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Impact of common gastrointestinal disorders in elderly on *in vitro* meat protein digestibility and related properties

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Abstract

This study aimed to *in vitro* evaluate the impact of common gastrointestinal (GI) alterations appearing with aging on protein digestibility, and functional related properties, in four different meats (chicken, turkey, pork and beef). Thus, three elderly digestion models were stated as long as altered GI conditions affected at oral (E1), oral and gastric (E2) and oral, gastric and intestinal stages (E3). Healthy adult GI conditions were also mimicked as standard control model (C). A notable trichloroacetic acid (TCA) soluble protein and the free amino acids (FAA) release reduction ($p<0.05$) were found under intestinal suboptimal conditions (E3), being more accused in beef than in other meats. Thus, chicken intake would be more advisable, than other meats, in terms of protein digestibility; while beef would provide a greater net supply of FAA under E3 model conditions. Gastric altered conditions, seem to favor protein solubility. Finally, while gastric and intestinal suboptimal conditions diminish the angiotensin converting enzyme (ACE) inhibition of meat digesta, their antioxidant activity was only negatively affected by intestinal altered conditions.

Keywords: Meats; Gastrointestinal digestion; Aging; Proteolysis; ACE inhibition; Antioxidant activity

1. INTRODUCTION

It is estimated that by 2050 a huge amount of the global population (2.1 billion) will be > 60 years old (United Nations, 2019b). Also, an increased life span implies a corresponding increase in aging-related disorders such as cardiovascular associated-diseases, cancer, obesity or diabetes (Plante et al., 2020). The increased oxidative stress as well as abnormalities in inflammatory responses, seem to drive the main etiologies of these aging-related diseases (Chakrabarti et al., 2014). Thus, not only increasing life expectancy but also healthy ageing are of growing global concern (United Nations, 2019a). Several factors affect how people get older, the role of diet being widely stated. The European Society for Clinical Nutrition and Metabolism advises elders to increase the consumption of rich-protein foods (Volkert et al., 2018), and especially those rich in essential amino acids such as leucine or tryptophan (Morley, 2016). Meat is one of the major protein sources providing all the body's essential amino acids, but it is also rich in some relevant micronutrients such as iron, zinc, selenium, and vitamins B6 and B12. Meat and its derivates generally provide high-quality protein with digestible indispensable amino acid scores (DIAAS) >100 regardless of processing (Bailey et al., 2020).

Nevertheless, the nutritional quality of proteins is also determined by its digestibility in the gastrointestinal tract, i.e. its protein digestion rate, short-chain peptides and amino acids bioavailability and functionality (Bax et al., 2012). However, a decline of certain gastrointestinal (GI) functions (i.e. reduction or alteration of enzyme secretions, luminal electrolyte composition, motility and bile secretion, among others) could lead to macronutrient maldigestion and malabsorption, among which sarcopenia or protein deficit, stands out (Shani-Levi et al., 2017). Besides, the poor oral health of elderlies can lead to inefficient mastication and the formation of oral boluses with bigger particles, which in the worst case can difficult swallowing and further digestion (Mioche et al.,

2004). The end-digestion products of proteins, mainly peptides, may have the ability to exert antihypertensive, antioxidant, antimicrobial, opioid, immunomodulatory and antithrombotic activities (Ding et al., 2018; López-Expósito et al., 2007; Toldrá et al., 2018). However, peptides bioactive effect keeps latent until they would be motivated by the GI digestion or food processing, i.e., drying, curing, fermentation and enzymatic hydrolysis (Xing et al., 2019). Within the functional properties of bioactive peptides, the antihypertensive activity is assessed by the Angiotensin I-converting enzyme (ACE), a trans membrane peptidase, which is a key enzyme influencing the regulation of blood pressure (Xing et al., 2019). The antioxidant potential of peptides is dependent on their size as well as on the amino acidic composition (Toldrá et al., 2018). These compounds would help to avoid the problems caused by oxidation and inflammation such as the developing of chronic diseases including cardiovascular disease, type II diabetes, hypertension and obesity (Xing et al., 2019).

In this context, this study aims at assessing proteolysis, the angiotensin converting enzyme inhibition and antioxidant activity of peptides and free amino acids released after in vitro digestion of different types of meat (chicken, turkey, pork and beef) mimicking the most common gastrointestinal disorders appearing with ageing.

2. MATERIAL AND METHODS

2.1. Materials

The raw meats (chicken breast, turkey breast, pork loin and beef entrecote) were purchased from a local store in Valencia (Spain). These selected meat types (two poultry meats and two mammalian meats) represent the most consumed meats in Spain, followed by other meats such as rabbit, lamb, sheep and goat (Alcalde et al., 2013; Escriba-Perez et al., 2017). Pepsin from the porcine gastric mucosa (3200-4500 U/mg, 3602 U/mg), pancreatin (8 x USP, 5.4 TAME U/mg) from porcine pancreas, bile bovine (dried,

unfractionated), analytical grade salts (potassium chloride, potassium dihydrogen phosphate, sodium bicarbonate, sodium chloride, magnesium chloride, ammonium carbonate, calcium chloride and potassium sulfate), boric acid, hydrochloric acid (37%), sulfuric acid (95-97%), sodium hydroxide, Angiotensin Converting Enzyme (ACE) from rabbit lung (≥2.0 units/mg protein), N-Hippuric-His-Leu hydrate (HHL), ethyl acetate, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Also, petroleum ether (VWR Chemicals, VWR International Pty. Ltd., Murarrie, Queensland, Australia), dichloromethane (HPLC grade > 99.8%, Honeywell Fluka, Morris Plains, NJ, USA) and EZ-Faast amino acid kit (Phenomenex, Torrance, CA, USA) were used.

2.2. Sample preparation

Sliced meats (50 ± 0.5 g) were microwave cooked on an extended plate with a lid without additional fat in a household microwave oven (model GW72N, Samsung) at 12 ± 1 W/g. Microwave processing was selected to avoid the incorporation of additional ingredients (e.g., oils) for cooking. This processing method presents similarities with other conventional methods such as grill, oven and water bath. The microwave cooking time was previously established according to the time required to reach an internal temperature of 70 ºC, resulting in 120 s for chicken, turkey and pork and 75 s for beef. Food composition influences heating rate and temperature uniformity (Fakhouri & Ramaswamy, 1993). Thus, the higher fat content in beef resulted in a shorter microwave cooking time.

Meat cooking was performed in at least three independent slices for each type of meat.

2.3. Physicochemical characterization of cooked meats

Moisture, ash, fat and protein contents were determined in cooked meats according to the official methods 934.01, 942.05, 920.39 and 960.52 (AOAC, 2000), respectively. In addition, cooked samples (1.5 cm cubes) were analyzed through a texture profile analysis (TPA) using a TA.XT (Stable Micro System Ltd., God-alming, Surrey, UK) with a 50 kg load cell and an SMS P/75 probe by compressing 80%. Hardness, cohesiveness, springiness, adhesiveness and chewiness were calculated based on the force-time deformation curves (Pematilleke et al., 2020). Determinations were performed by triplicate in at least three independent slices for each type of meat.

2.4. *In vitro* digestion simulation

Cooked meats were *in vitro* digested under four GI conditions (Table 1). Three digestion models were defined to mimic the GI alterations in elderlies at oral (E1), oral and gastric (E2), and oral, gastric and intestinal stages (E3) (Hernández-Olivas, Muñoz-Pina, Andrés et al., 2020). Besides, healthy adult GI conditions were also simulated as control (C) model (Minekus et al., 2014). Concretely, altered gastric and intestinal conditions in elderlies were stated according to Shani-Levi et al. (2017). Oral stage was, however, *in vivo* performed by a volunteer with healthy dentition. 30 mastication cycles were established to reach a bolus with similar consistency to that of a tomato or mustard paste (Jalabert-Malbos et al., 2007). Once established, this parameter was reduced to 50% to mimic the most suboptimal oral conditions given in elderlies which results in large particle size of the bolus and making food digestion more difficult (Le et al., 2004; O'Keeffe et al., 2019). Three independent digestion experiments were parallelly performed for each gastrointestinal condition (C, E1, E2 and E3).

Just before digestion experiments, gastric (SGF) and intestinal (SIF) digestion fluids were prepared from stock solutions and the enzymatic activity of pepsin and pancreatin previously tested according to Minekus et al. (2014). Aliquots were taken, if needed, after

gastric digestion. After intestinal digestion, digesta was kept in an ice bath for 10 min to slow down the enzymatic activity before bioaccessible fraction separation (liquid phase) from the remaining solids by centrifugation at 4000 g-force for 5 min at 10 °C.

2.5. Analytical determinations in meat digesta

2.5.1. TCA soluble protein

Protein hydrolysis was evaluated by measuring the soluble protein fraction in trichloroacetic acid (TCA) according to Lamothe, Azimy, Bazinet, Couillard, and Britten (2014). Briefly, 500 μL of 36% TCA was added to 1000 μL of the bioaccessible fraction to reach a final concentration of 12% (w/w). The protein extract was prepared by mixing, incubating at 25 ºC for 15 min on an Eppendorf Thermomixer Comfort (Eppendorf AG 22331, Hamburg, Germany), and centrifuging at 1200 g-force for 10 min. The supernatant was collected and diluted in 50 mM EDTA, 8 M urea, pH 10 buffer. The ratio supernatant: buffer (v:v) was 1:9 and 1:99 extract for gastric and intestinal samples, respectively. Soluble protein in TCA was determined in triplicate by measuring absorbance at 280 nm against a blank prepared with appropriate digestion fluids of each digestion model. TCA soluble protein (g/100 g of crude protein in cooked meat) was calculated by means of a calibration line of bovine serum albumin (BSA) as standard (assuming it were 100% pure) and agreed to Equation 1.

$$\boldsymbol{TCA\ soluble\ protein}\ (\%) = \frac{(\boldsymbol{g\ TCA\ soluble\ protein\ in\ bioccessible\ fraction})}{(\boldsymbol{g\ crude\ protein\ in\ undigested\ cooked\ meat})} \times \mathbf{100} \quad (\mathbf{1})$$

2.5.2. Free amino acids released

Free amino acids (essential and non-essential amino acids (EAA and NEAA)) resulting from protein digestion were determined in triplicate through the protocol published by Peinado, Koutsidis, and Ames (2016) with some amendments. Thus, 100 μL of post-intestinal bioaccessible fraction were derivatized using the EZ-Faast amino acid kit

(Phenomenex, Torrance, CA, USA) and analyzed by GC-MS (Agilent Technologies, Injector 7683B series, Network GC System 6890N series, Inert Mass Selective Detector 5975 series, MSD ChemStation software). Derivatized amino acid solution (2 µL) was injected at 250 °C in split mode (1:15) onto a 10 m × 0.25 mm × 0.15 µm Zebron ™ ZB-AAA GC column (Phenomenex, Torrance, CA, USA). The oven temperature was 110 °C for 1 min, then increased at 30 °C/min to 320 °C, and held at 320 °C for 2 min. The transfer line was held at 320 °C, and the carrier gas was helium at a constant flow rate of 1.1 mL/min. Norvaline was used as internal standard and the free amino acids (FAA) released (%) during digestion calculated according to Equation 2:

$$FAA´s\ released\ (\%) = \frac{(g\ FAA\ in\ bioaccessible\ fraction)}{(g\ crude\ protein\ in\ undigested\ cooked\ meat)} \times 100 \quad (2)$$

Where: FAA´s corresponds to the sum of the free amino acids in the bioaccessible fraction.

2.5.3. Angiotensin Converting Enzyme Inhibitory activity (ACE ia (%))

ACE ia (%) after gastric and intestinal digestion were measured in triplicate according to Akillioğlu and Karakaya (2009) with slight modifications. ACE reactive (25 mU/mL) and Hip-His-Leu (5 mM) as substrate were used for such purpose. Both solutions were prepared in 0.15 M Tris base buffer, containing 0.3 M NaCl and a pH adjusted at 8.3. Both digested samples (40 µL) and ACE reactive (100 µL) were incubated at 37 °C for 5 min and 100 µL substrate was added. Incubation was continued for 30 min at the same temperature. Three controls (100 µL ACE + 40 µL water; 140 µL water; 40 µL digesta + 100 µL water) were also incubated as the digested samples. To stop the reaction, 150 µL of 1 M HCl was added and mixed vigorously for 5 min. Ethyl acetate (1000 µL) was added into tubes, and tubes were vortexed and centrifuged at 1200 g-force for 10 min, then 750 µL of the supernatant were collected and put into clean tubes. Tubes were slowly

shaken at 80 °C to evaporate ethyl acetate (approximately 20 min). Solid hippuric acid remained in tubes was dissolved in 1 mL deionized water, and absorbance was measured at 228 nm.

2.5.4. Antioxidant activity (2,2-diphenyl-1-pricrilhidayil (DPPH))

The antioxidant activity was measured in digesta in triplicate according to Calvo-Lerma, Paz-Yépez, Asensio-Grau, Heredia, and Andrés (2020) with slight modifications. Briefly, 200 and 400 µL of gastric and intestinal bioaccessible fractions, respectively, were mixed with 1000 µL of 80:20 methanol:deionized water and shaked at 800 rpm on an Eppendorf Thermomixer Comfort (Eppendorf AG 22331, Hamburg, Germany) for 60 min at 25 ºC. After that, the methanolic extract was centrifugated at 1200 g-force for 10 min. Parallelly, 2,2-diphenyl-1-pricrilhidayil (DPPH) solution was prepared at a concentration of 35 mg/L to reach an absorbance of 1.1 ± 0.02. Following, 500 µL of methanolic extracts were added to 1500 µL of DPPH solution and allowed to react for 60 min with light absence. Finally, the absorbance was measured at 515 nm and antioxidant activity expressed as mg trolox equivalent (TE)/g meat on a dry basis with the aid of a calibration curve of Trolox. Distilled water was used as the negative control and BHT as a positive control (Mora et al., 2017).

2.6. Statistics

Data were subjected to an analysis of variance (ANOVA) and the homogeneous groups were identified between *in vitro* models and type of meat by the LSD (Less Significant Difference) Fisher test. Pearson's test was used to find the correlation between protein digestibility evaluated by the two analytical methods (TCA-soluble protein and free amino acids). A principal component analysis (PCA) was also performed to understand the descriptive relationship among digestion-end-parameters (TCA soluble protein, data

200 related to free amino acids released, ACE inhibitory activity and antioxidant activity),
201 meat origin (chicken and turkey, pork and beef) and host GI conditions (those of standard
202 healthy adult (C) and of elderlies (E1, E2 and E3). Statgraphics Centurion XVII was used
203 with a confidence level of 95% ($p < 0.05$)

204 3. RESULTS AND DISCUSSION

205 3.1. Proximal composition and mechanical parameters of cooked meats

206 The proximal composition of cooked meats in terms of water, protein, fat and ash contents
207 (g/ 100 g) is shown in Table 2. In general, values of proximal composition agreed with
208 those reported in literature (AESAN/BEDCA, 2010; Bohrer, 2017; U.S. Department of
209 Agriculture - Agricultural Research Service, 2019). Higher water content ($p<0.05$) was
210 found in poultry meats (63.65 and 66.91 g/100 g for chicken and turkey, respectively) as
211 compared to pork and beef (59.97 and 57.93 g/100 g, respectively); as refers to ash
212 content, pork, which has been reported to be a good source of iron, zinc and potassium,
213 among others minerals (Macharáčková et al., 2021) presented the highest value (1.90
214 g/100 g) ($p<0.05$). In addition to ash content, the main differences in terms of composition
215 were found in fat content. Beef entrecote presented the highest ($p<0.05$) fat content (10.04
216 g/100 g) compared to the other studied cut of meats. Concretely, turkey breast resulted as
217 the lowest ($p<0.05$) fat content (0.61 g/100 g). The protein/fat ratio is also found in Table
218 2, resulting the highest ($p<0.05$) value for turkey (52.16), without differences ($p>0.05$)
219 between the medium values of chicken and pork (7.91 and 11.32, respectively) and the
220 least value for beef (2.95).

221 The values of hardness, cohesiveness, springiness, adhesiveness and chewiness of the
222 cooked meats (Table 2) resulted in the same range to those reported for turkey, beef and
223 pork meats (Goli et al., 2014; Martinez et al., 2004; Pematilleke et al., 2020). No
224 differences ($p>0.05$) were found on hardness, adhesiveness, and chewiness among meats.

225 Chicken meat resulted in the least ($p<0.05$) cohesiveness value (0.61) and turkey and pork
226 were the most ($p<0.05$) cohesive meats (0.69 and 0.73). For the springiness parameter,
227 chicken and beef significantly presented the lowest ($p<0.05$) values (0.53 and 0.58),
228 compared to turkey and pork meats (0.71 and 0.70, respectively). Meat composition
229 (water, protein and fat) along with some cooking events such as water loss and fat
230 drainage (Pematilleke et al., 2020), muscle fiber shrinkage and protein coagulation
231 (Vasanthi et al., 2007), could impact on textural properties in different extent. In this
232 study, the greater protein along with the low water contents of turkey could be responsible
233 of its higher cohesiveness. Actually, the protein/fat ratio resulted much greater in turkey
234 than for the other type of meats, having thus, a correlation with the adhesiveness. Besides,
235 Pematilleke et al. (2020) reports a lineal correlation between hardness and chewiness,
236 suggesting that the number of chewing cycles required during mastication increases as
237 long as the hardness does. Accordingly, the same number of cycles were stated for *in vivo*
238 oral stage as statistical differences were found on neither the hardness nor the chewing as
239 function on meat origin. Changes undergone by meat muscle during mastication such as
240 particle size reduction pattern and saliva secretion, among others, are critical for protein
241 digestibility (Cichero, 2016).

242 **3.2. Digestive alterations in elders and meat protein digestibility**

243 The hydrolysis of meat proteins by gastro-intestinal enzymes was assessed after gastric
244 and intestinal stages by measuring TCA soluble protein (mainly, smaller peptides and
245 free amino acids) (Figure 1A). Additionally, as the post-intestinal amino acid profile was
246 determined by GC-MS (Table 3), the percentage of amino acids released after the
247 gastrointestinal digestion is also presented in Figure 1B. Data reported in Figure 1 were
248 normalized with respect to the protein content of the undigested cooked meats. As shown
249 in Figure 1A, proteolysis mostly occurred at intestinal stage. After gastric digestion under

C model, values ranged from 12 to 17 g TCA soluble protein/100 g protein for chicken and turkey ($p<0.05$), respectively. These values correspond to 16-29% of the total proteolysis achieved at the end of the GI digestion. Yin, Zhou, Pereira, Zhang, and Zhang (2020) and Martini, Conte, and Tagliazucchi (2019) found similar values in post-gastric digesta for the same type of meat. Partly, the low efficiency of pepsin could be consequence of the effect of cooking on meat muscle, since higher values of proteolysis in stomach has been found in raw meat (Bax et al., 2012). Thus, high cooking temperatures may promote protein aggregation and decrease protein hydrolysis by pepsin (Bax et al., 2012). Indeed, *in vitro* static model can be also responsible of the poor gastric proteolysis, since gastric proteolysis extent achieved *in vivo* studies have been reported to be higher than *in vitro* ones (Wen et al., 2015). Solubility of proteins highly depends on meat origin; while some proteins are highly soluble at normal gastric pH, others could interact with other macromolecules, forming aggregates and becoming insoluble, slowing the protein breakdown and release (Dekkers et al., 2016; Peram et al., 2013). Moreover, it has been reported that at normal gastric pH the acid present some ineffectiveness to open the structure to solubilization and enzyme action (Luo et al., 2015). The highest ($p<0.05$) protein digestibility was achieved in chicken (76.28 g of TCA soluble protein/ 100 g of protein) at the end of digestion, while pork protein resulted to be the least ($p<0.05$) digestible (45.96 g of TCA soluble protein/ 100 g of protein) under C conditions. Rates of meat protein digestibility up to 95% have been reported in previous studies (Bax et al., 2012). However, the hydrolysis of proteins depends on many meat factors such as matrix structure (Reynaud et al., 2020), the secondary structure of proteins resulting after processing (more β-sheet structure lead into lower digestibility), hydrophobicity (given by protein aggregation) or the possible disrupted cleavage sites of digestive enzymes (because lysine and arginine oxidation) which can enhance or limit proteolysis (Yin et

al., 2020). Also, lipid oxidation products (i.e., aldehydes), or reducing sugars could interact with proteins by means of Schiff bases (Bax et al., 2012), and further impact the hydrolysis of proteins. The differences of protein solubility between meat types are coherent with FAAs values (g/ 100 g of protein) (Figure 2B) and agree with those previously reported by Martini et al. (2019). A significant Pearson correlation (0.58 with a p value of 0.0174) was found when both results of the protein digestibility were analyzed. Thus, beef exhibited a significantly higher amount of FFAs released (66 g FAA/ 100 g protein) compared to pork, turkey and chicken (40.3, 53 and 43 g FAA/ 100 g protein, respectively) under C conditions. Gastric and duodenal enzymes degraded beef proteins more efficiently than proteins from pork, chicken and turkey. From Figures 1A and 1B, it is possible to affirm that beef and pork end-digestion products were mostly found as free amino acids, while smaller peptides (29-34% of total proteolysis in C and E1, 25-30% in E2 and 12-24% in E3) would find in chicken and turkey intestinal digesta, together with free amino acids. Previous studies found that myofibrillar proteins (55-60%), particularly actin, titin and myosin, are hydrolyzed more easily than sarcoplasmic (25-30%) or stromal (10-15%) proteins during *in vitro* digestion (Xiong, 2018). According to literature (Elkhalifa et al., 1988; Kauffman, 2001; Lawrie, 1961; Mudalal et al., 2014; Sorapukdee et al., 2013), pork has less myofibrillar proteins (44%, compared to 51-63% in other meats). Therefore, the protein composition of meat (myofibrillar:sarcoplasmic:stromal ratio) could be related to the lowest protein digestibility in pork ($p<0.05$).

In vitro simulation of altered GI conditions of elderlies discloses interesting information of protein hydrolysis of meats under this physiological scenario. Unexpectedly, 50% of chewing cycles reduction did not exert a statistically significant effect ($p<0.05$) on protein digestion (comparison of C and E1). Apparently, particle size distribution decreases along

digestion (Sicard et al., 2018), digesta reaching a very similar particle size in stomach regardless the differences of the bolus in particle size. In this sense, Zou et al. (2018) report similar particle size distribution after *in vitro* gastric and intestinal digestion of different bolus with different particle size distribution from three types of pig muscles with different composition. With regards to the impact of gastric alterations on gastric proteolysis, it was also expected that a pH increasing from 3 to 6 together with a pepsin concentration reduction to 75% (1500 U/mL), lessened the protein breakdown into smaller peptides and free amino acids. However, gastric alterations of elderlies mimicked in this study (model E2 and E3) resulted in a significant increase ($p<0.05$) of gastric proteolysis in poultry and pork meats, especially in chicken. These results were not expected since pepsin has maximal hydrolytic activity between pH 1.5 and 2.5 and activity is below 5% of the maximum above pH 5. The isoelectric point of the proteins must also be considered and is perhaps one of the key factors behind these results. As the pH of the digesta approaches the isoelectric point of the proteins, aggregation and precipitation occurs, decreasing the solubility of the proteins and hindering the access and efficiency of pepsin to the substrate (Reynaud et al., 2020). At more alkaline pH, for example at 6 (gastric pH in models E2 and E3) or 7 (intestinal pH), proteins are increasingly negatively charged due to ionization of the carboxyl groups and deprotonation of the amine groups. As a result, electrostatic repulsion is enhanced, increasing protein-water interactions, and thereby protein solubility. Even though the minimum solubility of proteins occur at the isoelectric point of proteins (Cercel et al., 2015), it has been reported that the solubility of myofibrillar proteins in chicken breast (the most abundant type) experiment a remarkable increase (from 10 to 80%) when the pH rise from 5.5 to 6 (Xiong, 1992). Reasonably, the variation in the amount of myofibrillar proteins among the types of meats (greater being for poultry meats) could be

responsible for the greater gastric protein digestibility in chicken and turkey, than beef and pork. On the other hand, at pH values lower than 4.5, and therefore at 3, proteins are positively charged and electrostatic repulsion increased as well. pH buffering capacity of meats which is highly determined by food intrinsic factors (consistency, particle size, origin, protein and amino acid content and acid and base groups (such as salts and organic acids)) has also to be accounted (Mennah-Govela et al., 2020; Mulet-Cabero et al., 2020; Sicard et al., 2018). Like manner, food composition also impacts buffering capacity (i.e. foods with high fat and low protein contents lead to lower buffering capacity) (Mennah-Govela et al., 2020). Reasonably, beef highly differs from the other studied meats at fat content. This difference in composition could impair differences in terms of buffer capacity. It was noted that pH was more stable along digestion time in beef than in the other meats. The contribution of fat to buffering capacity of meats has been previously reported (Tan et al., 2014). The higher lipidic content also could determine the action of micellization and emulsification promoting greater digestion of nutrients, not only of lipids but also of proteins (Salvia-Trujillo et al., 2017).

Regarding the proteolysis occurring later in the intestinal stage, the altered gastric model (E2) did not have a negative impact (Figure 1A and 1B). Pork resulted with the lowest significant value ($p<0.05$) (52.17 g/100 g protein) and chicken meat the highest (79.90 g/100 g protein) for TCA soluble protein. Moreover, for the release of free amino acids under E2 model, pork and turkey meat were the meats with the lowest (44.37 and 48.44 g/100 g protein) and beef the highest (79.07 g/100 g protein) ($p<0.05$). Denis et al. (2016) found a delay of protein digestion kinetics but not on its extent, being even higher under *in vitro* senior GI conditions. The activity of pancreatic proteases might compensate the gastric suboptimal conditions (E2) with the proteins conversion into peptides and free amino acids (Hernández-Olivas et al., 2020).

Finally, reduction of both pancreatic (50 U/mL) and bile salts (5 mM) concentration, together with an extended duration (4h) of intestinal stage (model E3), significantly dropped ($p<0.05$) proteolysis in all meats (Figure 1A and 1B). However, digestibility was reduced in a variable extent depending on the type of meat. TCA soluble protein in intestinal digesta that informs about short-chain peptides and free amino acids with potential functional activities, experimented a significant reduction ($p<0.05$) of 26, 26, 15 and 28% in chicken, turkey, pork and beef, respectively. If only FAAs released are considered, reduction of up to 16, 10, 5 and 27% in chicken, turkey and beef, was found respectively. Thus, the altered intestinal conditions have a higher impact on short-chain peptides than on free amino acids released of chicken, turkey and pork meats.

A decrease in pancreatic enzymes secretion have been stated to lead with poor digestion and consequently to protein malabsorption causing nutritional deficiencies (Rémond et al., 2015). Again, it is important to note that *in vivo* proteolysis extent could be higher than *in vitro* static models, because of end-digestion products, not only from proteins but also from lipids, are not removed from the system. This effect being more noticeable as long as the intestinal time increases, and therefore in E3 model than in the others.

The individual amino acid contents (g amino acids/ 100 g protein) as well as the essential amino acids (EAA)/non-essential amino acids (NEAA) ratio in the post-intestinal digesta are gathered in Table 3. It is remarkable the great contents of free lysine, leucine and tyrosine, in the meat post-intestinal digesta (beef and chicken > turkey and pork). Leucine serves as substrate for the synthesis of new muscle proteins and as a signal to initiate the rate-limiting translation initiation step of MPS (Crozier et al., 2005). Lysine participates building muscle tissue but also collagen (an important constituent of cartilage, connective tissue and skin). Moreover, it is involved in the production of carnitine, which help to burn long-chain fatty acids producing energy. (Liao et al., 2015). Tyrosine has numerous

375 functional roles such as the synthesis of neurotransmitters (catecholamines), alleviation
376 of mental anxiety and depression and neutralization of free radicals (Fernstrom &
377 Fernstrom, 2007).

378 Literature reports EAA/NEAA ratios between 0.6 and 0.9 depending on the type of meat
379 and processing (Brzostowski et al., 2008; Domínguez et al., 2015; Kim et al., 2013; Li et
380 al., 2020; Xu et al., 2019). However, no values of this ratio after digestion were found in
381 the literature. The EAA/NEAA ratio of cooked meats digested under C GI conditions
382 were 1.68, 2.13, 2.24 and 2.87 for beef, turkey, chicken and pork, respectively, with EAA
383 release being much more favored than NEAA release. The ratio EAA versus NEAA kept
384 similar under E1 (oral alteration) and E2 (oral and gastric alterations) GI conditions.
385 Nevertheless, a considerable rise in the EAA/NEAA ratio value was found in samples
386 digested mimicking the most suboptimal GI conditions given in elderlies (E3 model). So,
387 even when the extent of proteolysis (for both TCA soluble protein and for the sum of the
388 FAA) was limited under the E3 model, elderly GI conditions might enhance the EAA
389 release in a greater extent than the NEAA. The specificity of pancreatic enzymes for
390 certain peptide bonds (Aderinola et al., 2018) could be responsible of these results, being
391 this chemical preference more noticed under suboptimal pancreatic concentrations. Most
392 of amino acids involved in muscle synthesis are essential ones (Volpi et al., 2003), making
393 these results of great interest to dietitians when addressing recommendations to elderlies
394 and other individuals susceptible to suffer of sarcopenia.

395 Besides, amino acids have been chemically classified as hydrophobic amino acids (HAA=
396 Ala, Val, Ile, Leu, Tyr, Phe, Trp, Pro, Met, Cys), positively charged amino acids (PCAA =
397 Lys, His), negatively charged amino acids (NCAA = Asp, Asn, Glu, Gln), aromatic amino
398 acids (AAA = Phe, Trp, Tyr) and sulfur-containing amino acids (SCAA = Cys, Met) and
399 their values reported as well. According to the obtained results, meat digesta were found

400 to be richer in HAA than PCAA, AAA, NCAA and finally SCAA. The highest amount
401 of HAA, PCAA and NCAA (mg/ 100 g of protein) ($p<0.05$) was reported in digested
402 chicken and beef, regardless the GI conditions; while very similar values of AAA and
403 SCAA were found for all meat digesta.

404 With regards to the effect of GI conditions on the different amino acid chemical groups,
405 significant reduction ($p<0.05$) was found under E3 GI conditions in chicken and beef
406 compared to the C model. Concretely, the release of amino acids belonging to HAA,
407 NCAA and SCAA were highly compromised by the suboptimal conditions at intestinal
408 stage. Specifically, a decrease up to 21, 40 and 43% (respectively for HAA, NCAA and
409 SCAA) was noticed in beef under E3 with respect to those values achieved under C. On
410 the other hand, the concentration of the different amino acid groups, excepting NCAA,
411 were similar in turkey and pork digesta in the C and E3. The beneficial effect of amino
412 acids, peptic fractions built-up of them, on consumer's health have been stated to be
413 dependent on amino acids chemical classification (Xing et al., 2019). Particularly, end-
414 digestion protein products can exert as hypertensive inhibitor, antioxidative, glucose
415 uptake stimulating peptide, antithrombotic, anti-amnestic, dipeptidyl peptidase IV
416 inhibitor, stomach mucosal membrane activity, regulators, dipeptidyl carboxypeptidase
417 inhibitor. Both angiotensin converting enzyme inhibition and antioxidant activity have
418 been analyzed in this study and are discussed henceforth.

419 **3.3. Antioxidant activity and angiotensin converting enzyme (ACE) inhibition of**
420 **meat bioaccessible fractions obtained under control and elderly GI conditions**

421 Bioaccessible fractions of post-gastric and post-pancreatic meat digesta were analyzed
422 for their ACE-inhibitory (%) and DPPH antioxidant (mg TE/g meat dry basis) activities
423 (Figure 2). According to the obtained results under C GI conditions, only turkey digesta,
424 both gastric and intestinal, would exert lower Angiotensin Converting Enzyme (ACE)

425 inhibitory activity, compared to the other meats. The correlation between the release of
426 health-promoting peptides and amino acids and angiotensin converting enzyme inhibition
427 has been reported (Escudero et al., 2014; Sangsawad et al., 2017). In this sense, it has
428 been reported that ACE-inhibitory activity increases as long as proteolysis progresses
429 being higher at the end of digestion compared to post- gastric digesta. Nevertheless, the
430 enzymatic action of pepsin, together with the optimal pH, can be considered the key-
431 mechanism for bioactive peptides release along digestion as it can be deduced from the
432 drastic reduction of ACE inhibitory capacity of gastric digesta in all meats when gastric
433 conditions were suboptimal. Even if TCA soluble protein values after gastric digestion
434 were similar in all meats, except for chicken (Figure 1A), regardless the simulated GI
435 conditions, the peptide profile and their molecular weight, both parameters involved in
436 the biological activities, seems to be different under healthy and suboptimal GI
437 conditions. At this point, the determination of peptidic fractions would be interesting as
438 a reduction of molecular mass distribution of peptides from 5 kDa to 1 KDa, or lower,
439 has been reported to increase the ACE inhibitory activity (Sangsawad et al., 2017). Most
440 blood pressure-lowering peptides have been found to be short sequences of 2–12 amino
441 acids with Pro, Lys, Leu or aromatic residues preferably in any of the three positions close
442 to the C-terminal site (Mora et al., 2017). In contrast, larger peptides have been shown to
443 exhibit difficulties in binding to the ACE active site, resulting in decreased inhibitory
444 capacity (Natesh et al., 2003). The ACE inhibitory peptides contain hydrophobic amino
445 acid at the N-terminal, as well as Trp at the C-terminal tripeptide sequence, which may
446 contribute to ACE inhibitory activity. The hydrophobicity of peptides is assumed to
447 contribute to their ACE-inhibitory activity and, furthermore, to their bioavailability
448 (Foltz, van Buren, Klaffke, & Duchateau, 2009). On the other hand, only ACE inhibitory
449 (%) of intestinal digesta of beef experimented an additional significant reduction under

E3 model compared to values achieved under E2 model. Therefore, the positive health-related benefits obtained from meat intake, excepting from beef, would be more compromised in elderlies with gastric suboptimal conditions compared to those elders suffering from intestinal insufficiency.

Concerning the antioxidant activity of digesta (Figure 2B) turkey, followed by pork, achieved the highest values (mg of TE/ g dry matter) ($p<0.05$) at the end of digestion standard under healthy GI conditions (C). Protein hydrolysates might present different affinities for radicals resulting leading to synergistic and antagonistic effects at antioxidant level depending on meat origin (Serpen et al., 2012). Thus, Martini et al. (2019) reported the highest anti-peroxidative activity against linoleic acid auto-oxidation, ABTS and hydroxyl radical scavenging for turkey and pork post-intestinal digesta; while beef digesta presented the least values. Moreover, it has been reported that in fatty-meats, such as beef, some peptides can be involved in the prevention of essential fatty acids peroxidation resulting in a reduced total antioxidant activity (Kitts & Weiler, 2005).

The relevance of the digestion events occurring at gastric stage is also notable on the antioxidant activity of post-gastric digesta which underwent a drastic decrease under suboptimal gastric conditions, compared to under standard conditions.

As explained for the ACE-inhibitory capacity, gastric digesta obtained under C and E1 GI conditions would present peptides with improved inhibitory potentials against the DPPH radicals compared to those obtained from E2 and E3 models. Bioactive peptides displaying antioxidant properties contain HAA and PCAA (notably, Tyr, Met, His and Lys). Also, aromatic amino acids (AAA) such as tryptophan, as well as those with positively charged character (PCAA) like histidine, exhibit high antioxidant capacity as hydrogen donors due to the presence of indolic and imidazole groups in AAA and PCAA, respectively. Since pepsin presents high preference for the N-terminal of AAA, it is

475 expected that this chemical group were hydrolyzed at gastric level being available for
476 bioabsorption before others. Certain PCAA seem to enhance the up-regulation of genes
477 involved in the mitochondrial biogenesis, as an alternative pathway for long-chain fatty
478 acids oxidation and glucose metabolism in insulin-sensitive tissues (Wu, 2010).
479 Similarly, methionine (belonging to SCAA) besides histidine, serine and glycine are the
480 main contributors of 1-carbon groups (Wu, 2010). Actually, NCAA, PCAA and SCAA
481 (e.g. glutamine, arginine and N-acetyl-cysteine, respectively) are known to significantly
482 contribute to the oxidative defense and immune function (Wu, 2010).

483 Besides, gastric digesta from chicken exerted the highest antioxidant activity ($p<0.05$). In
484 this sense, chicken meat has been reported to be higher in bioactive imidazole dipeptides
485 anserine (β-alanyl-L-histidine) and carnosine (N-β-alanyl-1-methyl-L-histidine) which
486 display high antioxidant capacity (Arihara, 2006; Nagasawa et al., 2001; Sarmadi &
487 Ismail, 2010; Young et al., 2013). Even though the effect of gastric suboptimal conditions
488 on gastric digesta's antioxidant activity, only when alterations are also mimicked at
489 intestinal level, a reduction of this property is found at the end of digestion. Therefore,
490 the antioxidant activity of the potential bioabsorbable fraction would decline when
491 disfunctions appeared at both gastric and intestinal stages, their effect being more acute
492 in poultry than mammals' meat. The hydrophobic properties of some amino acids can
493 improve, or decrease, the antioxidant effect of peptides because of their interactions with
494 lipids among others (Aderinola et al., 2018). An increase of the digestion time could be
495 responsible of a promotion of greater number of these reactions.

496 **3.4. Principal Component Analysis (PCA) applied to the obtained data**

497 Figure 3 shows the biplot coming from PCA and applied to the data obtained after gastric
498 and intestinal digestion of the four meats under the different GI conditions (C, E1, E2 and

E3). As it can be seen in Figure 3A, the two main components explain 88.930% of the variance of data at gastric stage (PC1: 58.227% and PC2: 30.703%). PC1 clearly distinguishes between GI conditions, showing digesta of meat obtained under C and E1 GI conditions presented both higher ACE inhibitory activity and DPPH antioxidant activity than digested obtained under E2 and E3, except for turkey samples. Likewise, PC2 distinguishes among meats, being greater the TCA soluble protein and DPPH antioxidant activity in poultry than in pork or beef digesta. On the other hand, Figure 3B explains the 73.756% of the variance of data obtained at the end of intestinal digestion. PC1 (50.995%) highlights the closed relationship and higher values of TCA soluble protein, free amino acids and ACE inhibitory activity found for chicken and beef when intestinal conditions remain standard. Besides, DPPH antioxidant activity seems to be positively linked to the EAA/NEAA ratio. PC2 only represent the 22.801% of the variance of data but evidences different among poultry and mamals' meat intestinal digesta as for gastric digesta.

4. CONCLUSIONS

Among the simulated gastrointestinal alterations that appear with aging, intestinal conditions had the most significant negative effect on the digestibility of meat protein, this effect being dependent on the type of meat. Reductions of up to 28 and 27% of TCA soluble protein and free amino acid released, respectively, were found in beef compared with total extents achieved under standard intestinal digestion conditions. Besides and unexpectedly a 50% reduction of chewing cycles did not hinder meat digestibility. Gastric alterations neither affected the protein breakdown, even being favored, mainly in chicken meat. According to that, chicken meat consumption would be more advisable than other meats to maximize the TCA soluble protein, while beef intake would result in more FAA release under elderly GI conditions.

A notable increase in the release of essential amino acids, compared with the non-essential ones, was also noticed under simulated elderly GI conditions. Regarding the functional properties related to the protein end-digestion products, meats are highly recommended for their antioxidant activity and angiotensin converting enzyme inhibition. Both the gastric elderly alterations and the intestinal ones resulted in high reductions of meat digesta functionalities.

For those elderly subjects with specific oral, gastric and intestinal disorders, more personalized dietary recommendations could be established. In subjects with oral disorders, mammalian meats will favor a greater inhibition of ACE, turkey and pork meats for their antioxidant potential and chicken and beef meats for their protein digestion. On the other hand, if elderly subjects present both oral and gastric alterations, beef will be more recommendable than the other meats. In addition, for subjects with suboptimal oral, gastric and intestinal condition, chicken and beef meats should be prioritized for maximizing protein digestibility, pork meat to ensure maximum ACE inhibition against hypertensive diseases in the elderly, and turkey meat to exert more antioxidant benefits in those over 65 years of age.

Therefore, this study provides a better understanding of protein digestion according to the type of meat, together with the functional properties related to the hydrolysis of proteins, under oral, gastric and intestinal suboptimal conditions of elderlies. This data may contribute to the establishment of more accurate dietary recommendations concerning meat consumption and addressed to this population group.

Conflict of interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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Tables

Table 1. GI parameters established at oral, gastric and intestinal stages for the control (C) and elderly models (E1, E2 and E3).

Table 1

Digestion model	Oral	Gastric	Intestinal
Control (C)	5 g of food sample + 5 g human salivary fluid Chewing until a consistency like a tomato or mustard paste (30 for all samples).	Oral bolus + 10 mL SGF pH 3 Pepsin (2000 U/mL) 2 h 55 rpm 37 °C	Gastric chime + 20 mL SIF pH 7 Bile (10 mM) Pancreatin (100 U/mL) 2 h 55 rpm 37 °C
Elderly (E1, E2 and E3)	5 g of food sample + 5 g human salivary fluid **50% of the Control chewing cycles**	Oral bolus + 10 mL SGF **pH 6** **Pepsin (1500 U/mL)** 2 h 55 rpm 37 °C	Gastric chime + 20 mL SIF pH 7 **Bile salts (5 mM)** **Pancreatin (50 U/mL)** **4 h** 55 rpm 37 °C

Amendments included in the *in vitro* digestion models for elderlies, with respect to the control model (C), are highlighted in bold. E1 (alterations at oral stage); E2 (alterations at oral and gastric stages); E3 (alterations at oral, gastric and intestinal stages). SGF: Simulated gastric fluid; SIF: Simulated intestinal fluid.

Table 2. Proximal composition (g /100 g of wet basis) and mechanical parameters of microwave-cooked chicken, turkey, pork and beef entrecote obtained from Textural Profile Analysis (TPA).

Table 2

	Chicken	Turkey	Pork	Beef	RMSE	P-value
Nutrient content						
Water (g/100 g)	63.65^{b}	66.91^{b}	59.97^{a}	57.93^{a}	2.16	**
Protein (g/100 g)	28.46^{a}	31.60^{a}	34.29^{b}	29.50^{a}	1.71	**
Fat (g/100 g)	3.60^{b}	0.61^{a}	3.03^{b}	10.04^{c}	1.11	***
Ash (g/100 g)	1.10^{b}	0.83^{a}	1.90^{c}	1.26^{b}	0.10	***
Protein/fat ratio	7.91^{b}	52.16^{c}	11.32^{b}	2.95^{a}	3.99	***
Mechanical parameter					0.00	
Hardness (N)	237.85^{a}	212.71^{a}	238.77^{a}	279.23^{a}	36.66	***
Cohesiveness	0.61^{a}	0.69^{b}	0.73^{b}	0.65^{ab}	0.03	***
Springiness	0.53^{a}	0.71^{b}	0.70^{b}	0.58^{a}	0.06	***
Adhesiveness (Ns^{-1})	-0.07^{a}	-0.05^{a}	-0.07^{a}	-0.08^{a}	0.02	***
Chewiness (N)	78.01^{a}	104.88^{a}	122.25^{a}	106.62^{a}	21.48	***

The data shown are mean values from triplicates. ^{abc} Different lowercase letters indicate significant differences between meats, with a significance level of 95% ($p<0.05$). RMSE: root mean square error; P-level: NS: not significant; *:$p<0.05$; **:$p<0.01$; ***: $p<0.001$.

Table 3. Amino acids profile (g/100 g protein) of intestinal digesta of chicken, turkey, pork and beef obtained under *in vitro* simulation of control (C) and elderly GI conditions (E1, E2 and E3).

Amino acid	Chicken				Turkey				Pork				Beef				RMSE	P-value	
	C	E1	E2	E3	C	E1	E2	E3	C	E1	E2	E3	C	E1	E2	E3		Meat origin	GI conditions
Ala	1.87^{bC}	1.84^{bC}	2.15^{cC}	1.61^{aC}	1.45^{bB}	1.468^{bB}	1.90^{cB}	1.31^{aB}	1.21^{cA}	1.13^{bA}	1.36^{dA}	1.04^{aA}	3.40^{cD}	3.00^{bD}	3.11^{bcD}	2.17^{aD}	0.22	***	***
Gly	0.68^{bC}	0.70^{bC}	0.82^{cC}	0.50^{aC}	0.53^{bB}	0.57^{bB}	0.69^{cB}	0.41^{aB}	0.40^{bA}	0.42^{bA}	0.49^{Ca}	0.31^{aA}	0.97^{bD}	0.90^{bD}	0.92^{bD}	0.60^{aD}	0.06	***	***
ABA	n.d.	n.d.	n.d.	n.d.	0.11^{aB}	0.12^{aB}	n.d.	n.d.	0.08^{aA}	0.10^{bA}	0.13^{cA}	0.13^{cA}	0.18^{bcC}	0.18^{bC}	0.20^{cB}	0.13^{aA}	0.02	***	***
Val	2.99^{abB}	3.05^{bC}	3.45^{cC}	2.87^{aD}	2.28^{aA}	2.35^{aB}	2.95^{bB}	2.43^{aB}	2.19^{aA}	2.06^{aA}	2.49^{bA}	2.10^{aA}	4.2^{cD}	3.90^{bD}	3.9^{bcD}	2.68^{aC}	0.33	***	***
Leu	7.02^{abB}	7.18^{bB}	7.92^{cB}	6.77^{aB}	5.39^{aA}	5.58^{aA}	6.70^{cA}	6.19^{bA}	5.51^{abA}	5.38^{aA}	6.42^{cA}	5.91^{bcA}	8.30^{cC}	7.62^{bC}	7.93^{bcB}	6.12^{aA}	0.57	***	***
Ile	2.70^{abB}	2.81^{bC}	3.15^{cB}	2.64^{aC}	2.02^{aA}	2.09^{aB}	2.59^{bA}	2.25^{aA}	2.09^{abA}	1.96^{aA}	2.35^{bA}	2.04^{abA}	3.61^{bC}	3.26^{bD}	3.31^{bB}	2.43^{aB}	0.26	***	***
Thr	1.48^{bC}	1.48^{bC}	1.69^{cC}	1.25^{C}	1.20^{bB}	1.23^{bB}	1.54^{cB}	1.03^{aB}	1.07^{bA}	1.02^{bA}	1.21^{cA}	0.93^{aA}	2.62^{bD}	2.38^{bD}	2.40^{bD}	1.74^{aD}	0.17	***	***
Ser	1.22^{aC}	1.18^{aC}	1.34^{bB}	n.d.	1.07^{bB}	1.06^{bB}	1.33^{cB}	0.44^{aA}	0.75^{cA}	0.70^{bA}	0.84^{dA}	0.52^{aA}	2.34^{cD}	2.08^{bD}	2.10^{bcC}	0.58^{aB}	0.26	***	***
Pro	0.40^{aC}	0.42^{aC}	0.50^{bC}	0.39^{aC}	0.32^{bB}	0.34^{cB}	0.44^{dB}	0.30^{aB}	0.23^{bA}	0.23^{bA}	0.29^{cA}	0.18^{aA}	0.60^{bcD}	0.54^{bD}	0.69^{cD}	0.41^{aC}	0.05	***	***
Asn	1.10^{aB}	1.05^{aB}	1.10^{aB}	n.d.	1.06^{aB}	1.06^{aB}	1.31^{bC}	n.d.	0.81^{cA}	0.72^{bA}	0.82^{cA}	0.40^{aA}	2.31^{bC}	2.07^{bC}	2.06^{bD}	0.58^{aB}	0.29	***	***
Asp	0.92^{bC}	0.91^{bC}	1.03^{cC}	0.04^{aA}	0.79^{bB}	0.76^{bB}	0.93^{cB}	0.27^{aB}	0.60^{bA}	0.66^{cA}	0.73^{dA}	0.47^{aC}	1.91^{bD}	1.92^{bD}	1.77^{bD}	0.54^{aD}	0.32	***	***
Met	1.80^{bB}	1.86^{bB}	2.05^{cB}	1.71^{aB}	1.34^{aA}	1.46^{aA}	1.76^{bA}	1.21^{aA}	1.47^{abA}	1.42^{aA}	1.70^{bA}	1.49^{abA}	2.26^{cC}	2.06^{bC}	2.24^{bcB}	1.84^{aB}	0.16	***	***
Glu	3.62^{bB}	3.14^{bB}	3.42^{bC}	2.24^{aC}	2.20^{bA}	2.00^{bA}	2.40^{bB}	1.61^{aB}	1.90^{bA}	1.73^{bA}	1.83^{bA}	1.14^{aA}	8.04^{cC}	6.82^{bC}	6.82^{bcD}	5.13^{aD}	0.53	***	***
Phe	3.65^{bBC}	3.82^{cC}	4.10^{cB}	3.41^{aB}	2.87^{aA}	3.08^{aA}	3.48^{bA}	3.40^{bAB}	2.90^{aA}	3.00^{aA}	3.52^{bA}	3.26^{bA}	3.54^{bB}	3.32^{abB}	3.40^{abA}	3.12^{aA}	0.22	***	***
Gln	n.d.	n.d.	n.d.	n.d.	2.31^{aA}	2.38^{aB}	n.d.	n.d.	2.23^{cA}	2.07^{bA}	2.31^{cA}	1.92^{aA}	4.04^{aB}	3.70^{aB}	3.61^{Ab}	3.62^{aB}	0.38	***	NS
Orn	0.51^{bcD}	0.50^{bC}	0.51^{cC}	0.44^{aD}	0.43^{bB}	0.43^{bcB}	0.45^{cA}	0.40^{aC}	0.38^{bA}	0.38^{bA}	0.41^{cA}	0.37^{aB}	0.37^{bA}	0.36^{abAB}	0.37^{abA}	0.33^{aA}	0.03	***	***
Lys	11.22^{cB}	8.61^{aB}	9.92^{abcA}	9.19^{bC}	8.01^{bcA}	7.60^{bA}	8.60^{cA}	7.12^{aA}	9.13^{bA}	7.71^{aAB}	8.63^{bA}	8.96^{bB}	10.44^{aB}	9.74^{aC}	10.11^{aB}	10.62^{aD}	0.86	***	***
His	2.54^{bC}	2.68^{cC}	3.01^{dC}	2.36^{aC}	2.00^{aB}	2.15^{aB}	2.53^{bB}	2.43^{abC}	1.62^{aA}	1.61^{aA}	2.05^{cA}	1.81^{bB}	1.90^{Bab}	2.14^{abABC}	2.11^{bA}	1.44^{aA}	0.21	***	***
Tyr	4.71^{bC}	4.60^{bC}	4.58^{bC}	3.72^{aAB}	3.81^{aB}	4.12^{abBC}	4.24^{abC}	4.59^{bC}	3.02^{aA}	3.09^{aA}	3.41^{bB}	3.77^{cB}	2.31^{aA}	2.32^{aA}	2.31^{aA}	3.12^{aA}	0.45	***	NS
Trp	2.76^{aC}	2.99^{bC}	3.17^{dC}	2.60^{aB}	2.22^{aB}	2.43^{aB}	2.71^{bB}	2.62^{abB}	1.893^{aA}	1.99^{aA}	2.38^{cA}	2.15^{bA}	2.32^{aB}	2.44^{aABC}	2.50^{aAB}	2.31^{aAB}	0.18	***	***
C-C	1.63^{bC}	1.87^{cB}	2.02^{dC}	1.20^{aB}	1.66^{bC}	1.83^{cB}	1.90^{cC}	1.05^{aA}	0.76^{aA}	0.87^{aA}	1.04^{bA}	n.d.	1.02^{aA}	1.32^{abAB}	1.49^{bB}	n.d.	0.10	***	***
EAA/NEAA ratio	2.24^{aB}	2.22^{aC}	2.32^{aB}	3.54^{bB}	2.13^{aB}	2.09^{aB}	2.18^{aB}	2.80^{bA}	2.87^{aD}	2.69^{aD}	2.84^{aC}	3.64^{bB}	1.72^{aA}	1.75^{aA}	1.78^{aA}	2.5^{bA}	0.16	***	***
HAA	27.91^{abB}	28.5^{bC} (0)	31.14^{cC} (0)	26.4^{aB} (5)	21.64^{aA}	22.89^{abB} (0)	26.71^{cB} (0)	24.50^{bB} (0)	20.53^{aA}	20.32^{aA} (1)	23.76^{bA} (0)	21.60^{abA} (0)	30.52^{cC}	28.54^{bC} (7)	29.32^{bC} (4)	24.13^{aB} (21)	1.76	***	***
PCAA	13.72^{bC}	11.52^{aB} (16)	13.04^{abB} (5)	11.92^{aC} (14)	10.02^{abA}	9.28^{aA} (7)	11.18^{bAB} (0)	9.17^{aA} (8)	10.67^{bA}	8.82^{aA} (18)	10.72^{bA} (0)	10.60^{bB} (1)	12.32^{bB}	11.81^{aB} (4)	12.21^{bB} (0)	12.02^{abC} (2)	0.85	***	***
NCAA	5.64^{bAB}	5.02^{bA} (10)	5.52^{bAB} (1)	2.12^{aA} (63)	6.29^{cB}	6.19^{cB} (2)	4.66^{bA} (26)	2.05^{aA} (68)	5.54^{cA}	5.18^{bA} (6)	5.65^{bcB} (0)	3.91^{aB} (29)	16.20^{cC}	14.51^{bC} (11)	14.19^{bC} (13)	10.03^{aC} (40)	1.10	***	***
AAA	11.12^{bC}	11.40^{bcC} (0)	11.91^{cC} (0)	10.02^{aB} (10)	8.88^{aB}	9.61^{abB} (0)	10.40^{bB} (0)	10.90^{bB} (0)	7.82^{aA}	8.11^{aA} (0)	9.32^{bB} (0)	9.14^{bA} (0)	8.13^{aA}	8.02^{aA} (0)	8.22^{aA} (0)	8.51^{aA} (0)	0.74	***	***
SCAA	3.43^{bC}	3.73^{cC} (0)	4.07^{dC} (0)	3.02^{aC} (12)	3.01^{bB}	3.29^{bB} (0)	3.66^{cB} (0)	2.21^{aB} (25)	2.23^{bA}	2.30^{bA} (0)	2.72^{cA} (0)	1.47^{aA} (34)	3.21^{bB}	3.35^{bB} (0)	3.66^{cB} (0)	1.82^{aB} (43)	0.25	***	***

Data shown are mean values from triplicates. Not detected is indicated as "n.d.". Values in parentheses represent the percentage (%) of reduction of elderly GI conditions (E1, E2 and E3) with respect to the control (C). Different lowercase letters indicate significant differences between digestion models and different capital letters indicate significant differences between meat origin, with a significance level of 95% ($p<0.05$). RMSE: root mean square error; P-level: NS: not significant; *:$p<0.05$; **:$p<0.01$; ***:$p<0.001$. Ala: alanine; Gly: glycine: ABA: α-aminobutyric acid; Val: valine; Leu: leucine; Ile: isoleucine; Thr: threonine; Ser: serine; Pro: proline; Asn: asparagine; Asp: aspartic acid;

Met: methionine; Glu: glutamic acid; Phe: phenylalanine; Gln: glutamine; Orn: ornithine; Lys: lysine; His: histidine; Tyr: tyrosine; Trp: tryptophan; C-C: cystine; Essential amino acids / Non-essential amino acids ratio: EAA/NEAA ratio; Hydrophobic amino acids (HAA)= Ala, Val, Ile, Leu, Tyr, Phe, Trp, Pro, Met, Cys; Positively charged amino acids (PCAA) = Arg, Lys, His; Negatively charged amino acids (NCAA) = Asp, Asn, Glu, Gln; Aromatic amino acids (AAA) = Phe, Trp, Tyr; Sulfur-containing amino acids (SCAA) = Cys, Met.

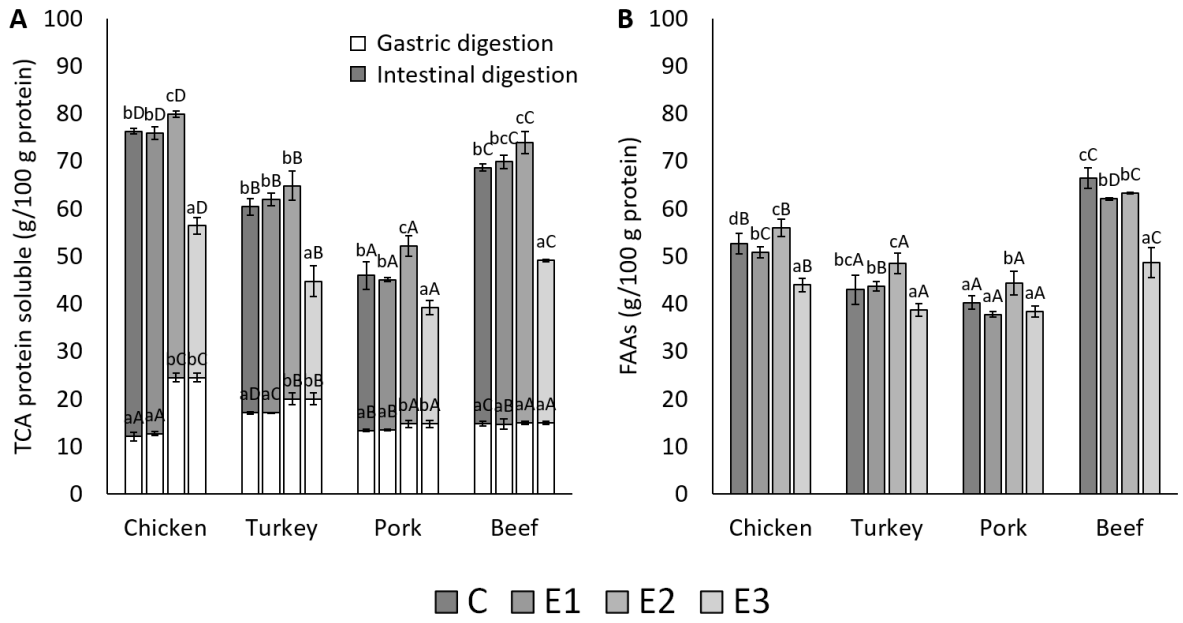
Figure legends

Figure 1. TCA soluble protein (g/100 g protein) of the bioaccessible fractions of gastric and intestinal digesta **(A)** and the FAA´s of bioaccessible fraction of intestinal digesta (g/ 100 g protein) **(B)** found in chicken, turkey, pork and beef *in vitro* digested under C (control), E1 (Elderly 1), E2 (Elderly 2) and E3 (Elderly 3) GI conditions. The data shown are mean values from triplicates and the standard deviation. Different lowercase letters indicate significant differences between digestion models and different capital letters indicate significant differences between meat origin, with a significance level of 95% ($p < 0.05$).

Figure 2. ACE inhibitory activity (%) (A) and DPPH antioxidant activity (mg TE/ g meat d.b.) (B) of the bioaccessible fractions of gastric and intestinal *in vitro* digesta of chicken and turkey, pork and beef under the elderly (E1, E2 and E3) and the standard GI conditions. The data shown are mean values from triplicates and the standard deviation. Different lowercase letters indicate significant differences between digestion models and different capital letters indicate significant differences between meat origin, with a significance level of 95% ($p<0.05$).

Figure 3. Biplot obtained by means of a principal component analysis (PCA) of the different gastric (A) and intestinal (B) end-digestion protein products and properties (FAA, EAA/NEAA ratio, HAA, PCAA, NCAA, AAA, SCAA, TCA soluble protein as well as ACE inhibition and DPPH antioxidant activities), and their association with the binomial meat type (chicken and turkey, beef and pork)-GI host conditions (C, E1, E2 and E3).

875 **Figure 1.**



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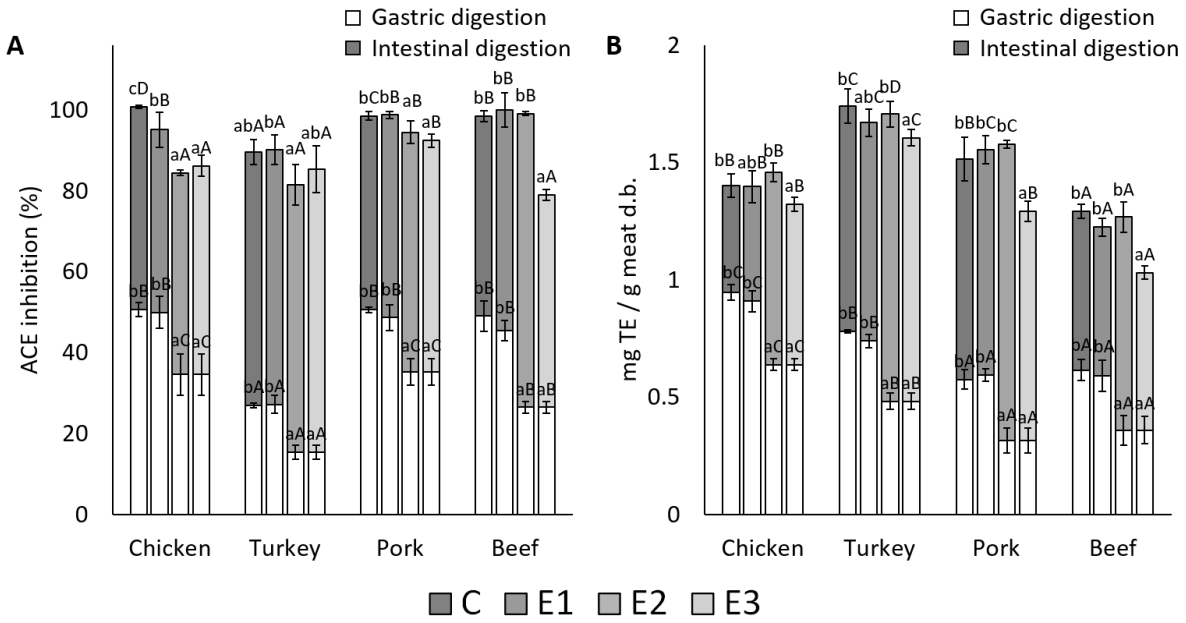
878 **Figure 2**



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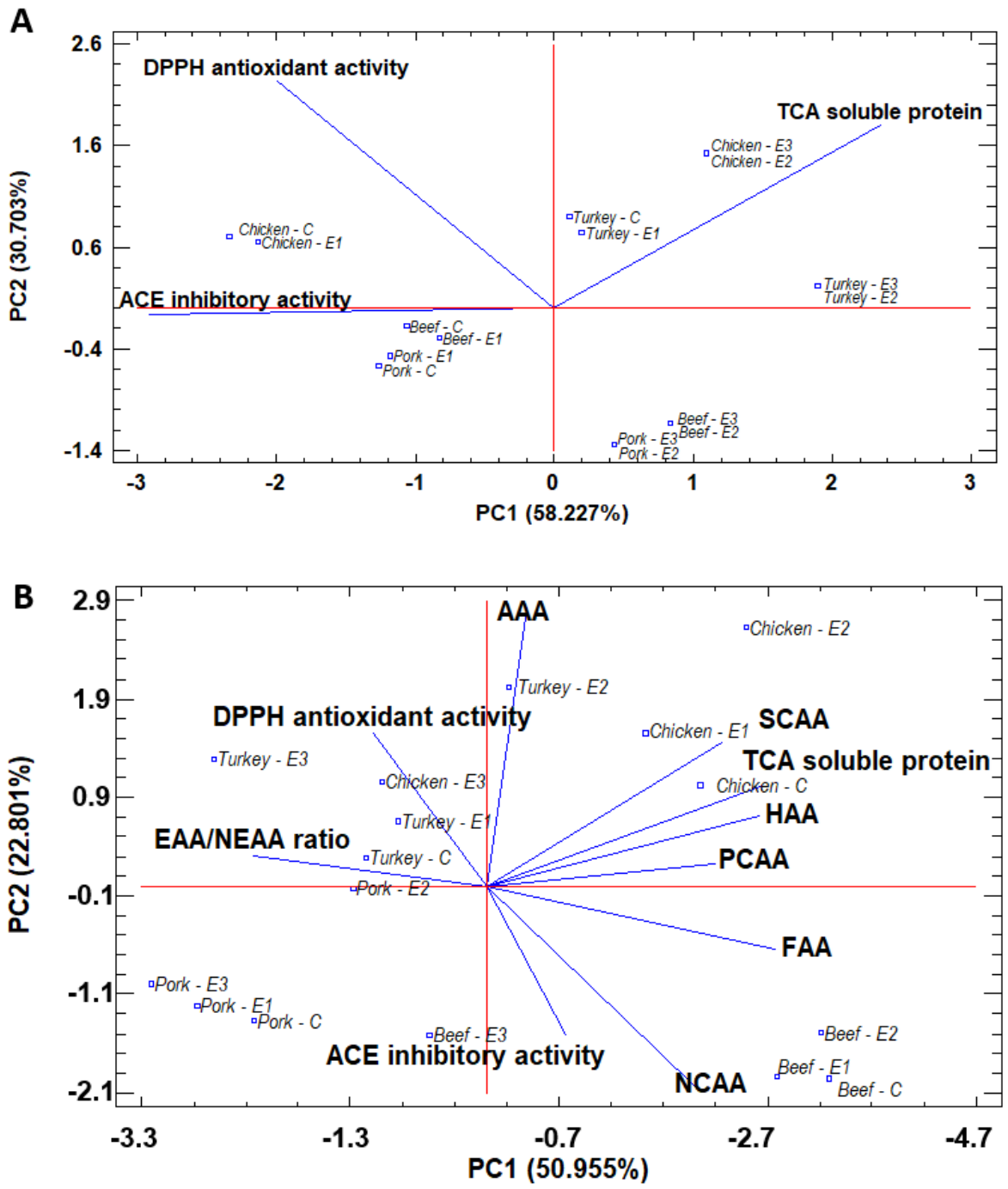
881 **Figure 3**



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