA) was used, equipped with a PLC61/PU40 parallel-plate geometry with a Peltier system to control the temperature at 37 °C, and rSpace software for data processing. Samples ($n = 6$) were subjected to 10 compression (up to 25% of their height) and decompression cycles in order to simulate the upward/downward movement of the tongue against the palate.

2.5. In vitro gastrointestinal digestion

Three simulated models were employed to evaluate the effect of different GID alterations possibly given in the elderly on the protein digestibility of meat samples. In duplicate, the standard gastrointestinal digestion (GID) was simulated according to the INFOGEST protocol (Minekus et al., 2014) whereas the altered GID conditions were established according to Denis et al. (2016) and Shani-Levi et al. (2017). Samples were mixed during the process at 40 rpm and 37 °C using a rotary mixer (Intell-MixerTM RM-2, ELMI Ltd., Riga, Latvia) within an incubator chamber (JP Selecta, S.A., Barcelona, Spain). Samples before digestion (S_0) were obtained by mixing the sample with water (1:1, *w/v*).

For standard GID conditions (S_{gi}), the oral phase (pH 7) was simulated by mixing each sample (1:1 w/v) with simulated salivary fluid and α-amylase (75 U/ mL) for 2 min. In the gastric phase (pH 3), the oral bolus was mixed (1:1, *v*/v) with simulated gastric fluid and pepsin (2000 U/mL) and incubated for 2 h. In the intestinal phase (pH 7), the gastric chyme was mixed (1:1, v/v) with simulated intestinal fluid, pancreatin (100 U/mL trypsin activity), and bile (10 mM) for 2 h.

For simulating GID alterations appearing with aging, the elderly model with altered gastric phase (A_g) was performed at pH 6 and the pepsin activity was reduced to 1500 U/mL. In the elderly model with altered both gastric and intestinal phases (A_{gi}), in addition to the conditions of A_g, pancreatin activity and bile concentration were reduced to 50 U/mL and 5 mM, respectively, and intestinal incubation was maintained for 4 h.

After the different GID models, samples were subjected to heat shock (98 °C, 5 min) for stopping enzymatic reactions and then cooled in an ice bath, centrifuged (8000 *g*, 4 °C, 10 min), and stored at −20 °C for subsequent analysis.

2.6. Protein digestibility

The protein digestibility of the samples before and during GID conditions was determined as described by Gallego, Arnal, Barat, and Talens (2021). For that, the contents of total soluble proteins (assayed by the Bradford assay), peptides soluble in 5% TCA, and free amino groups (assayed by the TNBS method) were evaluated. Measurements were done in triplicate, and the results were expressed as mg/g of sample.

2.7. Statistical analysis

Statistical analysis was performed using the Statgraphics Centurion 18 software (Statgraphics Technologies, Inc., The Plains, VA, USA). The data were expressed as the mean of replicates ± standard error (SE), and one-way analysis of variance (ANOVA) model and Tukey's honest significance test were used to compare means obtained from the measured physicochemical and texture parameters, instrumental mastication assay, and protein digestibility. Treatments (control and different enzyme-treated meats) were included as fixed effect and the replicates as random effect. Differences were considered statistically significant at $P < 0.05$.

3. Results and discussion

3.1. IDDSI tests on texture-modified meats

The texture-modified meat samples were subjected to the IDDSI methods including the spoon tilt test, fork drip test, fork and spoon pressure tests, and fork separation test (Table 1). The samples failed to meet the requirements for levels 4 and 5 of the IDDSI framework. The fork pressure test confirmed that the three enzyme-treated meats would be categorised at level 6 (soft and bite-sized food) of dysphagia diet. The compression of the samples with the base of a fork made the thumb nail blanch noticeably to white, which corresponds to a typical tongue force used during swallowing (around 17 kPa) (Cichero et al., 2017). Moreover, the samples squashed and did not return to their original shape when the pressure was released, and they were mashed/broken down with the pressure from fork or spoon. So, samples would be soft, tender and moist throughout but with no separate thin liquid, would not require biting but chewing, and tongue force would be needed to move the bolus for swallowing (IDDSI, 2019). Thus, the IDDSI level 6 is considered safe for dysphagic people who do not have a sufficiently strong biting ability to cut a piece of food by clamping teeth but require their chewing ability to crush the food by repeatedly opening and closing the jaws. It should also be mentioned that the fork and spoon pressure tests were introduced by IDDSI to easily measure food hardness for level 6 in aged care facilities and clinical settings, as the use of texture analysers in practical scenarios requires specialised trained personnel and high analysis time (Pematilleke, Kaur, Wai, Adhikari, & Torley, 2021).

3.2. Physicochemical characteristics of meat samples

Meat samples (control and samples treated with bromelain, papain, and flavourzyme) were characterised in terms of different physicochemical parameters, and the obtained results are shown in Table 2.

The moisture content of the meat samples ranged from 71.7 to 67.6%, with the highest values for the control and flavouzyme-treated samples. Regarding the weight loss and diameter reduction of meat medallions in comparison to raw samples, the control samples showed values of 30. 2 ± 0.6% weight loss and 15.0 ± 2.4% diameter reduction while enzymatic treatments led to an important increase in both parameters. In fact, the bromelain-treated samples reached values up to 70.4 ± 1.2% weight loss and 40.3 ± 1.9% diameter reduction. These results might be explained by the protein fragmentation caused by the enzymatic treatments as well as the myofibrillar shrinkage, protein denaturation, and water movement from the myofilaments into the extracellular region of the muscle during cooking. This would result in decreased moisture content and water holding capacity, hence greater weight loss and diameter reduction. (Botinestean et al., 2018; Maqsood, Manheem, Gani, & Abushelaibi, 2018).

The pH of meat products determines their stability to autolytic and microbial degradation as well as greatly influences on water holding capacity, tenderness, and juiciness of meat (Goli, Abi Nakhoul, Zakhia-Rozis, Trystram, & Bohuon, 2007). Meat samples presented pH values between 5.81 and 6.12, with lower values for the enzyme-treated samples than control although representing non-significant differences ($P > 0.05$). Treatment of beef meat with papain enzyme did not have significant effect on pH value of meat (Barekat & Soltanizadeh, 2019), whereas a reduction in pH was observed in different muscle foods such as beef, chicken, squid, and pork hydrolysed with bromelain enzyme compared to non-treated samples, observing lower pH as the enzyme concentration increased (Ketnawa & Rawdkuen, 2011; Saengsuk et al., 2021). Enzymes would cleave the peptide bonds of meat proteins into small peptides and free amino acids, releasing carboxyl and amino groups that are in their dissociated or protonated forms depending on the medium pH. Thus, slight changes in pH values may be attributed to the effect of the enzymes on the ionic strength of the meat (Abdel-Naeem

Table 1
Categorisation of the texture-modified meats within the IDDSI framework by performing the different testing methods for levels 4–7.

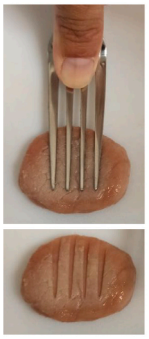
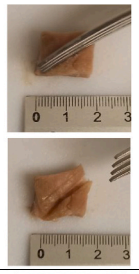
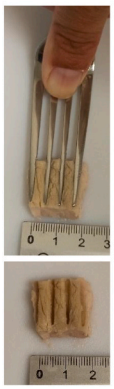
LEVELS 4/5			
	Bromelain	**Papain**	**Flavourzyme**
Spoon tilt test			
Fork drip test			
Fork pressure test			
LEVELS 6/7			
	Bromelain	**Papain**	**Flavourzyme**
Fork separation test			
Fork pressure test			
Spoon pressure test			

Table 2
Physicochemical parameters of the meat samples.

Parameter		Samples			
		Control	Bromelain	Papain	Flavourzyme
Moisture content (%)		71.6 ± 0.6^{a}	67.6 ± 0.3^{b}	69.2 ± 0.5^{b}	70.9 ± 0.4^{a}
Weight loss (%)		30.2 ± 0.6^{b}	70.4 ± 1.2^{a}	60.7 ± 1.2^{a}	64.1 ± 0.9^{a}
Diameter reduction (%)		15.0 ± 2.4^{c}	40.3 ± 1.9^{a}	26.7 ± 2.2^{b}	33.3 ± 3.2^{ab}
pH		6.12 ± 0.09^{a}	5.81 ± 0.02^{a}	5.92 ± 0.04^{a}	6.08 ± 0.13^{a}
Colour	L^*	73.1 ± 0.4^{a}	65.3 ± 0.4^{b}	65.4 ± 0.3^{b}	54.4 ± 0.4^{c}
	C^*	13.5 ± 0.1^{b}	14.0 ± 0.2^{b}	14.4 ± 0.1^{b}	16.1 ± 0.6^{a}
	h^*	84.1 ± 0.5^{a}	73.8 ± 1.2^{c}	78.3 ± 0.7^{b}	63.2 ± 0.6^{d}
	ΔE^*	–	8.4 ± 0.4^{b}	8.0 ± 0.3^{b}	19.9 ± 0.4^{a}

Results are expressed as means ± standard error. Different letters indicate significant differences among samples ($P < 0.05$).

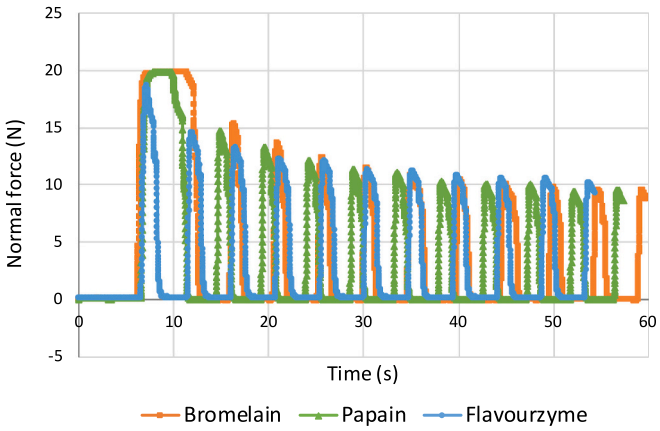


Fig. 1. Profile of the texture-modified meats during the 10 cycles of compression-decompression of the instrumental mastication assay.

& Mohamed, 2016; Wouters, Rombouts, Fierens, Brijs, & Delcour, 2016).

Colour determines consumers' acceptability, so it is a crucial parameter in food quality. The enzyme-treated samples showed lower L^* and h^* values than the control samples, which would be related to the reaction of enzymes on meat and metmyoglobin content. The samples treated with flavourzyme showed the lowest L^* and h^* values (54.4 ± 0.4 and 63.2 ± 0.6, respectively) and the highest C^* value (16.1 ± 0.6), thus they presented the highest ΔE^* (20.0 ± 0.4) with respect to the control. Moreover, the flavourzyme solution had a dark brown colour in comparison to the white to tan colour of the bromelain and papain solutions, which would also contribute to the high ΔE^* value in the first case.

3.3. Textural characteristics of meat samples

Food texture is an important factor to evaluate the suitability of foods for people with difficulties in chewing or swallowing, such as the elderly or dysphagia patients. Table 3 shows the textural parameters of the different meat samples obtained by the TPA test and punch test. TPA is a double compression test that mimics the biting motion during the early stage of oral processing, and it allows to measure multiple texture attributes in just one experiment (Pematilleke, Kaur, Adhikari, & Torley, 2021).). On the other hand, the punch test allows to measure in smaller areas of the sample and thus collects a greater variability within each one.

The TPA test showed a significant decrease in hardness in the enzyme-treated meats compared to control samples. Moreover, no significant differences ($P > 0.05$) were found between the three texture-modified meats, which fullfilled the purpose of the study that was to obtain similar hardness values regardless of the enzyme applied. Bromelain and papain enzymes are fruit-derived proteolytic enzymes with endoprotease action, so they hydrolyse proteins into peptides and disrupt the structure of fibers, showing a tenderising effect on meat (Botinestean et al., 2018; Gerelt, Ikeuchi, & Suzuki, 2000). Flavourzyme consists of a complex of fungal proteases, with both endoprotease and exopeptidase activities, which favour protein degradation into peptides and amino acids and thus soften meat texture (Zhang et al., 2017). This fact could lead to obtain the lowest hardness values in the flavourzyme-treated samples. Note that the TPA test only performed one compression cycle for control samples due to their high hardness, so the rest of parameters could not be calculated. The enzyme-treated samples showed low adhesiveness, with the highest values found for those treated with bromelain (−0.11 ± 0.02 N·s) and the lowest for flavourzyme (−0.04 ± 0.01 N·s). Although it is not the case, high values of this parameter could result in a serious problem for people with swallowing or chewing problems, since the meat would remain more attached to the palate, leading to possible choking problems. Regarding elasticity and chewiness, no significant differences ($P > 0.05$) were observed between bromelain- and papain-treated samples, whereas flavourzyme showed the lowest values (0.43 ± 0.02 and 64.2 ± 6.7 N, respectively). In terms of cohesiveness, the samples did not present significant differences ($P > 0.05$), although the highest values were found for bromelain-treated meats. Elasticity and cohesiveness attributes would be related to the strength of the internal bonds in the sample (Barekat & Soltanizadeh, 2017), so it is interesting to reduce their values when developing foods for people who require products with minimal chewing effort by compressing the tongue and palate and easy to swallow.

Like the TPA test, the punch test showed a significant decrease (3–4 times) in the hardness values for the enzyme-treated meats in comparison to the control, with no significant differences between the samples treated with bromelain, papain, and flavourzyme. Similar results were also found for the area values, which represents the area created in the compression cycle. The higher the values of both parameters, the greater the force to cut the meat during eating, which is detrimental for people with chewing or swallowing dysfunctions, such as the elderly.

Table 3
Texture parameters of the meat samples.

Parameter		Samples			
		Control	Bromelain	Papain	Flavourzyme
Texture Profile Analysis (TPA)	Hardness (N)	> 600^{a}	325 ± 17^{b}	309 ± 29^{b}	270 ± 18^{b}
	Adhesiveness (N·s)		−0.11 ± 0.02^{b}	−0.07 ± 0.01^{ab}	−0.04 ± 0.01^{a}
	Elasticity		0.69 ± 0.04^{a}	0.63 ± 0.03^{a}	0.43 ± 0.02^{b}
	Cohesiveness		0.57 ± 0.02^{a}	0.56 ± 0.02^{a}	0.56 ± 0.01^{a}
	Chewiness (N)		131 ± 19^{a}	111 ± 14^{ab}	64 ± 7^{b}
Punch test	Hardness (N)	36.03 ± 0.77^{a}	8.8 ± 0.5^{b}	14.9 ± 1.4^{b}	8.2 ± 0.4^{b}
	Area (N·s)	44.86 ± 0.99^{a}	12.8 ± 0.8^{b}	15.3 ± 6.9^{b}	11.4 ± 0.7^{b}

Results are expressed as means ± standard error. Different superscript letters indicate significant differences among samples ($P < 0.05$).

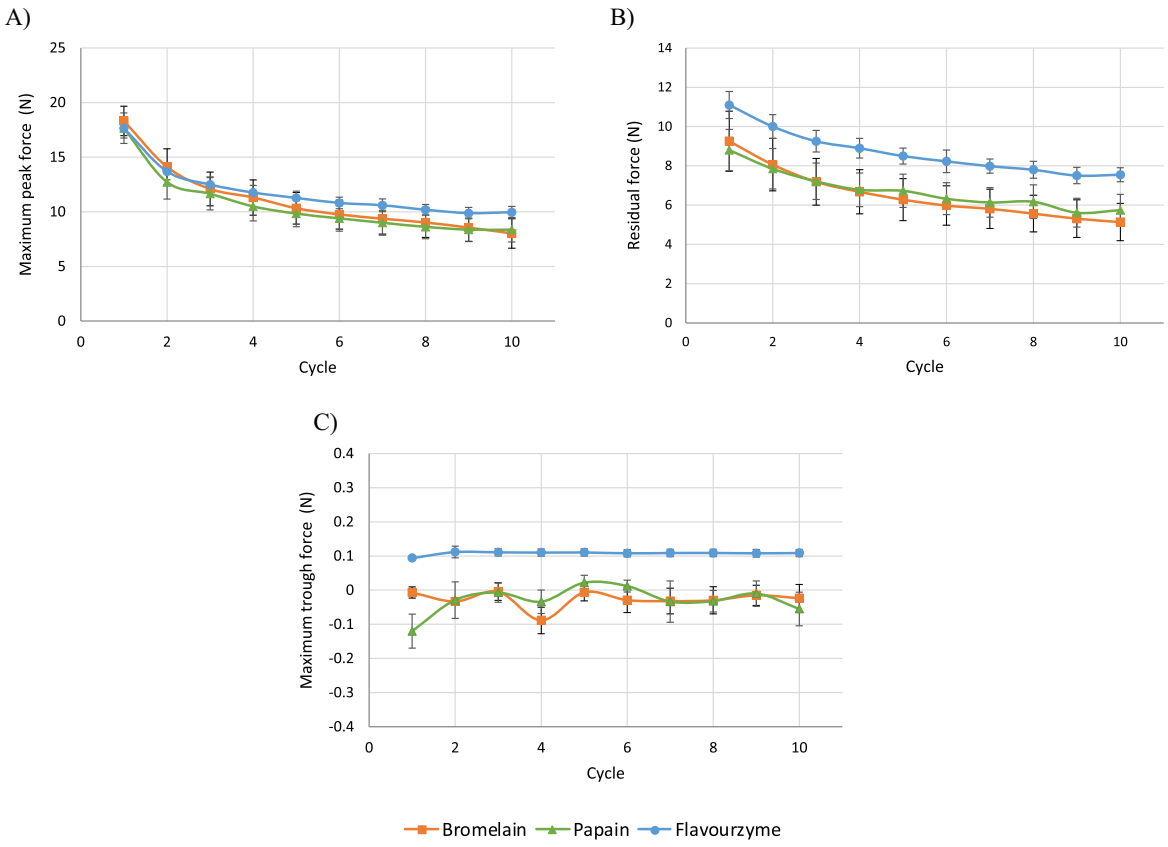


Fig. 2. Instrumental mastication assay of the texture-modified meats showing the values of A) maximum peak force during compression, B) residual force after compression, and C) maximum trough force during decompression during the successive compression-decompression cycles. Results are expressed as means ± standard error.

3.4. Instrumental mastication assay of texture-modified meats

The enzyme-treated meats were subjected to 10 compression-decompression cycles, showing differences in the mechanical responses of the samples when representing the profiles of the normal force *versus* time (Fig. 1). In addition, the changes in the average values of maximum peak force during compression, residual force after compression, and maximum trough force during decompression are shown in Fig. 2. These parameters are related to consistency, yield stress, and adhesiveness of the samples, respectively, thus they may relate to particular sensory responses during food consumption (Chung et al., 2012).

A decrease in the values of maximum peak force and residual force was observed in the texture-modified samples as the number of cycles increased, especially notable during the first cycles (Fig. 2A, B), suggesting the breakdown of the structure of the samples during mastication. No significant differences ($P > 0.05$) were observed between the three samples, but the flavourzyme-treated samples presented slightly higher consistency during the last masticatory cycles (Fig. 2A). Similarly, this sample presented the highest values of yield stress (Fig. 2B), indicating lesser structure breakdown due to shearing. Very low values of maximum trough force were observed for the three samples (Fig. 2C), which confirms their low adhesiveness, also shown through the TPA test.

3.5. Protein digestibility of meats under different elderly gastrointestinal conditions

The contents of total soluble proteins, peptides soluble in 5% TCA, and free amino groups were determined to evaluate the protein digestibility of meat samples under standard GID conditions and different GID alterations appearing with aging (Fig. 3).

Results of soluble proteins (Fig. 3A) showed values between 0.37 and 0.10 mg/g before digestion (S_0), with the highest values for the papain-treated samples. A sharp increase was observed in all the samples after the standard GID (S_{gi}), especially in the case of bromelain (>35 times). Digested bromelain- and flavourzyme-treated samples showed a higher protein content than control samples, although non-significant differences ($P > 0.05$) were found between them. However, in the GID model with altered gastric phase (A_g), bromelain- and papain-treated samples presented the highest values (4.47 and 3.97 mg/g, respectively), which in the case of papain was higher (83%) than that of the standard GID. After altered gastric and intestinal phases (A_{gi}), the values of all the samples decreased (between 1.6 and 3.2 times depending on the sample) in comparison to the A_g model, with the lowest values for the control and bromelain-treated meats. The products of gastric and intestinal proteolysis are small peptides and free amino acids that are not covered when measuring soluble proteins, so soluble peptides and free amino groups were also evaluated to obtain broader information on the protein digestibility of meats.

Peptides soluble in 5% TCA would include small peptides (< 10 residues) and free amino acids (Chen, Shih, Chiou, & Yu, 2010). As shown in Fig. 3B, flavourzyme-treated samples presented the highest values before digestion (6.86 mg/g), followed by bromelain (3.46 mg/g), papain (1.22 mg/g), and control samples (0.54 mg/g). Both endoprotease and exopeptidase activities of flavourzyme on meat, generating small peptides and free amino acids; the endoprotease activity of bromelain and papain enzymes, which hydrolyses proteins into peptides; and no enzymatic hydrolysis in control samples (except for endogenous muscle enzymes), would explain these results. The action of gastrointestinal enzymes led to a large increase in the content of soluble

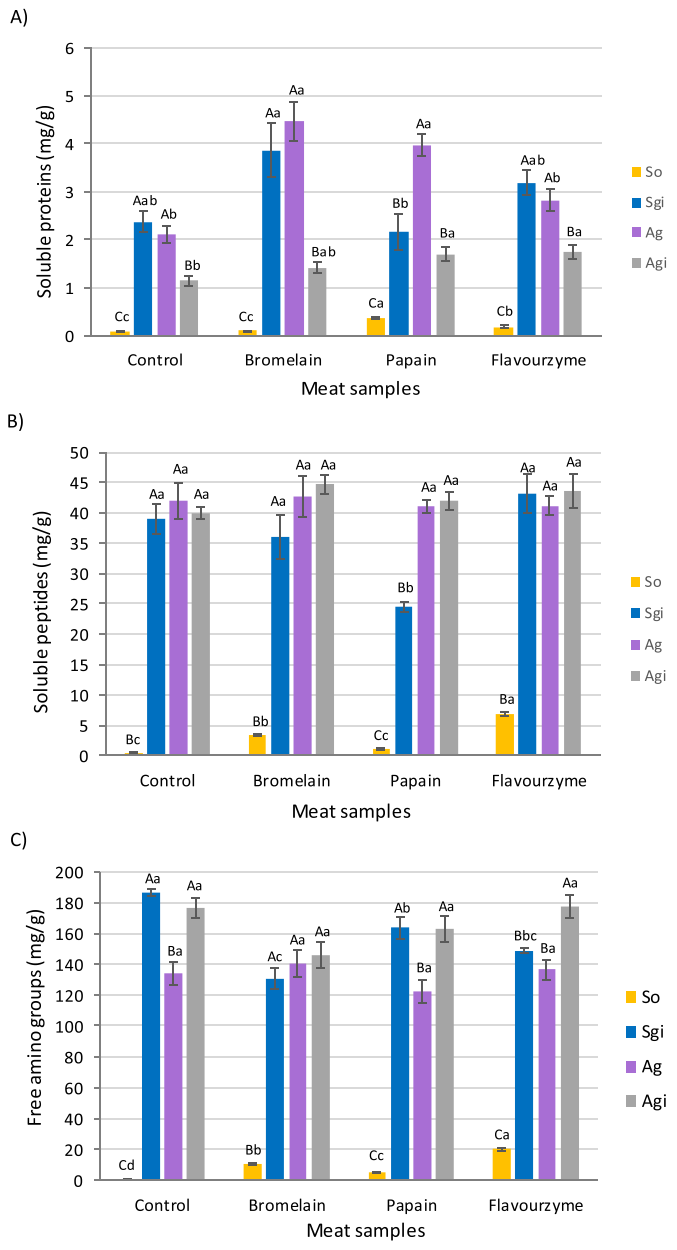


Fig. 3. Protein digestibility determined as the content of A) soluble proteins, B) soluble peptides, and C) free amino groups of the meat samples before digestion (S_0), after standard gastrointestinal digestion conditions (Sgi), and after altered elderly conditions at the gastric phase (A_g) and at both gastric and intestinal phases (A_{gi}). Results are expressed as means ± standard error. Capital letter indicates significant differences between digestion conditions (S_0, Sgi, A_g, and A_{gi}) within the same meat sample ($P < 0.05$), whereas lowercase letter indicates significant differences between meat samples within the same digestion condition ($P < 0.05$).

peptides in all the samples. Digested papain-treated samples showed the lowest content (24.55 mg/g) whereas non-significant differences ($P > 0.05$) were found between the other samples, with values between 36.09 and 43.17 mg/g. After the altered GID conditions, both A_g and A_{gi} models, the content of soluble peptides did not show significant differences ($P > 0.05$) with the standard model in any of the samples except for papain-treated samples, in which the values increased by around 70% in comparison to S_{gi}.

The content of free amino groups includes the available amino groups present in the hydrolysed proteins, as well as those in the peptides and amino acids generated, which is used to evaluate the extent of proteolysis (Adler-Nissen, 1979). A similar trend to the soluble peptides results was observed before GID, with flavourzyme-treated samples presenting the highest values (Fig. 3C). Control samples showed the greatest increase after standard GID, reaching the highest value (186.33 mg/g), whereas enzyme-treated samples presented between 130.61 and 163.84 mg/g of free amino groups. In the A_g model, the values of control and papain-treated samples decreased (around 26%) compared to the S_{gi} model, while increased (about 32%) in A_{gi}. Bromelain-treated samples did not present significant differences ($P > 0.05$) between the three models, whereas flavourzyme-treated samples after altered both gastric and intestinal digestion increased the content of free amino groups (around 1.2 times) with respect to S_{gi} and A_g.

The impact of elderly GID conditions on protein digestibility might depend on food matrix properties and structure, which determine the solubilisation, release, and hydrolysis of proteins. Before digestion, the softer texture and the presence of smaller proteins and peptides in the enzyme-treated samples compared to the control samples could maximize the protein surface contact, enabling a better accessibility of gastrointestinal enzymes to cleavage sites (Paz-Yépez, Peinado, Heredia, & Andrés, 2019). In fact, it has been suggested that ingestion of protein hydrolysates, as opposed to intact proteins, could accelerate protein digestion and absorption from the gut as well as increase the availability and incorporation rate of amino acids into skeletal muscle proteins (Koopman et al., 2009). However, this fact was not observed in the obtained results, since in general the digested control samples showed a greater increase in protein digestibility for any model than the enzyme-treated samples (Fig. 3). Protein digestion of cooked meats would be reduced depending on the secondary structure of proteins (transformation of secondary structure from α-helix to β-sheet), exposure of hydrophobic groups due to protein unfolding (increased protein cross-linking and aggregation) or disruption of digestive enzymes recognition with their cleavage sites (due to arginine and lysine oxidation) (Yin, Zhou, Pereira, Zhang, & Zhang, 2020). Moreover, interactions between proteins and aldehyde products formed during lipid oxidation or from reducing sugars by means of Schiff bases would also impact protein digestibility (Bax et al., 2012).

Furthermore, it should be noted that protein digestibility was evaluated at the end of GID after intestinal stage in any model. In fact, a low efficiency of pepsin in digesting cooked meat during the gastric phase has been reported (Bax et al., 2012), which could be due to cooking promotes protein aggregation and the stomach acidic medium is somewhat ineffective in opening the structure for protein solubilisation and enzymatic action (Bax et al., 2012; Luo, Boom, & Janssen, 2015). In the A_g model, although lower pepsin activity and higher pH in stomach occur, the similar results obtained between this and the standard model, mainly for the bromelain- and flavourzyme-treated samples (Fig. 3), would indicate that the activity of pancreatic proteases could compensate the suboptimal conditions of the gastric phase with the hydrolysis of proteins into peptides and free amino acids (Hernández-Olivas, Muñoz-Pina, Andrés, & Heredia, 2020). Similarly, no significant differences between the S_{gi} and A_{gi} models, mainly for the control and bromelain-treated samples (Fig. 3 B,C), could be explained because the longer intestinal transit time could compensate the suboptimal gastric and intestinal activities. Denis et al. (2016) used a dynamic gastrointestinal TIM model and mass spectrometry analysis to evaluate the influence of adult and elderly conditions on digestibility and bioaccessibility of cooked beef meats. Results of that study showed no impact of aging on meat protein digestion and only 6 proteins out of 46 identified, mainly from the cytosol, were differentially hydrolysed under the adult and elderly digestive conditions. In addition, Wang et al. (2022) showed that the digestion profiles of meat (chicken, beef, and pork) proteins were mainly affected by the altered gastric conditions of elderly individuals with achlorhydria, but the differences between altered and standard conditions were gradually reduced in the intestinal phase. Proteomics analyses showed that myofibrillar proteins were more degraded under control than altered conditions, probably because increasing pH might

lead to changes in protein structure and thus differences in digestibility. Different results were obtained by Hernández-Olivas et al. (2022) when evaluating the impact of elderly GI alterations on protein digestibility of different meats (chicken, turkey, pork, and beef). Altered intestinal conditions had the most significant negative effect on the digestibility of meat proteins compared to standard conditions, with the highest reduction observed for beef samples. In chicken, turkey, and pork meats, the highest values of protein digestibility were found after altered gastric conditions, which was explained because the pH of the digesta moved away from the isoelectric point of the proteins, resulting in enhanced electrostatic repulsion and thus, increased protein-water interactions and protein solubility.

4. Conclusions

The application of bromelain, papain, and flavourzyme enzymes allowed the development of texture-modified meats categorised at level 6 (soft and bite-sized food) of the dysphagia diet, and which presented lower moisture content, weight, and diameter, as well as colour differences compared to the controls. Enzyme-treated samples had similar hardness and cohesiveness, low adhesiveness, and their structural integrity was broken down mainly during the initial cycles of mastication. However, flavourzyme-treated samples presented the least elasticity, and slightly greater consistency during mastication. GID increased the protein digestibility of the meats but, in comparison to standard conditions, the proteolysis decreased for the control and papain-treated samples in the altered gastric model and increased for the samples treated with flavourzyme in the altered both gastric and intestinal model. These results allow integrating knowledge to design texture-modified foods that better meet the needs of people with chewing or swallowing problems such as dysphagics or the elderly, as well as to obtain information to establish appropriate dietary recommendations regarding meat consumption aimed at these specific population groups. Further works are needed in order to evaluate the sensory characteristics of the designed products as well as to elucidate their behaviour during oral processing and suitability for dysphagics or elderly people.

CRediT authorship contribution statement

Marta Gallego: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Supervision. **Raúl Grau:** Project administration, Funding acquisition. **Pau Talens:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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.

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