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The impact of age-related digestive disorders on *in vitro* digestibility of macronutrients and bioaccessibility of minor components of chia seeds

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ABSTRACT

Gastrointestinal (GI) functions deteriorate with age, primarily affecting protein digestion. The consumption of chia seeds may be helpful for the elderly because they offer a vegetable-based source of proteins, healthy lipids, fibre and micronutrients. The impact of common age-related GI deterioration on chia seed digestibility was assessed using *in vitro* digestion models. The goal was to study the potential of chia seeds as part of the diet of seniors. Deterioration in the oral, gastric and intestinal stages of digestion was cumulatively assessed in three digestion models: E1 (deterioration in oral conditions), E2 (deterioration in oral and gastric conditions) and E3 (deterioration in oral, gastric and intestinal conditions). Less efficient chewing (E1) decreased proteolysis, lipolysis and antioxidant capacity ($p < 0.05$). In contrast, deterioration in gastric functions seemed to affect only total polyphenolic content. Finally, in the model simulating the greatest deterioration in digestive functions (E3), all measured variables were negatively affected (proteolysis, lipolysis, amino acid release, total phenolic content, antioxidant capacity and calcium). Calcium bioaccessibility fell by 24% with a decrease in pancreatic enzymes and bile secretion (E3). Age-related reduced digestive function did not affect the ratio of essential to non-essential amino acids in the digested samples in any case. However, under suboptimal GI conditions (E3), amino acids such as valine, leucine and isoleucine, which are important for sarcopenia prevention in the elderly, fell by 39%, 49% and 44%, respectively. These findings might be helpful for further *in vitro* studies of chia seeds as a possible food ingredient. They may also be useful for the development of more targeted nutrition strategies in the elderly.

Keywords: ageing; protein hydrolysis; amino acid release; lipid hydrolysis; antioxidants

1. Introduction

Population growth, mainly due to increased life expectancy, is a major global concern. It is estimated that the global population in the year 2050 will exceed 9.7 billion. The population aged over 60 years old is expected to reach 2 billion by 2045 (UN, 2019). One of the major challenges facing the scientific community and health professionals involves ensuring the quality of life of the elderly. Therefore, it is crucial to minimise the impact of chronic diseases and to curb the deterioration of their physical condition. Ageing affects gastrointestinal (GI) function. It reduces sensory perceptions, salivation, oral health and other aspects such as enzymatic and bile production, fluid secretion at different stages, mobility and transit time (Rémond et al., 2015; Shani-Levi et al., 2017). Diminished GI function commonly hinders macro and micronutrient release and hydrolysis. It therefore hampers bioabsorption in the elderly. Hence, it is important to ensure the correct intake of macro and micronutrients with high digestibility to avoid malnutrition associated with low absorption (Shani Levi et al., 2017). A lack of protein ingesta or protein absorption is associated with sarcopenia and asthenia. Crucially, however, the nutritional quality of proteins depends not only on the dietary source but also on digestibility, bioavailability and amino acid profile (Bax et al., 2012). The European Society for Clinical Nutrition and Metabolism emphasises the importance of consuming foods that are especially rich in essential amino acids such as leucine and tryptophan (Morley, 2016; Volkert et al., 2018). Chia seeds (*Salvia hispanica* L.) contain around 15% to 24% protein, which is more than all cereals and most legumes (Hernández-Olivas, Muñoz-Pina, et al., 2021; Orona-Tamayo et al., 2017; Rodríguez et al., 2020). They offer not only a variety of essential amino acids but also a potential source of bioactive peptides. These bioactive peptides are associated with lower levels of cardiovascular disease, type 2 diabetes mellitus, obesity and low-density lipoprotein (LDL) cholesterol (Sá et al., 2019). Chia seeds are also a good supply of complex carbohydrates such as fibre (26%–41%) and fat (25%–40%), as well as minor components such as phenolic compounds, vitamins and minerals (Ayerza & Coates, 2011). Regarding their lipidic fraction, chia

seeds have a high essential fatty acid content, of which almost 55% to 60% are α-linolenic acid (ω-3), 18% to 20% are α-linoleic acid (ω-6) and 6% are monounsaturated (ω-9). They also have a low percentage (10%) of saturated fatty acids (Orona-Tamayo et al., 2017). Chia seeds are a good source of not only macronutrients but also a variety of compounds that can have beneficial functions. Examples include some phenolic acid compounds such as chlorogenic, caffeic ferulic and rosmarinic acids, flavanols such as myricetin, quercetin and kaempferol, and tocopherols (Valdivia-López & Tecante, 2015). Finally, its calcium content makes chia one of the most important vegetable sources of calcium, along with sesame seeds. Chia has a calcium content of about 600 mg per 100 grams, up to six times more than milk (Muñoz-Tébar et al., 2019). These properties have led chia to be considered a health food product. Chia has become a highly used ingredient in different formats. For instance, it is used as a topping in salads, cereals, desserts, yogurts and smoothies. Nonetheless, the digestibility of chia seeds' macro and micronutrients may vary depending on the influence of different degrees of deterioration in GI functions, such as those that occur in the elderly.

This study evaluates the impact of common age-related deterioration in GI functions on proteolysis, amino acid release and lipolysis in chia seeds. The study also examines the impact on calcium bioaccessibility, the release of total polyphenols and these polyphenols' antioxidant capacity in chia seeds. The analysis was performed using a static *in vitro* digestion model.

2. Materials and methods

2.1. Materials

Pepsin from porcine gastric mucosa (3200–4500 U/mg), porcine pancreatin (8 x USP), bovine bile (dried, unfractionated), other analytical grade salts and reagents, and chromatographic grade solvents were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). The EZ-Faast amino acid kit (Phenomenex, Torrance, CA, USA) was used. The chia seeds (*Salvia hispanica* L., Hacendado®, Valencia, Spain) were purchased at a local supermarket in Valencia (Spain). The entire study was performed with the same batch.

2.2. Chemical characterisation of chia seeds

Water, ash, lipid, fibre and crude protein (Kjeldahl factor of 5.71) contents were characterised in the samples following the Association of Official Analysis Chemists International (AOAC International, 2000) official methods 934.01, 942.05, 920.39, 962.09 and 960.52, respectively. Chia's initial sugar content was also determined by quantifying glucose using the DNS colorimetric method described by Armellini et al. (2019). Ash was dissolved in a 20% (v/v) nitric acid solution, and La (III) was added to 0.1% (w/v) to determine calcium content using an iCE 3000 Series flame atomic absorption spectrometer (Thermo Scientific, Waltham, MA, USA). A ratio of air to acetylene of 11.5:1.5 L/min was used in the flame. Samples were measured at 422.7 nm. $CaCO_3$ was used to obtain a calibration curve of calcium from 0 to 10 mg/L (Noël et al., 2008).

Cold lipid extraction was performed to analyse the chia seeds' lipid profile through proton nuclear magnetic resonance (^{1}H NMR) using a Bruker Ascend 400/R (Bruker Avance, BioSpin, Inc., Billerica, MA). This procedure used a spectral width of 6410 Hz, relaxation delay of 3 s, 64 scans, acquisition time of 4.819 s and pulse width of 90º (Hernández-Olivas, Muñóz-Pina, et al., 2021; Nieva-Echevarría et al., 2018). FT-IR spectra from the extracted lipids were recorded in attenuated total reflectance (ATR) mode at room temperature using a JASCO-4100 FT-IR spectrometer (Japan, Tokyo, provided by JASCO Co., Ltd, Shanghai, China). The spectra were taken at 4 cm^{-1} resolution in a wavelength range of 650 to 4000 cm^{-1}. On average, the minimum number of scans was 32. Finally, total polyphenol content was quantified (Muñoz-Pina et al., 2022), and antioxidant capacity was determined using 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and ferric reducing antioxidant power (FRAP) methods (Sánchez-García et al., 2022).

2.3. Static *in vitro* digestion

Chia seeds were digested under a control model (C) corresponding to the standard GI conditions of a healthy adult (Minekus et al., 2014). In parallel, three models of elderly GI functions simulated the cumulative deterioration that appears with ageing. These models were Elderly 1 (E1), which simulated deterioration in the oral stage of digestion, Elderly 2 (E2), which simulated deterioration in the oral and gastric stages of digestion, and Elderly 3 (E3), which simulated deterioration in the oral, gastric and intestinal stages of digestion (E3). Table 1 shows the specific conditions of each digestion model. Specific digestive conditions in the elderly were established (Hernández-Olivas, Muñoz-Pina, et al., 2021) based on the research of Shani-Levi et al. (2017), Denis et al. (2016) and (Russell et al., 1993). Gastric digestion time was established based on a preliminary study and the indications of Denis et al. (2016). Intestinal digestion time was stated as by Shani-Levi et al. (2017) with slightly modifications (4 h instead of 3 h trying to simulated the worst scenario). Gastric pH was raised considerably to simulate achlorhydria/hyposecretors in the elderly (Hurwitz et al., 2003; Russell et al., 1993).

In a preliminary study, chewing (number of mastication cycles) was standardised (Jalabert-Malbos et al., 2007). It was performed *in vivo* using a healthy volunteer (male student, 30 years old) with good dentition. For the elderly, the number of chewing cycles was reduced to 50% (Hernández-Olivas, Muñoz-Pina, et al., 2021; Lee et al., 2004; O'Keeffe et al., 2019). To provide a degree of rupture similar to that of the previously described *in vivo* chewing, chia seeds can be crushed using a mechanical grinder (Taurus Aromatic SP-7407 50 Hz, Ø80 mm grinding dial at 1480 rpm; Lleida, Spain). To determine the particle size distribution, broken chia seeds were poured onto a stack of nine sieves with apertures of 1.4, 1.25, 1, 0.71, 0.5, 0.355, 0.250, 0.180 and 0.125 mm. The broken seeds were sieved using an electromagnetic Filtra Vibración IRIS FTS-0200 sieve shaker (Filtra Vibración, Spain). The particles retained on each sieve were weighed and the results expressed as a cumulative curve using the particle mass falling through each sieve (Peyron et al., 2011).

Independent digestions were performed in parallel at least three times using each model of GI functions (C, E1, E2 and E3). After the gastric digestion, pH was raised to 7 in order to inactivate pepsin and to prepare the environment of the small intestine (Brodkorb et al., 2019). At the end of the intestinal digestion, the digests were kept in an ice bath and the pH was adjusted before the fractions were separated and aliquots were taken and immediately frozen. In the event that some pepsin activity remains in conjunction with trypsin activity from pancreatin, pH 5 is the value at which both enzymes have the lowest activity at the same time (Piumetti et al., 2022).To separate the liquid fraction (bioaccessible fraction) from the remaining undigested solids, the sample was centrifuged at 4000 g-force for 5 min at 10 °C (5810R, Eppendorf, Hamburg, Germany). The supernatant was considered to be the bioaccessible fraction, providing the source for the aliquots used in the determinations. Aliquots were immediately frozen at -40 °C until analytical determinations. For lipid digestibility analyses, independent digestion tubes were run in parallel and used in full for cold lipid extraction and ^{1}H NMR and FT-IR analysis.

2.4. Protein digestibility

Protein hydrolysis was evaluated by measuring the soluble protein fraction in trichloroacetic acid (TCA). Measurement was based on the procedure described by Hernández-Olivas et al. (2022), with slight modifications. Briefly, 36% TCA (500 µL) was added to the bioaccessible fraction (1000 µL) to give a final concentration of 12% (w/w). The protein extract was prepared by mixing, incubating at 10 °C 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 and a pH 10 buffer. The ratio of supernatant to buffer (v:v) was 1:75 for intestinal digested samples. Soluble protein in TCA was determined from three replicates by measuring absorbance at 280 nm against a blank prepared with appropriate digestion fluids for each digestion model. TCA soluble protein (TCA s.p. %) was calculated using a calibration line of L-tyrosine as standard (Eq. 1).

158 $$TCA\ s.p.(\%) = \frac{g\ TCA\ s.p. in\ the\ bioaccessible\ fraction}{g\ crude\ protein\ in\ undigested\ chia\ seeds} \times 100 \quad (1)$$

159 Essential (EAA) and non-essential (NEAA) amino acids from protein digestion were determined
160 (Hernández-Olivas, Muñoz-Pina, et al., 2021). Briefly, the free amino acids (FAA) in 100 µL of the
161 bioaccessible fraction were derivatised at room temperature in aqueous solution using the EZ-
162 Faast amino acid kit. Samples were analysed using a gas chromatograph (7820 A, CG System,
163 Agilent Technologies, California, USA) coupled with a flame ionisation detector (FID). Derivatised
164 amino acid solution (2 µL) was injected at 250 °C in split mode (1:15) onto a 10 m × 0.25 mm ×
165 0.15 µm Zebron ™ ZB-AAA GC column (Phenomenex, Torrance, CA, USA). The oven temperature
166 was kept at 110 °C for 1 min. It was then increased by 30 °C/min to 320 °C and held at 320 °C for
167 2 min. The transfer line was held at 320 °C. The carrier gas was helium at a constant flow rate of
168 1.1 mL/min. Norvaline was used as an internal standard. The free amino acids (FAA) released
169 (%) during digestion with respect to the amount of crude protein in undigested chia was
170 calculated (Eq. 2).

171 $$FAA\ released(\%) = \frac{g\ FAA\ in\ the\ bioaccessible\ fraction}{g\ crude\ protein\ in\ undigested\ chia\ seeds} \times 100 \quad (2)$$

172 **2.5. Lipid digestibility**

173 Whole digesta samples were subjected to cold liquid–liquid extraction. The composition of the
174 lipids, in terms of molar percentages of monoglycerides (1-MG and 2-MG), diglycerides (1,2-DG
175 and 1,3-DG), triglycerides (TG) and fatty acids (FA), was determined by ^{1}H NMR following the
176 procedure previously described for the chemical characterisation of the chia seeds. Absorbable
177 and non-absorbable lipid fractions, as well as the extent of lipolysis, were calculated according
178 to Eq. 3–5:

179 $$Absorbable\ lipid\ fraction\ (\%) = AG_{2-MG}\ \% + AG_{1-MG}\% + FA \quad (3)$$

180 $$Non-absorbable\ lipid\ fraction\ (\%) = AG_{1,2-DG}\ \% + AG_{1,3-DG}\% \quad (4)$$

181 $$Extent\ of\ lipolysis\ (\%) = AG_{1,2-DG}\ \% + AG_{1,3-DG}\% + AG_{2-MG}\ \% + AG_{1-MG}\% + FA \quad (5)$$

Here, AG corresponds to the acyl groups supported on the glyceryl backbone structures (TG, 1,2-DG, 1,3-DG, 2-MG and 1-MG) and fatty acids (FA). FT-IR spectra from the extracted lipids of the digested chia seeds under the different GI conditions were recorded as previously indicated for the extracted lipids for undigested chia seeds. The use of FT-IR spectra enabled qualitative analysis of lipid digestibility by examining the intensity and area under the curve (AUC) from the peaks corresponding to TG and FA.

2.6. Calcium bioaccessibility

An aliquot (4 mL) of the bioaccessible fraction was used for free calcium determination. Flame atomic absorption spectroscopy (FAAS) was applied following the same procedure as the one used to determine the total amount of calcium in undigested chia seeds. The bioaccessibility of calcium was estimated using Eq. 6.

$$Calcium\ bioaccessibility\ (\%) = \frac{mg\ Ca^{2+} free\ in\ bioaccessible\ fraction}{mg\ Ca^{2+}\ in\ undigested\ chia\ seeds} \times 100 \quad (6)$$

2.7. Total polyphenol content and antioxidant capacity

For the phenolic content and antioxidant capacity determinations, an aliquot (1 mL) of the bioaccessible fraction o no was taken. A methanolic extract was obtained by mixing 1:1 (v/v) with pure methanol. Incubation was performed at room temperature using 100 rpm on an Eppendorf Thermomixer Comfort (Eppendorf AG 22331, Hamburg, Germany) for 30 min and 10 min centrifugation at 6000 g-force (Minispin plus, Eppendorf, Hamburg, Germany). The determinations were performed for the supernatants following the procedures described earlier. Polyphenol bioaccessibility (%) was calculated using Eq. 7.

$$Polyphenol\ bioaccessibility\ (\%) = \frac{mg\ GAE\ in\ bioaccessible\ fraction}{mg\ GAE\ in\ undigested\ chia\ seeds} \times 100 \quad (7)$$

2.8. Statistics

The results of the analytical determinations were examined using analysis of variance (ANOVA). The least significant difference (LSD) Fisher test was performed to identify homogeneous groups between the *in vitro* models. The Pearson correlation test was used to find the correlation between protein digestibility according to the two applied analytical methods (TCA-soluble protein and free amino acids), between lipid digestibility according to the two applied analytical methods (^{1}H NMR and FT-IR) and between antioxidant capacity according to DPPH, ABTS and FRAP. Principal component analysis (PCA) was also performed to understand the descriptive relationships between the digestion parameters (TCA soluble protein, free amino acids, extent of lipolysis, calcium bioaccessibility, total polyphenol and antioxidant capacity) and the host GI conditions of a standard healthy adult (C) and the elderly (E1, E2 and E3). Statgraphics Centurion XVII was used at a confidence level of 95% ($p < 0.05$).

3. Results and discussion

3.1. Physicochemical characterisation of chia seeds

Table 2 presents the proximate composition of chia seeds in terms of crude protein, lipids, ash, fibre and sugar contents. The values are similar to those previously reported (Gómez-Favela et al., 2017; Olivos-Lugo et al., 2010; Orona-Tamayo et al., 2017). Based on their protein, lipid fibre contents and their low water content, chia seeds are described as a high density food (Kulczyński et al., 2019). Chia seeds provide more protein than most traditionally used vegetable foods such as wheat (14%), corn (14%), rice (8.5%), oats (15.3%) and barley (9.2%; Sandoval-Oliveros & Paredes-López, 2013). However, the protein content of chia seeds is comparable to that of other seeds such as flax (22%–33%) and sesame (17%; Bolaños et al., 2016). The protein profile of chia seeds has high biological value given the high presence of essential amino acids. These amino acids are mainly found in peptide sequences of globulins (52%–54%), albumins (17.3%–18.6%), glutelin (13.6%) and prolamin (17.9%; Orona-Tamayo et al., 2017). The lipid content of chia seeds (43%) has high amounts of polyunsaturated fatty acids.(Kulczyński et al., 2019).

Table 2 also displays values for some minor constituents of chia seeds, as well as their antioxidant capacity. The calcium content found in this study (780 ± 2 mg/100 g) is consistent with the values reported for chia seeds in previous research. It is about twice as high as for golden flax seeds (U.S. Department of Agriculture, 2019). The calcium content of chia seeds is above that found in other vegetable sources (Pająk et al., 2019). One serving of chia seeds (30 g) could provide around 20% of the recommended daily intake of 1200 mg for elderly persons (Severo et al., 2009). Finally, chia seeds were found to provide large amounts of polyphenols (more than 3 mg/g), corroborating the values reported by Menga et al. (2017), Ghafoor et al. (2022) and Pająk et al. (2019). Caffeic acid, ferulic acid, chlorogenic acid, p-Hydroxybenzoic acid, vanillin and vanillin acid, among other phenols, are reported to make the largest contribution to chia's antioxidant capacity (Menga et al., 2017). Chia has a similar polyphenol content to that of other seeds such as sesame, flax and hemp (Chen et al., 2020; Teh & Birch, 2014). The antioxidant capacity of chia seeds according to DPPH, ABTS and FRAP is consistent with the values reported by Pająk et al. (2019). The lowest values of mg TE/g were observed using DPPH, followed by ABTS and FRAP.

To understand the influence of chewing on further nutrient digestion and the release of bioactive compounds from the matrix, the particle size distribution of chia seeds was assessed. Figure 1 shows the particle size distribution of raw and broken seeds under the control and elderly chewing processes. The fraction of unbroken seeds retained on the 1 mm sieve was 98% of the raw chia seeds. After the control chewing simulation, 50% of the seed matter was larger than 1 mm. The other fraction consisted of the broken seeds, which had a length of 0.18 mm to 0.7 mm. The simulated elderly chewing process resulted in fewer broken seeds (28% of particles smaller than 1 mm). A difference of 20% was observed between broken chia seeds under control and elderly conditions. Thus, a half and a third of disruption post-treatment could be ascribed to the C and E1 chewing conditions, respectively.

3.2. The impact of age-related deterioration in GI functions on protein digestibility of chia seeds

The results of protein digestibility (TCA soluble protein) ranged from 31 ± 2 g to 18.7 ± 0.5 g tyrosine/100 g protein (Figure 2A). Two-fold values of *in vitro* protein digestibility have been reported for isolated protein fractions in different chia cultivars (Sá et al., 2020). In this study, the highest value was observed under standard GI conditions (C). This value is consistent with the value reported by Monroy-Torres et al. (2008) for ground Mexican chia seeds (29%). Under E1, there was a significant decrease in protein digestion of 11% with respect to C, indicating that reductions in chewing cycles decrease protein digestion. Seemingly, if the particle size remains larger, there is less enzyme accessibility because of the lower surface area, which decreases protein digestibility. Similar results have been found for other foods such as nuts, cheese and fish (Hernández-Olivas, Muñoz-Pina, Andrés, et al., 2020; Hernández-Olivas, Muñoz-Pina, Sánchez-García, et al., 2020; Paz-Yépez et al., 2019). Protein digestibility in the E2 and E3 models was also significantly lower, suggesting that the elderly have poorer digestion (Hernández-Olivas et al., 2022; Hernández-Olivas, Muñoz-Pina, Andrés, et al., 2020; Hernández-Olivas, Muñoz-Pina, et al., 2021; Hernández-Olivas, Muñóz-Pina, et al., 2021; Hernández-Olivas, Muñoz-Pina, Sánchez-García, et al., 2020). The reduction in pepsin concentration and the rise in pH value seemed not to affect protein digestion negatively. However, because proteolysis was measured only after intestinal digestion, the standard intestinal conditions may have compensated for the deterioration in gastric functions. Specifically, a reduction of 41% was observed in the E3 model with respect to the control model (C).

Some elderly oral deteriorations such as tooth loss, salivary defect and motor impairment increase the risk of aspiration and choking (Peyron et al., 2017). These complications are commonly caused by an inability to reduce the particle size of foods after chewing. This inability leads to impaired protein disruption and amino acid release and absorption. Impaired chewing

of chia seeds resulted in a reduction in the number of broken seeds. Proteins from plant foods have lower enzyme accessibility because of rigid cell walls and seed coats (Sá et al., 2019). However, despite fewer chewing cycles, significant differences were not found between the C and E1 models for the total release of free amino acids (Figure 2B). The hydrolysis from pepsin in the gastric stage and trypsin and chymotrypsin in the intestinal stage may have compensated for the negative impact of impaired chewing. This compensation may be important for the elderly because chewing problems are common. This factor determines dietary changes in elderly people, who seek smoother and easier-to-swallow foods to help with deglutition and increase the use of nutrients in specific physiological functions. Chia seeds are small and hard, with a resistant structure that makes them difficult to break. However, if chia seeds are processed (e.g. milled into a powder), the hydrolysis of chia proteins greatly increases (Calvo-Lerma et al., 2020). The total release of free amino acids (Figure 2B) from the chia seed matrix did not change significantly from E1 to E2. Contrary to expectations, the values resulting from a 75% reduction in pepsin concentration and an increase to pH 6 did not seem to affect proteolysis significantly. Similar behaviour has been reported in protein-rich foods of animal origin under the same digestive conditions (Hernández-Olivas et al., 2022). Even with a lower pepsin concentration, the higher pH value promotes greater protein solubilisation and consequently easier hydrolysis by enzymes. The E2 and E3 models were compared to observe age-related deterioration in GI functions due to reduced pancreatic and biliary capacity (with up 50% reductions in both). The results show a marked negative impact on the release of free amino acids after complete digestion.

The amino acid profile under the GI conditions of a healthy adult (C) is shown in Table 3. Amino acids are presented in order: first those considered essential (EAA), followed by those considered non-essential (NEAA). Regarding EAA, high contents of lysine (Lys) and leucine (Leu) were found. Together with the amino acids in collagen, Lys positively affects elderly health by building muscle tissue, cartilage, connective tissues and skin (Liao et al., 2015). In addition to the

synthesis of muscle mass, Leu contributes as a signal to initiate the rate-limiting translation initiation step of muscle protein synthesis (MPS; Crozier et al., 2005). An adequate dose of Leu in the absence of other amino acids has even been reported to stimulate MPS close to maximal levels (Wilkinson et al., 2018). However, low concentrations of two EAAs were found: methionine (Met) and threonine (Thr). These low concentrations may be of interest given that Met participates in the production of homocysteine, which, in low levels, could help prevent deterioration in cognitive function and dementia (Garcia & Zanibbi, 2004). Also, in addition to Lys, Phe and Ala, Thr has been reported as critical because its intake is important for the maintenance of cognitive function in seniors (Kinoshita et al., 2021).

Tyr was the most abundant NEAA (with 43.5 ± 1.8 mg/g protein) among the NEEA released under control (C) conditions. In contrast, Pro, Gly and Asn were the least abundant, with 4.6 ± 0.4 mg/g protein, 5.2 ± 0.4 mg/g protein and 9.3 ± 0.9 mg/g protein, respectively. The fact that chia seeds were deficient in prolamins (a type of protein mainly formed by Pro but also Gln) could be a positive factor because prolamins contribute to gluten intolerance. Hence, chia seed consumption may be recommended for subjects suffering from celiac disease (Hernández-Olivas, Muñoz-Pina, et al., 2021). Gly insufficiency may lead to a chronic shortage that causes suboptimal growth, impaired immune response and other adverse effects on health and nutrient metabolism (Wang et al., 2013). The Pearson correlation test revealed a high correlation of 0.9098 between the two methods for assessing protein end-digestion products. Thus, although the values for FAA were lower than for TCA soluble protein, they still accounted for a greater fraction of protein digestion.

Bile acts on the micellisation and emulsification of lipids, leading to greater digestion of not only lipids but also proteins (Salvia-Trujillo et al., 2017). When the GI conditions of elderly people were fully simulated (E3), there was a 37% reduction in the extent of proteolysis with respect to the control (C) conditions. Essential amino acids (EAA) were more affected than non-essential

amino acids (NEAA), as reported in Table 3, with a 39% and 35% reduction, respectively. Reductions of 20% to 50% were observed for individual free amino acids from the control (C) model to the model simulating the greatest deterioration in GI functions (E3). Phe, Leu, Ile and Met were most affected (50%, 49%, 44% and 40%, respectively). Among NEAA, Tyr was heavily affected, with a reduction of 41%.

The proportion of essential amino acids (EAA) to non-essential amino acids (NEAA) is considered an indicator of protein quality. This factor is important because age-related disorders could affect both the total release of amino acids and the type of protein essential units. The EAA to NEAA ratio (Table 3) was slightly greater than 1 (ranging from 1.07 to 1.13). It varied depending on the GI conditions. It was greater for the C and E1 models than for the E2 and E3 models. Only gastric atrophy in the E2 model seemed to have a negative impact on this ratio. However, it can be offset by intestinal enzymes and bile salts, still with reductions in E3. Lower amounts of EAA than NEAA have been reported for non-digested seeds such as chia and brown and gold flax seeds (Nitrayová et al., 2014; Olivos-Lugo et al., 2010). However, regardless of the digestive conditions, the results revealed that the release of EAA seems to be helped by GI digestion because the ratio remained above 1. These levels are lower than those for the majority of animal proteins. For example, the percentages of EAA with respect to the total amino acids for beef, eggs and cow's milk are, respectively, 52%, 55% and 54% (Olivos-Lugo et al., 2010).

In sum, chia seeds were observed to have a high essential amino acid (EAA) content with high bioaccessibility. Furthermore, chia seeds presented a higher scoring pattern in Trp, Val and aromatic amino acids (AAA) than the scoring pattern (mg/g protein) requirement for adults (FAO, 2011).

3.3. The impact of age-related deterioration in GI functions on lipid digestibility of chia seeds

354 The analysis of the ^{1}H NHR spectra provided molar percentages of acyl groups (AG) and fatty
355 acids (FA) derived from triglyceride hydrolysis (TG) in the non-digested (ND) chia seeds and
356 digesta (C, E1, E2 and E3) of chia seeds (Table 4). As expected, the total lipidic fraction of ND chia
357 seeds mainly consisted of triglycerides (99.7%), with an almost negligible amount of 1-
358 monoglycerides (1-MG). Nonetheless, TG decreased significantly, while FFA increased, as did
359 other hydrolysed lipidic fractions. The conversion of TG due to the hydrolytic action of pancreatic
360 lipase mainly resulted in FFA (16.5%), followed by 1,2-DG, 1,3-DG, 1-MG and 2-MG under control
361 (C) conditions. The extent of lipolysis in chia seeds was only 20%. This value is very low compared
362 to the value for other foods digested under the same GI conditions, for which values of above
363 70% have been reported (Hernández-Olivas, Muñoz-Pina, Andrés, et al., 2020; Hernández-
364 Olivas, Muñóz-Pina, et al., 2021; Hernández-Olivas, Muñoz-Pina, Sánchez-García, et al., 2020).
365 The structure of chia hinders the contact of lipids with enzymes and bile salts. It thus hinders the
366 solubilisation of fat in digestive fluids. Therefore, chia needs to be broken and opened to allow
367 this contact for hydrolysation (Calvo-Lerma et al., 2020). In the present study, only about one
368 third of seeds were broken or opened during chewing under normal conditions, which would
369 explain the low digestibility. As Table 4 shows, when the chia seeds were digested under E1
370 (deterioration in oral functions), there was a significant decrease in the absorbable fraction from
371 17.3% to 11.5%, as well as in the extent of lipolysis (from 19.9% to 13.6%). This result supports
372 the idea that in the case of lipid digestibility, breaking seeds is crucial to increase the release of
373 fat and thus ensure greater contact with digestive enzymes. This situation is in contrast to that
374 for protein. In the case of protein, it was not necessary to open the seeds because the high
375 solubility allowed the extraction of protein from the seed shell. Other factors, such as the high
376 concentration of calcium, might lower the digestion of the chia fat. Phospholipids have been
377 reported to be found in low levels of less than 1% in chia seed oil (Bushway et al., 1984). They
378 may interact with calcium ions, thus decreasing the release of FFA, which also impedes lipase

379 action. In this case, the high amount of calcium ions in the chia seeds makes this interaction
380 easier (Pizones Ruiz-Henestrosa et al., 2022).

381 Regarding gastric and intestinal deterioration in the elderly, there were no significant
382 differences ($p < 0.05$) between models E1 and E2 for any parameter. Seemingly, deterioration in
383 gastric functions did not affect lipidic digestibility at the end of luminal digestion. Nonetheless,
384 in the E3 model, there was a significant decrease ($p < 0.05$) with respect to the other models (C,
385 E1 and E2). Despite the longer intestinal transit time in the E3 model (4 h instead of 2 h), the
386 reduced pancreatic lipase and bile concentrations exerted a negative effect on lipid digestion.
387 In this scenario, only 7% of lipids were in the absorbable fraction. In the worst case scenario, the
388 extent of lipolysis would fall by 45% for chia seeds.

389 *In vitro* digested chia lipids under the different gastrointestinal conditions of elderly individuals
390 were also analysed using FT-IR. The aim of this analysis was to confirm the results from the ^{1}H
391 NMR analysis. The spectra (Figure 3) show a typical fat spectrum in the range of 4000 to 600 cm^{-1}.
392 The absorption bands at 1461 cm^{-1} and 1745 cm^{-1} in the CO were attributed to the vibration
393 bending of lipid CH_2 groups and the ester carbonyl stretching (C=O) of fatty acids (Shen et al.,
394 2018). There was an intense peak at 1200 cm^{-1} related to the asymmetrical stretching of C(=O)–
395 O–CH_3 (Kollar et al., 2017). However, the major changes observed between all the samples were
396 in the region between 1650 and 1800 cm^{-1}. More specifically, they were in the bands at 1706
397 cm^{-1} to 1741 cm^{-1}. The band at 1741 cm^{-1} (Fig. S2) was attributed to the stretching vibrations of
398 the carbonyl group of triglyceride esters (–C=O), whereas the band at 1706 cm^{-1} was related to
399 the fatty acids (Daoud et al., 2019; Kollar et al., 2017). In the non-digested sample, the band
400 related to the fatty acids was not present, whereas the band at 1741 cm^{-1} had the largest area,
401 in line with the NMR results. In contrast, after digestion, the control sample had a higher band
402 at 1706 cm^{-1} but lesser in the triglyceride band. The Pearson correlation test showed strong
403 correlation between the FA and TG values from ^{1}H NMR and FT-IR analysis, with r^2 values of

0.9918 and 0.997, respectively. This result indicates that the two methods offer alternatives to evaluate lipid digestibility and confirms the previous results for ^{1}H NMR. Nonetheless, even though FA represent the highest fraction of lipid digestibility, ^{1}H NMR analysis is preferable because it can provide deeper insight, revealing information about the entire lipid profile, including TG, DG, MG and FA.

3.4. Minor component bioaccessibility and antioxidant capacity of chia seed digesta

As already confirmed by the study, deterioration in GI functions significantly reduces the digestibility of macronutrients such as proteins and fat. This reduction could lead to lower release and solubilisation of some micronutrients and bioactive compounds. Table 5 shows that free calcium in the bioaccessible fraction was much lower than the total calcium content in the chia seeds (from 780 to 195 mg Ca^{2+}/100 g chia). It could be due to numerous reasons. First, the high lipidic fraction in the seeds may produce insoluble soaps with calcium, thereby lowering its bioaccessibility (Guéguen & Pointillart, 2000). Second, dietary fibre and the presence of phytic acid produce unabsorbable complexes with calcium and reduce the final soluble content (Ghavidel & Prakash, 2007). Regarding suboptimal digestion conditions, calcium bioaccessibility (%) did not significantly change under deterioration in oral functions (E1) or deterioration in gastric functions (E2) in elderly individuals. Differences in the particle size of chia seeds after chewing or when a mechanical disruption was applied did not seem to affect the release of calcium into the bioaccessible fraction. Calvo-Lerma et al. (2020) reported only a 10% increase in calcium bioaccessibility when chia flour was digested compared to when whole chia seeds were digested. Therefore, the difference of only 20% in the number of broken seeds (Figure 1) between C and E1 was not large enough to cause changes in the release of calcium. However, deterioration in intestinal functions (E3) in the elderly significantly reduced the free amount of calcium from 27% to 20%. In the E3 scenario, the total extent of non-digested proteins and lipids was significantly higher than in the C, E1 and E2 models. This situation may affect calcium

429 solubility, lowering its bioaccessibility. If the calcium in chia seeds were totally bioaccessible, a
430 30 g serving of chia seeds would provide 20% of the recommended daily intake. However, a
431 serving under E3 GI conditions would provide 4% of the recommended daily intake. Thus, chia
432 seeds can still be considered a complementary source of calcium in addition to conventional
433 calcium rich foods such as milk, dairy foods and other vegetable sources such as cabbages.

434 In addition to presenting data on calcium, Table 5 also shows polyphenol content and
435 antioxidant capacity in the bioaccessible fraction. According to the results, digestion favoured
436 polyphenol release and incorporation into the bioaccessible fraction, regardless of the GI
437 conditions, providing a bioaccessibility of more than 100%. The Folin–Ciocalteu reagent, used
438 for polyphenol determination, may react with protein end-digestion products, enhancing the
439 spectrophotometric signal. Other non-phenolic compounds released after digestion such as
440 reducing sugars, ascorbic acid, aromatic amines, organic acids and inorganic ions can also
441 undergo a redox process (Carmona-Hernandez et al., 2021; Lowry et al., 1951). The
442 bioaccessibility of total polyphenol fell with respect to the control when age-related
443 deterioration in GI functions was simulated (from around 199% to 140%). This result was
444 expected, given the impact of the E1, E2 and E3 models on macronutrient hydrolysis because
445 polyphenols are trapped and bound to the macronutrients (mainly proteins and complex
446 carbohydrates). Consequently, polyphenol release and bioaccessibility depend heavily on the
447 extent of macronutrient hydrolysis (Quiñones et al., 2012).

448 In chia seeds, both phenolic compounds and fat-soluble compounds have antioxidant capacity.
449 As shown, even though the free polyphenol content increased, the antioxidant capacity
450 (measured by either DPPH or FRAP) decreased after digestion under optimal (model C)
451 conditions (3.34 and 7.48 mg TE/g chia, respectively). This tendency became even stronger when
452 deterioration in the oral stage of digestion (model E1) was introduced (2.5 and 5.8 mg TE/g chia,
453 respectively). There were no significant changes in these parameters from the E1 to E2 models.

This result implies once again that breaking seeds is more relevant than gastric conditions in the release of bioactive compounds with antioxidant capacity. However, deterioration in intestinal functions in model E3 negatively affected antioxidant capacity, with reductions of up to 43%, 40% and 12% compared to the control model (C) for DPPH, FRAP and ABTS, respectively. In the case of the ABTS assay, an increase was observed from undigested to digested chia seeds. Similar results were reported by Pellegrini et al. (2018), who found that the GI process promoted the release of unextractable bioactive compounds from the food matrix and/or their chemical transformation into compounds with greater antioxidant capacity. Nevertheless, the decrease in antioxidant capacity observed in ABTS followed the same pattern as for DPPH and FRAP when there was deterioration in GI functions. The Pearson correlation test showed a significant correlation between DPPH, ABTS and FRAP, with r^2 values of more than 0.95.

3.5. Relationship between end-digestion products of chia seeds and the gastrointestinal conditions of healthy adults and the elderly

Principal component analysis (Figure 4) shows that 95.33% of the variance is explained by the analysis. PC1 (with 83.522%) differentiates E3 from the other GI conditions (C, E1 and E2). It also explains the relationships between EAA and NEAA because the chia seeds result in well-balanced amino acid profiles, regardless of the GI condition (C, E1, E2 and E3). The EAA/NEAA ratio is smaller in the C and E1 models than in the E2 and E3. The total release of polyphenols seems to be related to macronutrient hydrolysis because there was greater solubility after seed disruption. There was greater calcium bioaccessibility and total free amino acids in the E2 model, indicating better solubilisation. There seems to be a close relationship between the absorbable lipid fraction determined by ^{1}H NMR and free fatty acids determined by FT-IR, as well as between the three antioxidant capacity determinations (DPPH, ABTS and FRAP) and proteolysis analysis (sum of free amino acids and TCA s.p.).

4. Conclusions

In this study, the effects of age-related gastrointestinal deterioration were explored. Simulated oral and intestinal deterioration significantly affected the digestibility of chia seeds. Less efficient chewing and reduced biliary and pancreatic functions hindered the digestion of proteins and lipids, total phenolic compound release and antioxidant capacity. Chia seed digestion resulted in well-balanced amino acid profiles, regardless of the GI condition (C, E1, E2, and E3). From a qualitative perspective, chia seed digesta provided more EAA than NEAA. This factor became more heavily affected as gastric and intestinal disorders appeared. An approximately 40% reduction in some amino acids such as Val, Leu and Ile was observed in the model that simulated the greatest deterioration in digestive functions (E3). Lipolysis extent was also affected, falling 6 (E1) and 10 (E3) percentage points with respect to the control model (C). The bioaccessibility of calcium was only significantly affected by reduced biliary and pancreatic functions (E3). The release of polyphenols would seem to be connected to the hydrolysis of macronutrients. In conclusion, the results can support the development of more targeted dietary plans for the elderly. They can also provide a reference for future *in vitro* investigations of chia seeds as a food ingredient.

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Table captions

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

Table 2. Proximate composition (water crude protein, lipids, ash, fibre, sugar, starch, calcium and total polyphenol contents) and antioxidant capacity (DPPH, ABTS, FRAP) of chia seeds. Reported values are the mean and standard deviation of the three replicates.

Table 3. Amino acid profile (mg/g protein) of chia seed luminal digesta obtained under *in vitro* control (C) and different elderly GI conditions (E1, E2 and E3).

Table 4. Molar percentages of acyl groups (AG) supported on different glyceryl backbone structures (TG, 1,2-DG, 1,3-DG, 2-MG, 1-MG) and free fatty acid (FFA) and cholesterol content (mg/g Fat) present in non-digested (ND) and digested chia seeds under *in vitro* GI models: Control (C), Elderly 1 (E1), Elderly 2 (E2) and Elderly 3 (E3).

Table 5. Calcium release, total polyphenols and antioxidant capacity (by DPPH, FRAP and ABTS tests) of digested chia seeds under different *in vitro* GI conditions: Control (C), Elderly 1 (E1), Elderly 2 (E2) and Elderly 3 (E3).

772 **Figure captions**

773 **Figure 1.** Particle size distribution of raw chia seeds after control and (simulated) elderly
774 chewing.

775 **Figure 2.** A: TCA soluble protein (g tyrosine/100 g protein) and B: free amino acids of
776 bioaccessible fraction of intestinal digesta (g free AA/100 g protein) found in chia seeds under
777 *in vitro* digestion models of GI conditions: Control (C), Elderly 1 (E1), Elderly 2 (E2) and Elderly 3
778 (E3). The data shown are mean values from three replicates and the standard deviation.
779 Lowercase letters indicate significant differences between digestion models at a significance
780 level of 95% ($p < 0.05$).

781 **Figure 3**. FTIR spectra of non-digested and digested chia seeds. Inset (A) is an expanded view of
782 the spectral feature in the range of 1680–1800 cm^{-1}.

783 **Figure 4.** Biplot obtained from principal component analysis (PCA) of the digestion parameters
784 (TCA soluble protein, free amino acids, extent of lipolysis, calcium bioaccessibility, total
785 polyphenols and antioxidant capacity) from chia seed digestion and the association with the *in*
786 *vitro* GI conditions of standard healthy adults (C) and the elderly (E1, E2 and E3).

787 **Table 1.**

Digestion model		Oral	Gastric	Intestinal
Control (C)[a]	Input	5 g of chia seeds	Oral bolus	Gastric chime
	Digestive fluid	5 g human salivary fluid	10 mL SGF Pepsin (2000 U/mL)	20 mL SIF Bile (10 mM) Pancreatin (100 U/mL)
	Digestion conditions	2 min 37 °C	pH 3 2 h 37 °C	pH 7 2h 37 °C
	Mechanical force	Mechanical disruption to reach a bolus consistency suitable for safe swallowing (8 presses of the grind button)	Agitation head-over-heels at 55 rpm, holding 1.5 s every 180°	Agitation head-over-heels at 55 rpm, holding 1.5 s every 180°
Elderly (E1, E2 and E3)[b]	Input	5 g of food sample	Oral bolus	Gastric chime
	Digestive fluid	5 g human salivary fluid	10 mL SGF **Pepsin (1500 U/mL)**	20 mL SIF **Bile salts (5 mM)** **Pancreatin (50 U/mL)**
	Digestion conditions	2 min 37 °C	**pH 6** 2 h 37 °C	pH 7 **4 h** 37 °C
	Mechanical force	**50% of Control presses of the grind button**	Agitation head-over-heels, holding 1.5 s every 180° 55 rpm	Agitation head-over-heels, holding 1.5 s every 180° 55 rpm

788 Amendments included in the *in vitro* digestion models for elderly persons with respect to the control model (C) are
789 highlighted in bold. E1 (alterations at oral stage); E2 (alterations at oral and gastric stages); E3 (alterations at oral,
790 gastric and intestinal stages). SGF: simulated gastric fluid; SIF: simulated intestinal fluid. Pancreatin units are based
791 on trypsin activity. [a] Data from Jalabert-Malbos et al. (2007) and Minekus et al. (2014). [b] Data from Denis et al. (2016),
792 Russell et al. (1993) and Shani-Levi et al. (2017).
793

794

795 **Table 2.**

Major components	
Water (g/100 g)	6.1 ± 0.0
Crude protein (g/100 g)	17.4 ± 0.6
Lipids (g/100 g)	43 ± 2
Ash (g/100 g)	4.38 ± 0.04
Fibre (g/100 g)	30 ± 2
Sugars (g/100 g)	0.50 ± 0.07
Minor components and antioxidant capacity	
Calcium (mg/100 g)	780 ± 2
Total polyphenols (mg GAE/ g)	3.43 ± 0.12
Antioxidant capacity (mg TE/ g)	
- DPPH	4.7 ± 0.5
- ABTS	6.6 ± 0.3
- FRAP	15.8 ± 0.7

796 Data shown are mean values from three replicates and the standard deviation.

797

Table 3.

Amino acid (mg free AA/g protein)	Control (C)	Elderly 1 (E1)	Elderly 2 (E2)	Elderly 3 (E3)
Histidine (His)	13.3 ± 0.7^{b}	12.46 ± 1.06^{b}	12.5 ± 0.5^{b}	8.4 ± 0.2^{a}
Isoleucine (Ile)	11.1 ± 1.0^{b}	10.1 ± 0.3^{b}	10.0 ± 0.5^{b}	6.3 ± 0.2^{a}
Leucine (Leu)	22 ± 2^{c}	19.1 ± 0.8^{bc}	18.1 ± 0.9^{b}	10.9 ± 0.6^{a}
Lysine (Lys)	30 ± 4^{b}	31 ± 2^{b}	37.4 ± 1.2^{c}	22 ± 4^{a}
Methionine (Met)	6.1 ± 0.5^{b}	5.3 ± 0.2^{b}	4.97 ± 0.14^{c}	3.6 ± 0.2^{a}
Phenylalanine (Phe)	14.8 ± 1.4^{c}	13.0 ± 0.8^{b}	11.5 ± 0.6^{b}	7.4 ± 0.2^{a}
Threonine (Thr)	8.4 ± 0.5^{bc}	7.83 ± 0.14^{b}	9.0 ± 0.4^{c}	5.5 ± 0.3^{a}
Tryptophan (Trp)	13.63 ± 1.07^{b}	13.0 ± 0.9^{b}	12.9 ± 0.9^{b}	8.4 ± 0.2^{a}
Valine (Val)	14.7 ± 1.2^{c}	13.3 ± 0.3^{b}	14.0 ± 0.7^{bc}	9.0 ± 0.5^{a}
Alanine (Ala)	10.27 ± 1.04^{b}	9.4 ± 0.5^{b}	10.1 ± 0.4^{b}	6.7 ± 0.6^{a}
Asparagine (Asn)	9.3 ± 0.9^{b}	8.7 ± 0.3^{b}	9.6 ± 0.5^{b}	5.8 ± 0.4^{a}
Aspartic acid (Asp)	7.0 ± 0.6^{b}	6.60 ± 0.06^{b}	8.3 ± 0.5^{c}	4.7 ± 0.3^{a}
Cystine (C-C)	17.6 ± 0.9^{b}	17.5 ± 0.7^{b}	19.7 ± 0.9^{c}	10.8 ± 0.3^{a}
Glutamine (Gln)	12.8 ± 1.0^{bc}	10.4 ± 1.3^{b}	14 ± 2^{c}	7.0 ± 0.7^{a}
Glycine (Gly)	5.2 ± 0.4^{bc}	5.00 ± 0.14^{b}	5.6 ± 0.3^{c}	3.30 ± 0.10^{a}
Proline (Pro)	4.6 ± 0.4^{bc}	4.3 ± 0.2^{b}	4.8 ± 0.2^{c}	3.63 ± 0.10^{a}
Serine (Ser)	10.7 ± 0.6^{bc}	10.01 ± 0.09^{b}	11.6 ± 0.5^{c}	7.5 ± 0.6^{a}
Tyrosine (Tyr)	43.5 ± 1.8^{c}	39 ± 2^{b}	38.01 ± 1.06^{b}	24.2 ± 0.7^{a}
EAA/NEAA ratio	1.13 ± 0.20^{b}	1.13 ± 0.04^{b}	1.07 ± 0.08^{a}	1.07 ± 0.18^{a}

Data shown are mean values from three replicates. Lowercase letters indicate significant differences between digestion models at a significance level of 95% ($p < 0.05$). EAA/NEAA ratio: ratio of essential amino acids to non-essential amino acids.

Table 4.

GI conditions	AG_{TG} (%)	$AG_{1,2\text{-}DG}$ (%)	$AG_{1,3\text{-}DG}$ (%)	$AG_{2\text{-}MG}$ (%)	$AG_{1\text{-}MG}$ (%)	FFA (%)	Absorbable fraction (%)	Non-absorbable fraction (%)	Extent of lipolysis (%)
ND	99.7 ± 0.3	-	-	-	0.86 ± 0.12	-	0.86 ± 0.12	-	0.86 ± 0.12
C	80.1 ± 0.5^{a}	1.38 ± 0.04^{a}	1.2 ± 0.04^{a}	0.230 ± 0.005^{b}	0.58 ± 0.04^{b}	16.5 ± 0.5^{c}	17.3 ± 0.5^{c}	2.57 ± 0.08^{a}	19.9 ± 0.6^{c}
E1	86.4 ± 0.2^{b}	1.1 ± 0.2^{a}	1.0 ± 0.3^{a}	0.08 ± 0.04^{a}	0.391 ± 0.005^{a}	11.0 ± 0.3^{b}	11.5 ± 0.4^{b}	2.1 ± 0.5^{a}	13.6 ± 0.9^{b}
E2	86.4 ± 1.2^{b}	1.22 ± 0.17^{a}	1.2 ± 0.6^{a}	0.13 ± 0.02^{a}	0.32 ± 0.03^{a}	10.8 ± 0.3^{b}	11.2 ± 0.3^{b}	2.4 ± 0.7^{a}	13.6 ± 1.2^{b}
E3	91 ± 2^{c}	0.6 ± 0.8^{a}	1.26 ± 0.14^{a}	-	-	7.0 ± 1.3^{a}	7.0 ± 1.3^{a}	1.9 ± 1.0^{a}	9 ± 2^{a}

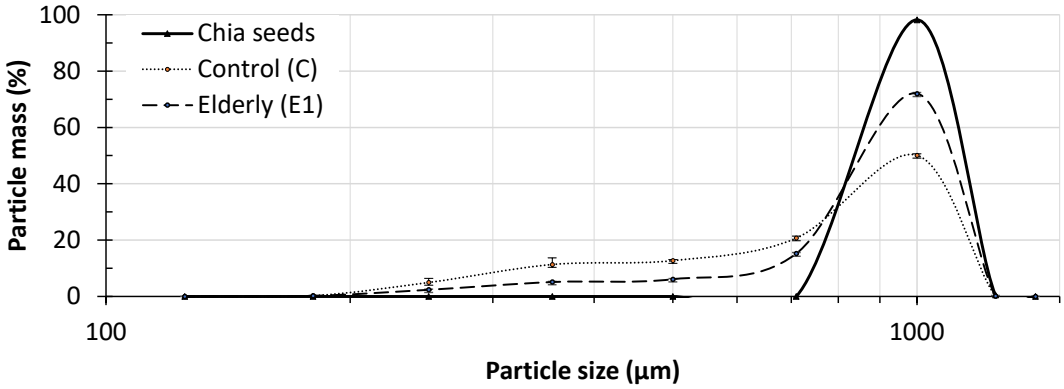
Data shown are mean values from three replicates. Lowercase letters in digested samples indicate significant differences between *in vitro* digestion models at a significance level of 95% ($p < 0.05$). TG: Triglycerides; DG: Diglycerides; MG: Monoglycerides; FFA: Free fatty acids. Absorbable fraction (%) is the sum of FFA, 1-MG and 2-MG; Non-absorbable fraction (%) represents the 1,2-DG and 1,3-DG. Extent of lipolysis is the sum of $AG_{1,2\text{-}DG}$, $AG_{1,3\text{-}DG}$, $AG_{2\text{-}MG}$, $AG_{1\text{-}MG}$ and FFA .

Table 5.

GI conditions	Calcium (mg Ca^{2+}/100 g)	Total polyphenols (mg GAE/g chia)	Antioxidant capacity (mg TE/g chia)		
			DPPH	FRAP	ABTS
C	195 ± 12^{b} (27)	7.0 ± 0.3^{c} (199)	3.34 ± 0.12^{c}	7.48 ± 0.15^{c}	14.9 ± 0.3^{c}
E1	212 ± 15^{b} (29)	6.6 ± 0.3^{bc} (191)	2.59 ± 0.12^{b}	5.77 ± 0.09^{b}	13.8 ± 0.2^{b}
E2	216 ± 9^{b} (30)	6.3 ± 0.3^{b} (184)	2.33 ± 0.19^{ab}	5.2 ± 0.4^{ab}	14.0 ± 0.2^{b}
E3	148 ± 2^{a} (20)	4.84 ± 0.13^{a} (140)	1.90 ± 0.12^{a}	4.46 ± 0.03^{a}	13.1 ± 0.2^{a}

Data shown are mean values from three replicates. Values in parentheses represent the bioaccessibility (%) of calcium and total polyphenols. Lowercase letters in digested samples indicate significant differences between *in vitro* digestion models at a significance level of 95% ($p < 0.05$). GAE: Gallic acid equivalents; TE: Trolox equivalents.

812 **Figure 1.**



813

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Figure 2.

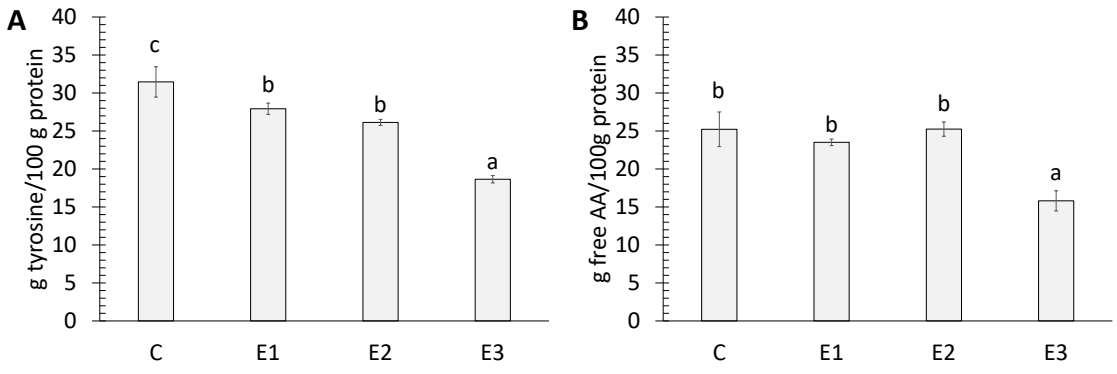
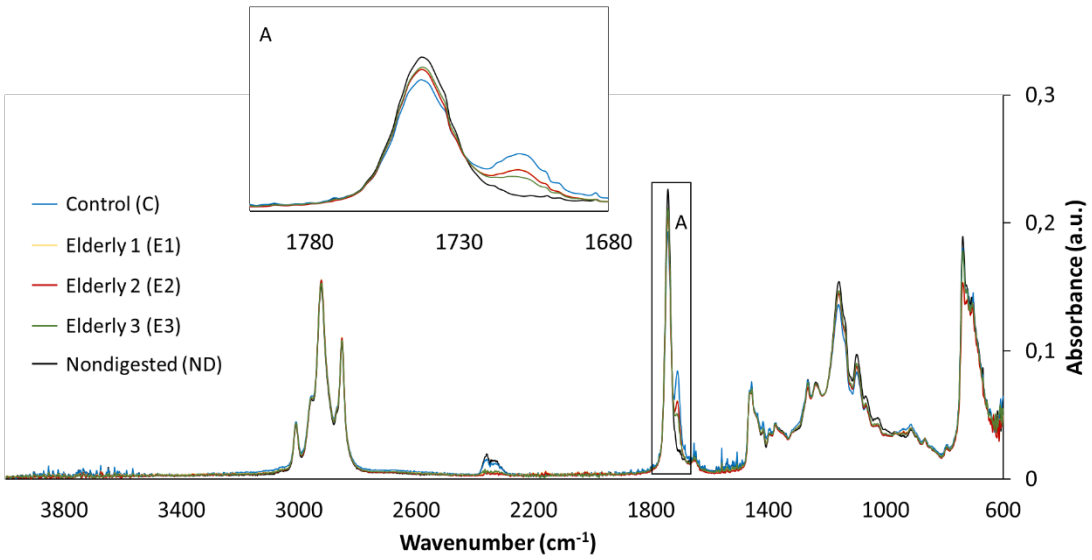
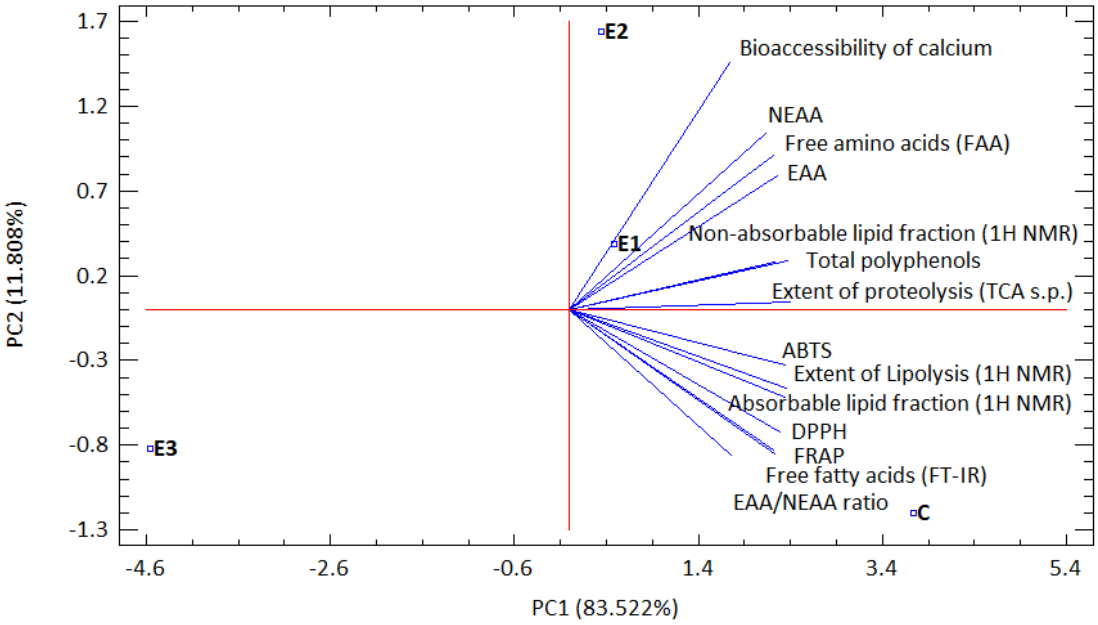

A
g tyrosine/100 g protein
40
35
30
25
20
15
10
5
0
c
b
b
a
C
E1
E2
E3
B
g free AA/100g protein
40
35
30
25
20
15
10
5
0
b
b
b
a
C
E1
E2
E3

Figure 3.


A
1780
1730
1680
A
Control (C)
Elderly 1 (E1)
Elderly 2 (E2)
Elderly 3 (E3)
Nondigested (ND)
0,3
0,2
0,1
0
Absorbance (a.u.)
3800
3400
3000
2600
2200
1800
1400
1000
600
Wavenumber (cm-1)

821 **Figure 4.**



822