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UNDERSTANDING THE ROLE OF FOOD MATRIX ON THE DIGESTIBILITY OF DAIRY PRODUCTS UNDER ELDERLY GASTROINTESTINAL CONDITIONS

Ever Hernández-Olivas, Sara Muñoz-Pina, Janaina Sánchez-García, Ana Andrés, Ana Heredia*

Instituto Universitario de Ingeniería de Alimentos para el Desarrollo (IUIAD-UPV). Universitat Politècnica de València, Camino de Vera s/n, 46022, Valencia, Spain.

*Corresponding author. E-mail: anhegu@tal.upv.es

ABSTRACT

This study aimed to evaluate the effect of some elderly in vitro gastrointestinal (GI) conditions on proteolysis and lipolysis extent, calcium, vitamins A and D bioaccessibility and lactose release in milk, yogurt, fresh and aged cheeses. To evaluate the impact of the some oral, gastric and intestinal disorders appearing with ageing on dairy digestion, three in-vitro elderly models were applied (E1 (oral altered conditions), E2 (oral and gastric altered conditions) and E3 (oral, gastric and intestinal altered conditions)) plus a healthy adult one as control. Proteolysis extent was significantly affected by elderly GI alterations ($p<0.05$) (around 40% of reduction compared to control), being fresh and aged cheese proteolysis the most affected with an important descrease in leucine release (18 and 25%, respectively). Calcium, vitamins A and D3 bioaccesibility and lactose release seemed not to be highly compromised in these models of elderly conditions; however, the micronutrients bioaccessibility was very dependent on dairy matrix's structure. Finally, the amount of the lipid hydrolyzed fraction of cheeses is not influenced in the investigated models.

Key words: dairy products; elderly in vitro digestion models; protein digestibility; fat digestibility; micronutrients bioaccessibility; lactose release

1. Introduction

Population group above 65 years old is growing, expecting to be in Europe more than one-quarter (27%) by 2050 (Chollet et al., 2014). Worldwide, it is expected that the number of people over 65 will exceed the number of children for the first time in 2045. Both lifestyle and diet present an impact on elderly wellness and therefore, on the prevalence of chronical diseases in this population group. Therefore, specific nutrition for elderly has been identified as one of the rising world's challenges (United Nations. Department of International Economic and Social Affairs. Population Division, 2015). Among the dietary recommendations addressed to individuals over 65 years by European Society for Clinical Nutrition and Metabolism (ESPEN), an intake of rich-protein foods is highly advisable (Volkert et al., 2018), and preferably with a protein profile rich in leucine (Morley, 2016). Among food categories contributing the most to protein intake through the diet, dairy products are highly consumed by elderly and more specifically, yogurt and cheese (Chollet et al., 2014). These products present a positive impact on cardiovascular health (Dehghan et al., 2018) and especially have shown to contribute to bone health in individuals over 65 years (McCabe et al., 2004), because of their protein, calcium and liposoluble vitamins content. A protein deficit in elderly has been associated with a loss of muscle mass (sarcopenia), asthenia, depression and weakness of the immune system(Rashid, Tiwari, & Lehl, 2019). Gastrointestinal disorders appearing along ageing could be partially responsible of this

48 protein deficit, because they frequently lead to less hydrolysis and absorption of
49 macronutrients, especially of proteins. Among them, secretion of digestive fluids and
50 enzymes, peristaltic contractions and chyme passage rates could be suboptimal
51 (Nagler & Hershkovich, 2005; Salles, 2007). Besides, micronutrients bioaccessibility is
52 often compromised, as it is the case of calcium and zinc, and/or some vitamins such as
53 B12, B6, A and D.
54 Besides to the host-related factors, it is expected that the characteristics inherent to
55 the food matrix (composition, structure, physicochemical properties or interactions
56 between macro and micronutrients within the same matrix, ...) also modulate
57 digestibility, resulting in different extents of hydrolysis under similar digestive
58 conditions. Nevertheless, these food-inherent and host-related factorsare barely
59 considered when addressing dietetic recommendations to elderly.
60 Given this scenario, it was considered of interest to carry out an in vitro digestion study
61 to assess the contributions of food-inherent and host-related factors to different dairy
62 products digestibility under altered digestion conditions frequently given in senior
63 population. The results might generate accurate dietetic recommendations for elderly
64 and open the door to the design of new functional products addressed to senior. In-
65 vitro digestion models allow simulating the digestion processes with a series of
66 advantages compared to in vivo ones. They are highly reproducible, easy to sampling
67 in the different stages of the digestive process and allow modifications of the
68 controlled digestion conditions, among others.
69 Thus, the aim of the present work is to assess, by means of a static in-vitro digestion
70 methodology, the influence of the most frequent elderly GI alterations according to
71 the model published by Shani-Levi et al. (2017) onto the digestibility of macronutrients

(proteins, fats and carbohydrates) and the bioaccessibility of micronutrients (calcium and vitamin A and D3) in four different dairy products (whole milk, yogurt, fresh and aged cheeses).

2. Materials and methods

2.1. Chemicals

Reagents for the in-vitro simulation of digestion fluids were pepsin from the porcine gastric mucosa (P6887), porcine pancreatin (P7545), bovine bile (B3883), potassium chloride, potassium dihydrogen phosphate, sodium bicarbonate, sodium chloride, magnesium chloride, ammonium carbonate, calcium chloride, hydrochloric acid, sodium hydroxide and potassium sulphate, all of them from Sigma-Aldrich (Sigma-Aldrich, USA).

For the analytical determinations, boric acid, tetrahydrofuran (HPLC grade), methanol (HPLC grade), retinol ≥99% (HPLC grade), cholecalciferol ≥98% (HPLC grade), sulfuric acid, glucose standard solution (1 mg/mL), potassium sodium tartrate tetrahydrate (ACS reagent, 99%) and 3,5-dinitrosalicylic acid were also provided by Sigma-Aldrich (Sigma-Aldrich, USA). Nitric acid (70%), lanthanum chloride heptahydrate and dichlorometane (HPLC grade > 99.8%) were acquired from Honeywell Fluka (Buchs, Switzerland) and petroleum ether from VWR International (VWR International, France). Sodium chloride and anhydrous sodium sulfate were supplied by Panreac (Panreac AppliChem, Barcelona, Spain). The EZ-Faast amino acid analysis kit for the analysis of amino acids by GC–MS was provided by Phenomenex (Torrance, CA, USA) and acetonitrile HPLC grade was acquired from JT Baker (Phillipsburg, NJ, USA)

The four selected dairy products for this study (whole milk, natural yogurt, 12-monthaged cheese and fresh cheese) were all exclusively of cow origin (100%) and acquired in a local store of the city of Valencia, Spain.

2.3. Compositional analysis of dairy products

Moisture, ashes, fat and protein contents were determined according to the official methods 934.01, 942.05, 920.39 and 960.52of the Association of Official Analytical Chemist (AOAC, 2000), respectively. For fluid matrices (milk and yogurt), the above-methodologies were carried out, excepting for the fat analysis that followed the methodology of the International Standard ISO 1211 | IDF 001: 2010, (ISO & IDF, 2010). Furthermore, lactose content(as glucose equivalent) was determined by the colorimetric method of dinitrosalicylic acid (DNS) (Armellini et al., 2019).

Calcium content was determined according to the methodology proposed by (Noël, Carl, Vastel, & Guérin, 2008) using a flame atomic absorption spectrometer at 422.7 nm (Termo Scientific, iCE 3000 Series), previous calcination of the sample.

Samples were also subjected to saponification and extraction of both vitamin A (retinol) and D3 (cholecalciferol) according to the protocol of Castaneda & Lee, (2019). Vitamins were first separated using a RP-HPLC (Waters e2695 Separation Module, Waters, Milford, MA, USA) with a Kinetex™C18 column 5µm, 100 Å, 150 x 4.6 mm (Phenomenex, Torrance, CA, USA). An isocratic separation was performed with 15% acetonitrile, 7% water and 78% methanol:tetrahydrofurane (90:10 v/v) during 10 min using a flow rate of 1 mL/min and an injection volume of 20 µL. Then, they were detected and quantified using a photo diode array detector (Waters PDA 2996) at 265 nm (vitamin D3) and 325 nm (vitamin A).

All above-mentioned macro and micronutrients were expressed per g of dairy product.

Finally, fresh and aged cheeses were subjected to cold liquid-liquid extraction to determine their lipidic profile by Proton Nuclear Magnetic Resonance (^{1}H NMR) using a BRUKER 400/R at 400 MHz (Nieva-Echevarría, Goicoechea, Manzanos, & Guillén, 2016). The lipidic profile provides information about the molar percentage of triglycerides (TG), diglycerides (1,2-DG and 1,3-DG), monoglycerides (1-MG and 2-MG) and free fatty acids (FFA) in the samples.

2.4. Static in-vitro simulation of the digestive process

The simulation of gastrointestinal digestion was carried out following the standardized method of static in-vitro digestion for a healthy adult, internationally agreed and published by Minekus et al. (2014). On the other hand, the specific gastrointestinal conditions of the elderly were established according to Shani-Levi et al. (2017).For the first step of the digestion, oral stage, it was decided to perform an *in vivo* simulation realized by a healthy subject, only in the case of solid food since in the case of milk and yogurt this stage was suppressed. As chewing is a complex process where parameters such as the number of cycles, chewing frequency and speeds depend on the food characteristics (Chen & Lolivret, 2011; Le Révérend, Saucy, Moser, & Loret, 2016; Peyron, Santé-Lhoutellier, François, & Hennequin, 2018), it is difficult to establish a chewing standard. Therefore, taken this into account and based on the publications of other authors, the number of chewing cycles needed to reach a bolus with similar physical characteristics to that of a tomato or mustard paste were determined for each solid product and considered the standard conditions of a volunteer adult with healthy dentition (Jalabert-Malbos, Mishellany-Dutour, Woda, & Peyron, 2007;

Minekus et al., 2014; Woda, Foster, Mishellany, & Peyron, 2006), and then to simulate the altered chewing conditions of a most critical oral elderly scenery the number of cycles was reduced by 50% in order to generate boluses with larger particle size and difficult to swallow (Hernández-Olivas, Muñoz-Pina, Andrés, & Heredia, 2020; Lee et al., 2004; O'Keeffe et al., 2019).

Four in-vitro models were designed to study the impact of different gastrointestinal alterations on the elderly population on the digestibility and bioaccessibility of dairy products: first one representing the standard GI conditions of a healthy adult (control (C)) and three models mimicking the accumulative alterations commonly observed with ageing (Elderly 1 (oral stage altered (E1), Elderly 2 (oral and gastric stages altered (E2)) and Elderly 3 (oral, gastric and intestinal stages altered) (E3))) (Figure 1).

In-vitro digestion was performed as follows:

Oral stage: in the case of fresh and aged cheese, 5 g of sample were chewed *in vivo* by the volunteer with normal dentition during 20 cycles simulating a healthy adult. In contrast, 10 cycles were performed to simulate an elderly. After chewing, food boluses were transferred to the falcon tubes to continue gastrointestinal digestion.

Gastric stage: food boluses of fresh and aged cheeses, or a direct aliquot of yogurt and milk, were mixed in a ratio 1:1 with SGF (v/v) and the pH and the pepsin concentration was adjusted according to the conditions to be tested (Figure 1). Subsequently, the samples were flipped from top to bottom at 55 rpm using an Intell-Mixer RM-2 (Elmi Ltd, Riga, LV-1006, Latvia) for 2 h at 37 °C in a chamber Selecta (JP Selecta SA, Barcelona).

Intestinal stage: After the gastric stage, SIF was incorporated in a proportion 1:1 (v/v) to each tube containing the gastric chime according to the conditions of the models (Figure 1). Samples were then being flipped from top to bottom at 55 rpm for another 2 or 4 h, depending on the model tested, at 37 °C. pH was monitored during the digestion process and readjusted if necessary, to keep it constant.

At the end of the digestion, samples were cooled down in ice bath during 10 min to reduce the digestion before phase separation and analytical determinations. Where needed, separation of the liquid phase from the solid phase resulting from the digestion process was performed by centrifuging at 4000 g-force during 20 minutes at 10 °C and the supernatant, considered as bioaccessible fraction, was collected for analytical determinations.

2.5. Analytical determinations in the digesta

2.5.1. Free amino acids

The determination of free amino acids resulting of protein digestion was performed using the EZ-Faast amino acid kit following the procedure proposed by (Peinado, Koutsidis, & Ames, 2016). First, aliquots of bioaccesible fraction (100 µL) were taken to be derivatized using EZ-Faast amino acid kit and then analyzed by gas chromatography-mass spectrometry (GC-MS) (Agilent Technologies, Injector 7683B series, Network GC System 6890N series, Inert Mass Selective Detector 5975 series. Data from both the calibration curve and the samples were analyzed using the MSD ChemStation software. The amino acid profile after digestion was expressed as mg free amino acid/ g product and proteolysis extent (%), with respect to the initial protein content, according to Eq 1:

$$\textbf{\textit{Proteolysis extent}}\ (\%) = \frac{(\textbf{\textit{total g of free amino acids}})}{(\textbf{\textit{g initial protein}})} \times \mathbf{100} \quad \textbf{(1)}$$

2.5.2. Lipidic profile determination

Digesta from both fresh and aged cheeses were subjected to same protocol for lipidic profile determination and described in section 2.2 for undigested cheeses.

2.5.3. Lactose released

Lactose content, expressed as mg glucose eq/ g of initial product, was determined in 0.5 mL of the bioaccessible fraction by the colorimetric method of Dinitrosalicylic Acid (DNS) (Armellini et al., 2019)). Lactose released (%), with respect to the initial content, was estimated according to Eq.2.

$$\textbf{\textit{Lactose released}}\ (\%) = \frac{(\textbf{\textit{mg glucose eq. released}})}{(\textbf{\textit{mg glucose eq. total in undigested food}})} \times \mathbf{100} \quad \textbf{(2)}$$

2.5.3. Calcium bioaccessibility

An aliquot of 4 mL was taken from the bioaccessible fraction and subjected to the same protocol explained in the section 2.2. Calcium content in the bioaccessible fraction was expressed as mg of Ca^{2+} / g of initial product and its bioaccessibility (%) calculated according to Eq. 3; where "free Ca^{2+} released" refers to the calcium content in the bioaccessible fraction and "Ca^{2+} in undigested food" to the total calcium content in the dairy product before digestion.

$$\textbf{\textit{Calcium bioaccessibility}}\ (\%) = \frac{(\textbf{\textit{mg }}\boldsymbol{Ca^{2+}}\textbf{\textit{released}})}{(\textbf{\textit{mg }}\boldsymbol{Ca^{2+}}\textbf{\textit{ in undigested food}})} \times \mathbf{100} \quad \textbf{(3)}$$

2.5.4. Vitamin A and D3 bioaccessibility

Vitamins A and D3 were determined in 20 mL of bioaccessible fraction according to the protocol described in the section 2.2 and expressed as μg/ g of initial product. Subsequently, their bioaccessibility was calculated according to Eq. 4 in which "vitamin released" refers to the vitamin content in the bioaccessible fraction, and "vitamin in the undigested food" to the vitamin content in dairy product before digestion.

$$\boldsymbol{Vitamin\ bioaccessibility}\ (\%) = \frac{(\mu g\ of\ vitamin\ released)}{(\mu g\ of\ vitamin\ in\ undigested\ food)} \times \mathbf{100} \qquad \textbf{(4)}$$

2.6. Statistical analysis

Data are reported as mean ± standard deviation (three replicates). The results obtained were statistically analyzed using Statgraphics Centurion XVII program with a 95% confidence level ($p < 0.05$) using a simple analysis of variance (one-way ANOVA) followed by the multiple range test LSD (Less Significant Difference) of Fisher test in order to identify homogeneous groups between models and dairy products. PCA was used an orthogonal transformation to convert the obtained data (proteolysis, lipolysis, lactose release and bioaccessibilities of calcium, vitamin A and D3) of possibly correlated variables into a set of values of linearly uncorrelated variables (called principal components). This transformation is defined in such a way that the first principal component has the largest possible variance (that is, accounts for as much of the variability in the data as possible), and each succeeding component in turn has the highest variance possible under the constraint that it is orthogonal to the preceding components.

3. RESULTS AND DISCUSSION

3.1. Nutritional composition of the samples

The nutritional contents of milk, yogurt, fresh and aged cheeses, expressed per 1 g of product, are gathered in Table 1. In general, protein, total fat and ashes contents were similar to those reported in literature for the same food matrices (Delgado, Salazar, & García, 2013; Mulet, Escriche, Rossello, & Tarrazó, 1999; Rinaldi, Gauthier, Britten, & Turgeon, 2014) and correspond to label declarations. As expected, both cheeses (0.16 and 0.29 g/g product, for fresh an aged cheese) presented higher protein content than yogurt and milk (around 0.03 g/g product). In terms of lipid content, dairy products ranged from 0.0287 to 0.288 g/g product, corresponding to yogurt the least content and to aged cheese the most. Thus, the processing (coagulation, pressing, salting and/or curing) resulting in different composition of matrices (Diana, Rafecas, Arco, & Quílez, 2014). With regard to calcium content of the different dairy products, results were consistent with those reported in the literature (AESAN/BEDCA, 2010; Segarra, 1999), reporting 1 g of cheeses provides more calcium mineral than the same amount of liquid or semi-liquid dairy products. Vitamins A and D3 contents were also in agreement with data found in the literature (AESAN/BEDCA, 2010; Segarra, 1999). According to these results, the studied dairy products can be considered as an important source of liposoluble vitamins, and especially of retinol. However, differences in terms of vitamins concentration were also noticed. Aged cheese presented notable higher content of both vitamins, A and D3, compared to the other dairy products. With respect to lactose content, milk presented the highest sugar content compared to the other products. As it is well-known, lactose consumption by lactic acid bacteria during fermentation results in lower lactose content in yogurt than milk. During cheese production, the whey removal (in which lactose is solubilized) after acidic or enzymatic coagulation, gives as a result low lactose content in fresh cheese;

while lactose conversion to lactate during the two weeks of ripening additionally decrease the residual lactose present in aged cheeses (Harju, Kallioinen, & Tossavainen, 2012). Of note, important differences exist among products in terms of protein and micronutrients contributions per serving to the daily diet. In fact, a serving of milk (averaged serving of 200 mL) or aged cheese (40 g) puts up to the diet with higher protein and liposoluble vitamins contents, than the intake of a serving of yogurt (125 g) or fresh cheese (40g); while the consumption of whatever of the cheeses is interesting in order to insure high calcium intake. Nevertheless, the affection of gastrointestinal alterations of elderly on macro and micronutrients digestibility and availability might be consider to address dietary recommendations.

3.2. Protein digestibility of dairy products under elderly GI conditions

Figure 2 shows the digested protein (mg of free amino acids/ g of product) and the proteolysis extent (%) of dairy products (milk, yogurt, fresh cheese and aged cheese) digested under standard (C) and elderly scenarios (E1, E2 and E3). Firstly, it can be noted that the amount of digested protein under standardized GI conditions (C) ranged between 31.3 to 131 mg free amino acids/g product (for yogurt and aged cheese, respectively) and proteolyisis extent from 50 to 100 %, depending of the food matrix. Nevertheless, higher values of proteolysis extent do not necessarily correspond to higher supplies of free amino acids per gram of product.

Dairy structure plays a key role on the solubilization, release and/or hydrolysis of caseins during the GI digestion (Rinaldi et al., 2014), being caseins taking part of solid structures (fresh and aged cheeses) less digestible than those present in liquids and semi-liquids products. Similar results were reported by Asensio-Grau, Peinado,

Heredia, & Andrés (2019) and Rinaldi et al. (2014). Besides, it is important to remark that ripened cheese often contains free amino acids and small peptides due to proteases activity during ripening (McSweeney, 2004). Proteolysis extent in 12-month ripened cheeses has been reported to range from 2 to 8 %, when no fungal microorganisms are involved (García-Palmer, Serra, Palou, & Gianotti, 1997; Kastberg et al., 2012). Therefore, proteolysis resulting from digestion in cheeses could be slightly lower than showed in Figure 2. Some studies have reported that the presence of products of hydrolysis such as free amino acids in ripened cheeses could enhance the breakdown of caseins during the posterior GI digestion because of their emulsifying capacity (Asensio-Grau et al., 2019; Maldonado-Valderrama, Wilde, MacIerzanka, & MacKie, 2011). However, no differences were found in terms of proteolysis extent among fresh, without ripening, and aged cheese in this study.

Regarding the effect of altered GI conditions of elderly on proteolysis, protein hydrolysis experimented an accumulative reduction as long as the GI conditions were altered from the oral to the intestinal stage in fresh and aged cheese and from the gastric to the intestinal stage in milk and yogurt. Hence, a proteolysis extent of 32 ± 3, 33 ± 3, 53 ± 7, 65 ± 8 % for aged cheese, fresh cheese, milk and yogurt were registered under the worst scenario of digestion for elderly E3). From standard (C) to elderly GI conditions, 65% of reduction was observed for solid and semi-solid dairy products and 50% for milk. Yogurt and milk presented the highest protein digestibility under all GI conditions, but lower amount of free amino acid supply than both cheeses. Therefore, the impact level of elderly GI conditions on the protein in-vitro digestion is dependent on the matrix-inherent properties. To deeper, C and E1 models differ in oral stage conditions (being major the breakdown in C than E1). Thus, the reduction of the food

298 particle size and a mixing with saliva is aimed in optimal conditions to swallow. In this
299 way, smaller particles maximize the protein surface contact, enabling better the
300 accessibility of enzymes to cleavage sites (Paz-Yépez, Peinado, Heredia, & Andrés,
301 2019). This fact could explain the impact of mastication level on proteolysis achieved
302 at the end of digestion in fresh cheese and aged cheese (Figure 2). The comparison of
303 the proteolysis achieved under E1 and E2 models for both cheeses, and between C and
304 E2 in milk and yogurt was aimed at evidencing the impact of gastric alteration in
305 proteolysis extent. However, it is necessary to point out that proteolysis is estimated
306 by free amino acids quantification at the end of luminal digestion, i.e. after intestinal
307 stage. Consequently, the products of gastric proteolysis are mainly peptides of low
308 molecular weight that cannot be detected by the analytical method. The results show
309 that gastric stage change would reduce protein digestibility measured after luminal
310 simulation in all the analyzed foods, but without significant difference in fresh cheese.
311 The isoelectric point of caseins is close to pH 6 ($4.5 < pH < 5.5$), and aggregates could
312 hinder the hydrolysis (Levi & Lesmes, 2014). Thus, if protein hydrolysis into peptides
313 decreases under E2 conditions, the analytical method was not able to register
314 completely that fact. In any case, the similar proteolysis extent achieved E1 and E2 in
315 cheeses, and C and E2 in milk and yogurt, indicates that the activity of pancreatic
316 proteases might compensate the suboptimal conditions of the gastric stage (E2) with
317 the hydrolysis of proteins into peptides and free amino acids.

318 Finally, a reduction in the pancreatic enzymes lead to maldigestion and malabsorption
319 of proteins causing nutritional deficiencies (Rémond et al., 2015). This fact agrees to
320 proteolysis extent resulted under suboptimal intestinal conditions (E3) compared with
321 the proteolysis extent achieved under non-altered intestinal conditions (E2).

322 Tables 2a and b gather the free amino acid profile (mg of free amino acid/ g of
323 product) resulting of proteolysis under standard healthy adult (C) and are consistent
324 with that reported by other authors (Ceballos et al., 2009; Diana et al., 2014; Germani
325 et al., 2014). As it can be observed, major free amino acids values correspond to lysine,
326 leucine, tyrosine, valine and phenylalanine, all of them essential ones. In Particular,
327 leucine, together with isoleucine and valine, is an amino acid of concern in the elderly,
328 because its participation in muscle protein synthesis (Rémond et al., 2015). Besides,
329 Table 2 show the free amino acid profiles obtained after digestion under elderly
330 conditions (E1, E2 and E3) and the reduction of each amino acid release, with respect
331 to the control (C), occurring as consequence of elderly GI alterations (E1, E2 and E3).
332 Thus, amino acids reduction ranged from 20 to 100 % under the worst digestion
333 conditions (E3), being glycine, cystine, asparagine, aspartic acid, threonine and alanine
334 the free amino acids experimenting the highest reductions. Among the essential amino
335 acids (valine, isoleucine, leucine, phenylalanine, tryptophan, histidine, lysine,
336 threonine and methionine), the reduction ranged from 20-60%, being the percentage
337 of reduction very dependent on the dairy matrix. Of note, a reduction of 18, 25, 25 and
338 44 % of leucine were found in aged cheese, fresh cheese, yogurt and milk digested
339 under E3, respectively. Similarly, the release of tryptophan, which is linked to
340 serotonin production and better sleeping, providing relief from anxiety and depression
341 reduction, was also compromised in elders with a higher reduction in digested milk
342 (52%), than in yogurt (25%), fresh (35%) and aged cheese (39%).

343 **3.3. Cheese-lipolysis under elderly GI conditions**

Fat digestibility was evaluated in fresh and aged cheeses, because of their considerable fat content, after the in-vitro digestion under control and elderly altered conditions. This analysis was carried out through the evaluation of the spectral data obtained from ^{1}H NMR. The spectra obtained were analyzed according to Nieva-Echevarría et al. (2016) for the quantification of the main products derived from triglyceride hydrolysis (TG) after digestion. Table 3shows molar percentages of acyl groups (AG) supported on the different glyceryl backbone structures (TG, 1,2-DG, 1,3-DG, 2-MG, 1-MG) and free fatty acids (FFA), present in the non-digested and digested (C, E1, E2, E3) of fresh and aged cheese. Thus, absorbable fraction by the intestinal epithelium consists of the molar percentage of FFA, 2-MG and 1-MG, after undergoing a micellization process thanks to the presence of bile salts (Salvia-Trujillo et al., 2017); while the non-absorbable fraction would be the sum of the remaining TG, 1,2-DG and 1,3-DG.The lipolysis extent corresponds to the sum of the molar percentage of 1,2-DG, 1,3-DG, 2-MG, 1-MG and FFA. As expected, almost all fat was present as TG (around98%), in both cheeses before digestion. After digestion under healthy standard GI conditions (C), a lipolysis extent of 89% in fresh cheese and 82% in aged cheese occur because of the hydrolytic action of pancreatic lipase, with a conversion of TG mainly into FFA (70 and 63% for fresh cheese and aged cheese, respectively), followed by 1,2-DG, 2-MG 1,3-DG and 1-MG.

With respect to the elderly GI conditions and their effect on fat digestibility, the absorbable fraction of fresh cheese was higher under intestinal altered conditions (E3) than under control ones. The decreased pancreatic lipases and biliar concentration in this model compared to control one, would not negatively affect the lipid digestibility because it is compensates by the longer intestinal time (Harper, 1998). Therefore, the

368 increase of intestinal residence time under model E3 would be the responsible ofthe
369 greater lipid hydrolysis achieved under these digestive conditions (Lamothe, Corbeil,
370 Turgeon, & Britten, 2012).

371 **3.4. Lactose release and calcium, vitamins A and D3 bioaccessibility under elderly**
372 **GI conditions**

373 A reduced digestion of macronutrients, such as proteins and lipids, could be coupled to
374 a deficient release and solubilization of micronutrients and/or lactose. Figure 3 shows
375 lactose (mg glucose eq./g product), calcium (mg Ca/g product), vitamin A (µg retinol/g
376 product) and D3 (µg cholecalciferol/g product) contents in the bioaccessible fraction as
377 well as their bioaccessibility (%) (at the bottom of the bars) with respect of the initial
378 content of each nutrient. Lactose content in the bioaccessible fraction ranged from 4
379 to 20 mg glucose eq./ g product for milk and aged cheese, respectively under the C
380 digestive conditions. In terms of lactose released (%), yogurt and aged cheese
381 presented the highest values compared to milk and fresh cheese, regardless the GI
382 conditions. Regarding the effect of elderly GI conditions on lactose released, no
383 statistically significant differences were found in the digesta of yogurt, fresh and aged
384 cheeses, even if the oral, gastric and intestinal were altered. Only elderly GI conditions
385 seemed to negatively the lactose release from milk, which possess the highest lactose
386 content among the studied dairy products. In fact, it exists a lack of data related to the
387 lactose release during luminal digestion process to support this behavior, even if it
388 seems to be related to structural matrix of the product. Wang, Ye, Lin, Han, & Singh,
389 (2018) reported that casein coagulation in dairy matrices might generate a complex
390 matrix that affect the enzyme cleavage site and nutrients releasing, such as lactose.
391 However, Figure 3 shows higher bioaccessibility from certain solid matrices such as

aged cheese than from liquid matrices as milk. This fact could be related to the acidic coagulation experimented by milk at stomach and thus, resulting also in a semi-solid matrix.

Calcium content in the bioaccessible fraction ranged from 0.6 to 2.1 mg Ca/g product in milk and aged cheese, respectively, under standard conditions of digestion (C). The bioaccessibility (%), however, was much higher in milk (43%), and especially in yogurt (67%), than in cheeses (11 and 16% for fresh and aged). In fact, Lorieau et al. (2018) reports greater calcium bioaccessibility in liquid matrices than in gel structured matrices. The higher bioaccessibility of calcium in yogurt than milk could be attributed to some dietary factors related to casein phosphopeptides (CPP), carbohydrates, Maillard reaction products, among others. Casein phosphopetides resulting from the enzymatic hydrolysis of caseins, can effectively bind calcium and inhibit formation of insoluble calcium phosphates (Etcheverry, Grusak, & Fleige, 2012). Yogurt present more CPP than milk due to the alteration in their micelle structure obtained during processing (Kawahara, Aruga, & Otani, 2005). Even if cheeses present lower bioaccessibility, aged cheese remains the highest supplier of bioaccessible calcium. On the other hand, no elderly GI alterations seem to highly compromise the release and solubilization of this mineral from dairy products, with the exception of from aged cheese. Even though, both cheeses remain an excellent source of bioaccessible calcium for lactase-deficient subjects such as most of elderly people, considering the calcium content (mg of Ca/ g of product) reported even under the worst GI conditions (E3). Diet recommendations addressed to elderly advice an increase of calcium intake, since bone density decreases with ageing, which can lead to osteopenia and, in extreme cases, osteoporosis, which is partly related to the consumption of dietary calcium

(McCabe et al., 2004). The latter is a significant health problem that contributes to disability and premature mortality among women and older men. Although genetic factors influence maximum bone mass, diet together with an active life style are clearly two of the modifiable risk factors for osteoporosis (Rémond et al., 2015).

Besides, vitamin A bioaccessibility (%) varied between 17 and 45 % under control GI conditions (C); while vitamin D3 bioaccessibility did from 24 and 39 % under the same GI model (Figure 3), milk being the most advantageous matrix for vitamins release and cheeses the least. However, the liposoluble vitamins content in the bioaccesible fraction of digested aged cheese is noticeable superior to other matrices. The differences in terms of release, solubilization and micellar incorporation of these vitamins among milk and dairy products could be attributed to the food matrix. Thus, it is found that when structured food matrices are more complex the minor the fat-soluble vitamins bioaccessibility (Borel, 2003). In fact, vitamins A and D3 exhibited the highest bioaccessibility in yogurt and milk, but lower net supply of these nutrients in their bioaccessible form, compared to cheeses, and specially aged one.

It is reported that digestion and absorption of the fat-soluble vitamins basically follow the same path as lipids (Rémond et al., 2015). However, it was observed in none of the cheeses. In these cases, vitamins A and D· experimented a significant reduction under E3; while fat digestibility was not affected. The suboptimal bile salts concentration given in E3 model could be, however, responsible of vitamins bioaccessibility detriment. Liposoluble vitamins are dependent on solubilization by bile acids, and an alteration in bile flow results in maldigestion and malabsorption (Werner, Kuipers, & Verkade, 2013).

3.5. Descriptive relationship among the digestion end-products

A PCA was conducted to evaluate the global relationship between products of digestion in the dairy products from a descriptive point of view. Figure 4 illustrates the loadings for the different products of the digestion (proteolysis, lipolysis, lactose release, calcium, vitamin D3 and A bioaccessibilities) as well as the scores of the different dairy products (milk, yogurt, fresh and aged cheese) under the different simulated GI conditions (C, E1, E2 and E3). The first two principal components of the analyses explain 77.264 % of the total variance of the percentage of macronutrient extents and percentage of micronutrients bioaccessibility of the samples (PC1: 58.813 % and PC2: 18.451%). In the score plot, proximity between samples indicates similar behavior in terms of digestibility. PC1 (59%) clearly differentiates between liquid and semi-liquid products (milk and yogurt), located at the right side of the plot, and solids ones (cheeses), located at the left side of the plot. Besides, PCA shows the narrow relationship between proteolysis, bioaccessibility of calcium and vitamin D3; while PC2 seems to distinguishyogurt and aged cheesefrom milk and fresh cheese in terms of vitamin A bioaccessibility (higher in milk than in the other matrices) and lactose release (higher in yogurt and aged cheese than in milk or fresh cheese).

4. CONCLUSIONS

This study contributes to a better understanding of dairy products (milk, yogurt, fresh and aged cheese) digestibility under elderly gastrointestinal conditions and depending on food matrix characteristics. The results report that proteolysis extent highly depends on the structural matrix of dairy products, ranging from 50 to 100 % under healthy gastrointestinal conditions (control) for cheeses and milk and yogurt, respectively. GI alterations appearing with ageing negatively affect the digestibility of

463 dairy proteins with a reduction around 50 %, compared proteolysis extent obtained
464 under control conditions. A notable decrease of some essential amino acids release
465 such as leucine, isoleucine, valine and tryptophan was also noticed under elderly GI
466 conditions. Nevertheless, absorbable fraction and lipolysis extent of cheeses seem to
467 be enhanced by the longer duodenal transit time given of elderly digestion. Finally,
468 calcium, vitamin D3 and proteolysis extent seem to be positively associated, especially
469 in milk and yogurt matrices. Liquid and semi-liquid matrices favour micronutrients
470 release in a greater extent to solid-matrices; however, the net supply of calcium,
471 vitamins A and D3 in their bioaccessible form (per g of product) is greater in cheeses
472 than milk or yogurt.

473 Therefore, the obtained results could be useful to establish accurate dietetic
474 recommendations addressed to elderly with regards to dairy products consumption.

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481 **Statement of Informed Consent, Human/Animal Rights**

482 No conflicts, informed consent, or human or animal rights are applicable to this study.

483 **Conflict of interest**

484 There are no conflicts to declare

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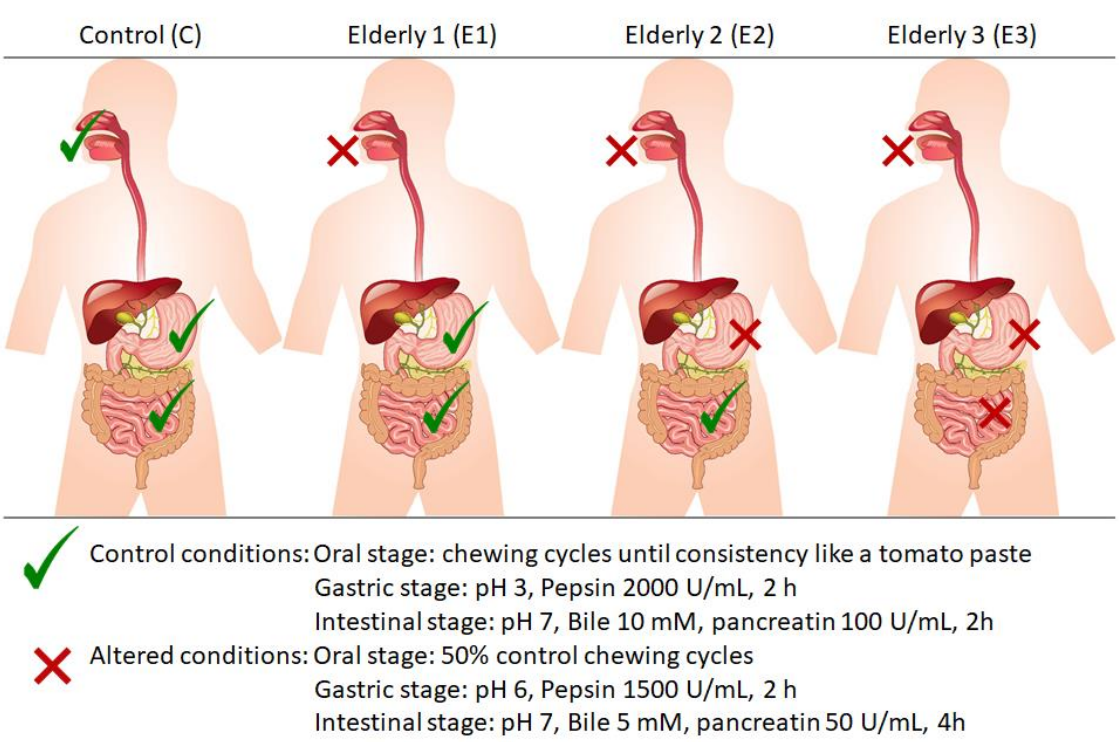
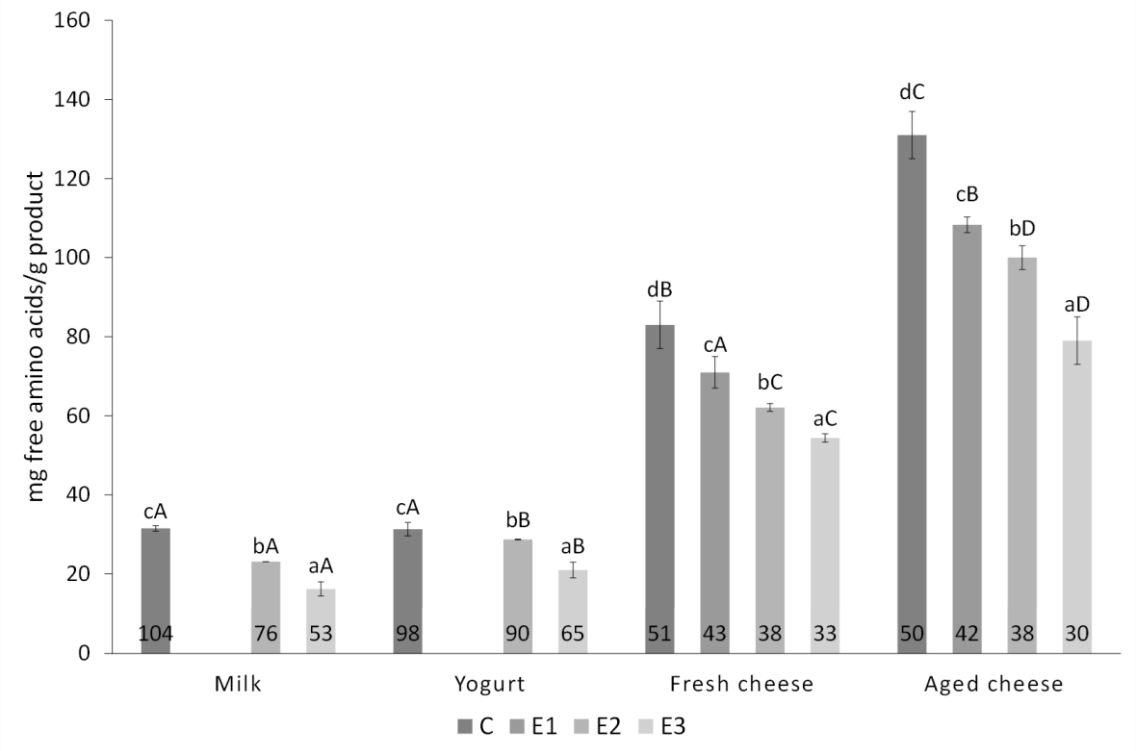


660

661 **Figure 1.** Specific gastrointestinal conditions of the four in vitro digestion models
662 applied to mimic healthy adult standardized conditions (C: control)) and elderly GI
663 alterations (E1: Elderly 1; E2: Elderly 2; E3: Elderly 3).

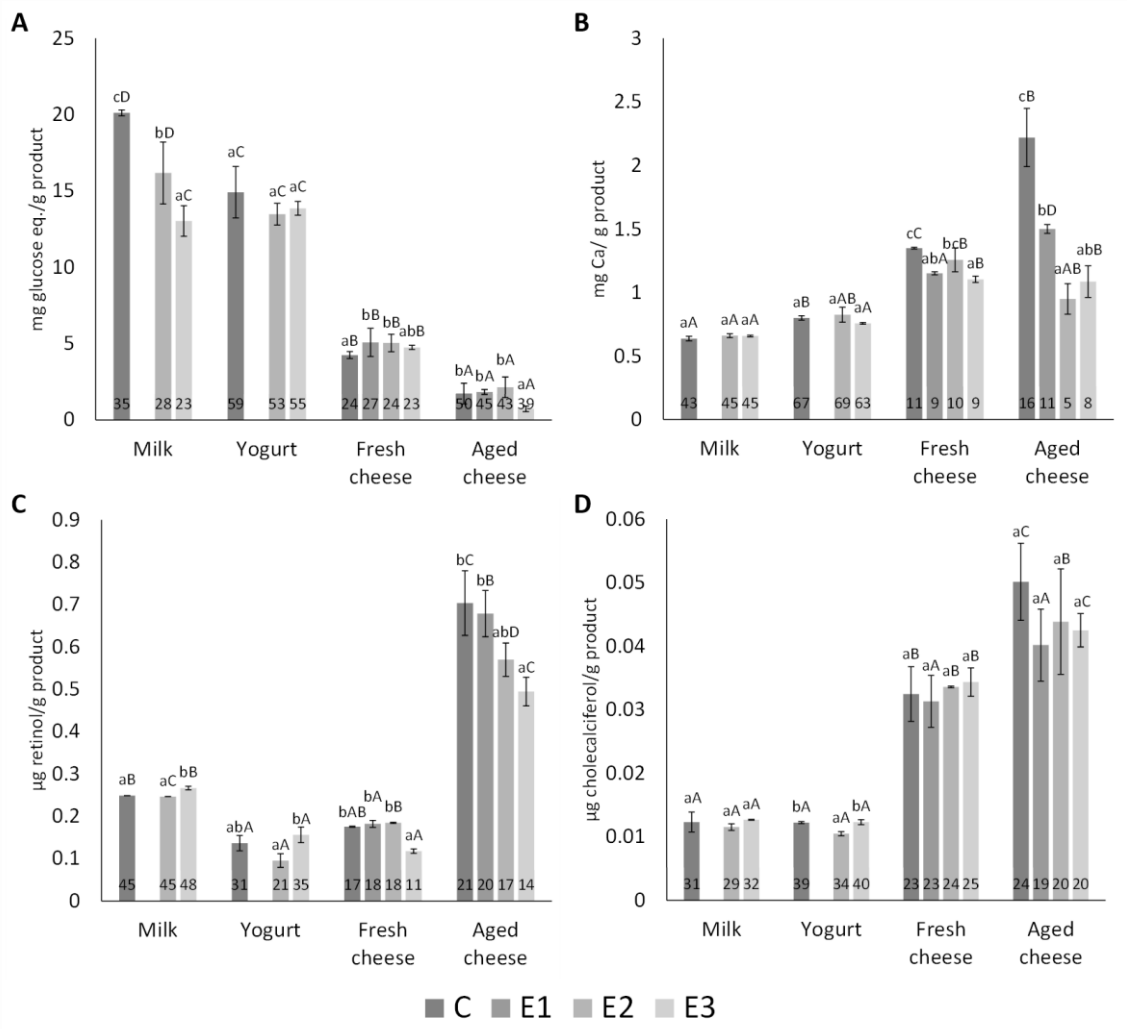
664



665

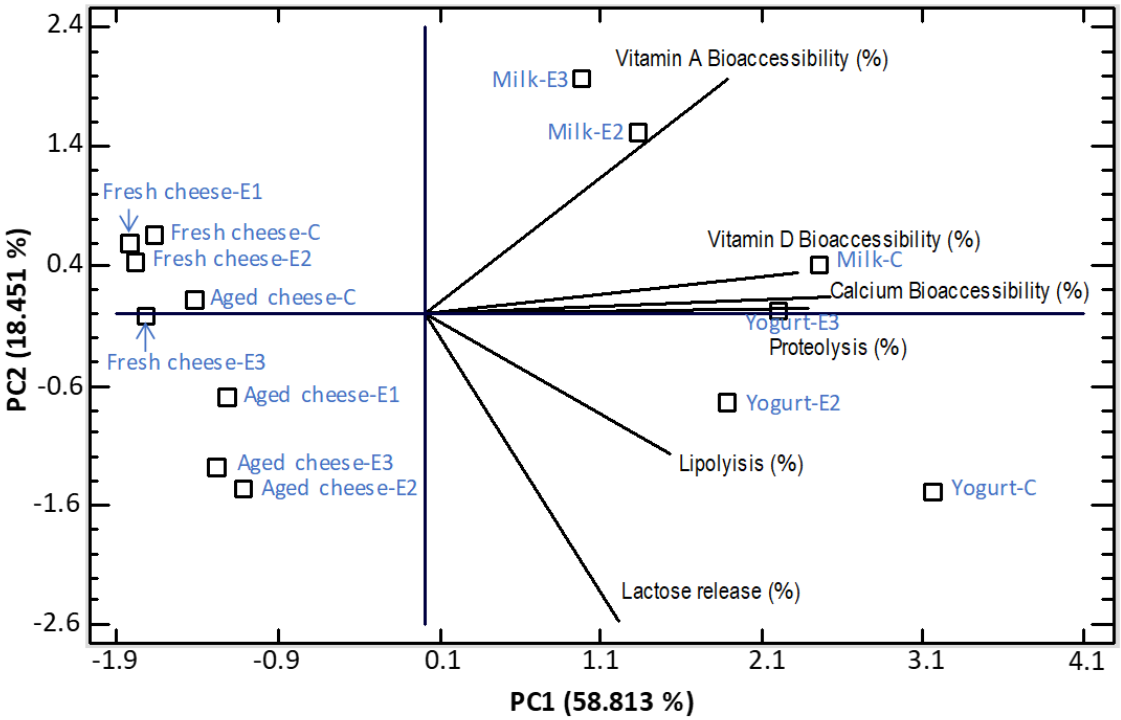
666 **Figure 2.** Digested protein (mg free amino acids/g product) of milk, yogurt, fresh
667 cheese and aged cheese under different in vitro digestion models (C: control; E1:
668 Elderly 1; E2: Elderly 2; E3: Elderly 3). Values at the bottom of the bars represent the
669 proteolysis extent (%) achieved after in vitro digestion. Oral alterations (E1) in milk and
670 yogurt were not evaluated because of the absence of mastication, and therefore the
671 low saliva secretion in the oral cavity.[a-d] different lowercase letters indicate significant
672 differences between models ($p < 0.05$). [A-D] different capital letters indicate significant
673 differences between products ($p < 0.05$).

674



675

676 **Figure 3.** A) Lactose (mg glucose eq./g product) and B) calcium (mg Ca/g product), C)
677 vitamin A (µg retinol/g product) and D) vitamin D3 (µg cholecalciferol/g product)
678 content in the biaccessible fraction from milk, yogurt, fresh cheese and aged cheese
679 digested under different in vitro digestion models (C: Control; E1: Elderly 1; E2: Elderly
680 2; E3: Elderly 3).Values at the bottom of the bars represents lactose released (%) and
681 bioaccessibility (%) of calcium, vitamin A and D3, with respect to the nutrient content
682 in the product before in vitro digestion. $^{a-c}$ different lowercase letters indicate
683 significant differences between models ($p < 0.05$). $^{A-D}$ different capital letters indicate
684 significant differences between products ($p < 0.05$).



685

686 **Figure 4.** Biplot of the different end-product resulting from digestion and their

687 relationship with the binomial dairy product (milk, yogurt, fresh or aged cheese)-GI

688 conditions (C: Control; E1: Elderly 1; E2: Elderly 2; E3: Elderly 3) obtained by means of

689 the principal components analysis (PCA).

690

691 **Table 1.** Macro and micronutrients contents in dairy products (milk, yogurt, fresh cheese and aged cheese) expressed per g of product.

Nutrient	Milk	Yogurt	Fresh cheese	Aged cheese
Moisture (g/ g product)	0.882 ± 0.002^{d}	0.895 ± 0.0009^{c}	0.618 ± 0.009^{b}	0.362 ± 0.012^{a}
Protein (g/ g product)	0.0303 ± 0.0012^{a}	0.0319 ± 0.0019^{a}	0.163 ± 0.008^{b}	0.29 ± 0.007^{c}
Fat (g/ g product)	0.035 ± 0.001^{b}	0.0287 ± 0.0012^{a}	0.202 ± 0.015^{c}	0.288 ± 0.012^{d}
Ashes (g/ g product)	0.0053 ± 0.0003^{a}	0.0073 ± 0.0006^{b}	0.0092 ± 0.0002^{c}	0.03 ± 0.003^{d}
Lactose (mg glucose eq./ g product)	57 ± 4^{d}	25.3 ± 1.5^{c}	20.7 ± 1.2^{b}	4 ± 0.6^{a}
Calcium (mg/ g product)	1.47 ± 0.09^{b}	1.19 ± 0.02^{a}	12.6 ± 0.5^{c}	14.1 ± 0.5^{d}
Vitamin A (µg/ g product)	0.55 ± 0.02^{b}	0.45 ± 0.04^{a}	1.03 ± 0.07^{c}	3.4 ± 0.2^{d}
Vitamin D3 (µg/ g product)	0.0397 ± 0.0013^{b}	0.031 ± 0.0013^{a}	0.138 ± 0.005^{c}	0.216 ± 0.014^{d}

692 Data shown are mean values from triplicates and the standard deviation. $^{a-d}$ Different lowercase letters indicate significant differences between foods (p
693 <0.05).
694
695

696 **Table 2a**. Amino acids profile (mg free amino acid/ g product) of milk and yogurt digested under control (C) and Elderly (E1, E2 and E3) GI conditions and
697 reduction (%) of amino acid released with respect to the control.

Amino acid	mg free amino acid / g product (Reduction with respect to the control (%))							
	Milk				Yogurt			
	C	E1	E2	E3	C	E1	E2	E3
Alanine	1.566 ± 0.014^{c}	-	1.29 ± 0.02^{b} (17)	0.91 ± 0.12^{a} (42)	1.5 ± 0.2^{b}	-	1.40 ± 0.14^{b} (12)	0.93 ± 0.09^{a} (39)
Glycine	0.98 ± 0.14^{b}	-	0.559 ± 0.006^{a} (43)	0.27 ± 0.08^{a} (72)	0.80 ± 0.13^{b}	-	0.787 ± 0.003^{b} (2)	0.31 ± 0.07^{a} (61))
Valine	2.20 ± 0.03^{c}	-	1.67 ± 0.04^{b} (24)	1.26 ± 0.15^{a} (43)	2.12 ± 0.09^{b}	-	2.004 ± 0.012^{b} (6)	1.72 ± 0.10^{a} (19)
Leucine	3.65 ± 0.18^{c}	-	2.537 ± 0.006^{b} (31)	2.1 ± 0.2^{a} (44)	3.4 ± 0.2^{b}	-	3.09 ± 0.03^{b} (8)	2.5 ± 0.2^{a} (25)
Isoleucine	1.40 ± 0.07^{c}	-	1.02 ± 0.04^{b} (27)	0.756 ± 0.113^{a} (46)	1.362 ± 0.110^{b}	-	1.400 ± 0.007^{b} (0.4)	1.18 ± 0.04^{a} (16)
Threonine	1.36 ± 0.08^{c}	-	0.89 ± 0.06^{b} (34)	0.54 ± 0.13^{a} (60)	1.20 ± 0.08^{b}	-	1.028 ± 0.014^{b} (15)	0.75 ± 0.06^{a} (37)
Serine	1.69 ± 0.06^{c}	-	1.27 ± 0.05^{b} (25)	0.75 ± 0.17^{a} (56)	1.73 ± 0.10^{b}	-	1.44 ± 0.04^{b} (17)	0.945 ± 0.114^{a} (45)
Proline	0.76 ± 0.03^{c}	-	0.476 ± 0.007^{b} (38)	0.34 ± 0.06^{a} (56)	0.95 ± 0.02^{b}	-	0.895 ± 0.013^{b} (6)	0.80 ± 0.06^{a} (16)
Asparagine	0.97 ± 0.05^{c}	-	0.62 ± 0.09^{b} (36)	0.35 ± 0.13^{a} (64)	1.04 ± 0.14^{b}	-	0.95 ± 0.04^{b} (11)	0.26 ± 0.08^{a} (75)
Aspartic acid	1.14 ± 0.05^{c}	-	0.81 ± 0.06^{b} (29)	0.34 ± 0.03^{a} (70)	1.17 ± 0.09^{b}	-	1.114 ± 0.006^{b} (5)	0.70 ± 0.10^{a} (40)
Methionine	0.79 ± 0.05^{b}	-	0.47 ± 0.02^{a} (40)	0.36 ± 0.06^{a} (54)	0.63 ± 0.05^{b}	-	0.6175 ± 0.0010^{b} (2)	0.50 ± 0.06^{a} (27)
Glutamic acid	1.77 ± 0.10^{b}	-	1.640 ± 0.009^{b} (7)	1.20 ± 0.02^{a} (33)	1.99 ± 0.13^{b}	-	1.83 ± 0.03^{b} (8)	1.49 ± 0.08^{a} (25)

Phenylalanine	2.17 ± 0.06^{c}	-	1.30 ± 0.02^{b} (40)	0.92 ± 0.12^{a} (58)	1.63 ± 0.12^{b}	-	1.51 ± 0.03^{b} (7)	1.147 ± 0.108^{a} (30)
Glutamine	2.06 ± 0.19^{b}	-	1.56 ± 0.18^{a} (24)	1.37 ± 0.19^{a} (34)	2.4 ± 0.2^{b}	-	1.79 ± 0.04^{b} (25)	1.6 ± 0.3^{a} (41)
Ornithine		-	-		-	-	-	-
Lysine	2.50 ± 0.19^{b}	-	2.568 ± 0.003^{b} (7)	1.9 ± 0.2^{a} (31)	4.0 ± 0.4^{b}	-	3.96 ± 0.02^{b} (2)	2.4 ± 0.5^{a} (42)
Histidine	1.09 ± 0.05^{c}	-	0.75 ± 0.05^{b} (31)	0.56 ± 0.05^{a} (48)	1.05 ± 0.06^{b}	-	0.76 ± 0.09^{b} (28)	0.77 ± 0.10^{a} (27)
Tyrosine	3.8 ± 0.2^{c}	-	2.65 ± 0.03^{b} (31)	1.62 ± 0.15^{a} (58)	3.4 ± 0.2^{b}	-	3.059 ± 0.005^{b} (10)	1.9 ± 0.3^{a} (43)
Tryptophan	1.56 ± 0.05^{c}	-	0.99 ± 0.03^{b} (36)	0.75 ± 0.03^{a} (52)	1.20 ± 0.07^{b}	-	1.08 ± 0.04^{b} (10)	0.90 ± 0.08^{a} (25)
Cystine	-	-	-	-	-	-	-	-

Data shown are mean values from triplicates and the standard deviation. Values in parentheses represent the percentage (%) of reduction of elderly GI conditions (E1, E2 and E3) with respect to the control (C). $^{a-c}$ Different lowercase letters indicate significant differences between models, with a significance level of 95% ($p < 0.05$).

Table 2b. Amino acids profile (mg free amino acid / g product) of fresh and aged cheese digested under different in vitro digestion models (C: control; E1: Elderly 1; E2: Elderly 2; E3: Elderly 3) and reduction (%) of amino acid released with respect to the control.

Amino acid	mg free amino acid / g product (Reduction with respect to the control (%))							
	Fresh cheese				Aged cheese			
	C	E1	E2	E3	C	E1	E2	E3
Alanine	3.5 ± 0.4^{c}	2.8 ± 0.2^{bc} (20)	2.3 ± 0.6^{b} (42)	1.5 ± 0.3^{a} (55)	4.391 ± 0.106^{c}	3.7 ± 0.2^{b} (16)	3.2 ± 0.2^{b} (27)	2.538 ± 0.006^{a} (42)
Glycine	1.7 ± 0.2^{c}	1.5 ± 0.2^{bc} (12)	1.25 ± 0.17^{b} (28)	0.51 ± 0.10^{a} (72)	2.6 ± 0.6^{b}	2.623 ± 0.106^{b} (12)	2.33 ± 0.06^{b} (22)	0.96 ± 0.07^{a} (68)
Valine	4.5 ± 0.3^{b}	4.2 ± 0.3^{b} (3)	3.234 ± 0.007^{a} (28)	2.96 ± 0.10^{a} (34)	7.9 ± 0.5^{b}	7.08 ± 0.07^{ab} (10)	6.24 ± 0.05^{a} (21)	6.3 ± 0.4^{a} (19)
Leucine	11.6 ± 0.9^{b}	10.96 ± 0.10^{b} (6)	8.9 ± 0.2^{a} (24)	9.0 ± 0.3^{a} (25)	16.765 ± 1.108^{b}	14.1 ± 0.2^{a} (16)	14.79 ± 0.12^{a} (12)	13.8 ± 0.5^{a} (18)
Isoleucine	2.55 ± 0.18^{c}	2.16 ± 0.18^{b} (19)	1.722 ± 0.106^{a} (33)	1.58 ± 0.09^{a} (38)	4.78 ± 0.12^{c}	4.1 ± 0.2^{ab} (14)	4.19 ± 0.03^{b} (12)	3.83 ± 0.17^{a} (20)
Threonine	2.45 ± 0.18^{b}	1.5 ± 0.4^{a} (309	1.31 ± 0.03^{a} (46)	1.18 ± 0.06^{a} (52)	3.5 ± 0.3^{b}	2.8 ± 0.2^{a} (19)	2.78 ± 0.16^{a} (20)	2.33 ± 0.17^{a} (33)
Serine	3.2 ± 0.5^{c}	2.09 ± 0.09^{b} (35)	1.77 ± 0.17^{ab} (45)	1.59 ± 0.15^{a} (53)	4.584 ± 0.017^{c}	3.9 ± 0.2^{bc} (14)	3.3 ± 0.3^{ab} (27)	3.1 ± 0.4^{a} (33)
Proline	1.06 ± 0.17^{c}	0.85 ± 0.06^{b} (23)	0.68 ± 0.05^{ab} (29)	0.51 ± 0.03^{a} (52)	3.47 ± 0.09^{c}	2.96 ± 0.17^{b} (15)	2.3 ± 0.2^{a} (34)	2.41 ± 0.13^{a} (34)
Asparagine	1.67 ± 0.03^{b}	1.53 ± 0.03^{b} (9)	0.61 ± 0.17^{a} (59)	0.597 ± 0.008^{a} (64)	3.6 ± 0.6^{c}	2.77 ± 0.14^{b} (22)	1.97 ± 0.06^{a} (45)	1.82 ± 0.17^{a} (19)
Aspartic acid	1.9 ± 0.2^{c}	1.87 ± 0.09^{c} (6)	1.26 ± 0.14^{b} (38)	0.815 ± 0.004^{a} (58)	3.6 ± 0.3^{b}	3.16 ± 0.04^{b} (13)	2.24 ± 0.04^{a} (38)	2.0 ± 0.2^{a} (45)
Methionine	2.35 ± 0.19^{c}	1.93 ± 0.10^{b} (18)	1.47 ± 0.02^{a} (38)	1.45 ± 0.06^{a} (38)	3.69 ± 0.07^{c}	3.24 ± 0.13^{b} (12)	3.07 ± 0.09^{ab} (17)	2.94 ± 0.07^{a} (20)
Glutamic acid	3.8 ± 0.2^{b}	3.47 ± 0.13^{b} (6)	3.49 ± 0.18^{b} (10)	2.85 ± 0.13^{a} (24)	5.9 ± 0.2^{c}	4.78 ± 0.06^{b} (19)	5.2213 ± 0.0113^{ab} (11)	4.2 ± 0.4^{a} (28)

Phenylalanine	7.9 ± 0.5^{c}	6.2 ± 0.4^{b} (23)	5.38 ± 0.17^{a} (32)	5.00 ± 0.15^{a} (36)	14.15 ± 0.06^{d}	11.52 ± 0.09^{c} (19)	9.7 ± 0.2^{b} (32)	7.6 ± 0.2^{a} (46)
Glutamine	6.2 ± 1.4^{a}	5.0 ± 0.5^{a} (15)	4.8 ± 0.7^{a} (28)	5.0 ± 0.3^{a} (22)	10.5 ± 0.7^{b}	8.0 ± 0.8^{a} (24)	7.1 ± 0.2^{a} (32)	6.9 ± 1.0^{a} (34)
Ornithine	-	-	-	-	0.970 ± 0.008^{c}	0.85 ± 0.03^{b} (13)	0.51 ± 0.04^{a} (47)	- (100)
Lysine	10.7 ± 0.3^{a}	9 ± 2^{a} (4)	9.5 ± 0.7^{a} (15)	9.2 ± 0.9^{a} (19)	17.9 ± 0.4^{b}	13.85 ± 0.14^{a} (23)	13.7 ± 1.0^{a} (21)	12.8 ± 1.0^{a}
Histidine	2.1 ± 0.3^{b}	1.68 ± 0.15^{a} (15)	1.74 ± 0.15^{ab} (20)	1.57 ± 0.14^{a} (24)	3.29 ± 0.03^{c}	2.72 ± 0.02^{ab} (17)	2.91 ± 0.13^{bc} (11)	2.4 ± 0.3^{a}
Tyrosine	9.19 ± 0.04^{b}	8.5 ± 0.3^{b} (8)	7.2 ± 0.6^{a} (25)	6.4 ± 1.0^{a} (36)	11.1 ± 0.7^{d}	9.2 ± 0.3^{c} (17)	8.0 ± 0.3^{b} (28)	5.2 ± 0.4^{a}
Tryptophan	4.5 ± 0.6^{b}	3.5 ± 0.3^{a} (25)	3.30 ± 0.07^{a} (26)	2.90 ± 0.14^{a} (35)	5.62 ± 0.07^{d}	4.7 ± 0.3^{b} (16)	4.70 ± 0.02^{b} (16)	3.45 ± 0.10^{a}
Cystine	2.42 ± 0.08^{b}	2.12 ± 0.04^{a} (12)	2.13 ± 0.06^{a} (12)	- (100)	2.8 ± 0.3^{c}	2.16 ± 0.06^{b} (23)	1.54 ± 0.05^{a} (45)	- (100)

Data shown are mean values from triplicates and the standard deviation. Values in parentheses represent the percentage (%) of reduction of elderly GI conditions (E1, E2 and E3) with respect to the control (C). $^{a-c}$ Different lowercase letters indicate significant differences between models, with a significance level of 95% ($p < 0.05$).

Table 3. Molar percentages of acyl groups (AG) supported on the different glyceryl backbone structures (TG, 1,2-DG, 1,3-DG, 2-MG, 1-MG) and free fatty acids (FFA), present in the non-digested (ND) and in vitro digested samples (C: Control; E1: Elderly 1; E2: Elderly 2; E3: Elderly 3) of fresh and aged cheese.

	In vitro digestion model	AG_{TG} (%)	$AG_{1,2\text{-}DG}$(%)	$AG_{1,3\text{-}DG}$(%)	$AG_{2\text{-}MG}$(%)	$AG_{1\text{-}MG}$ (%)	FFA (%)
Fresh cheese	Non digested	97.84 ± 0.12^{c}	1.3 ± 0.3^{a}	1.16 ± 0.14^{c}	0 ± 0^{a}	0 ± 0^{a}	0.4 ± 0.3^{a}
	C	11.09 ± 1.14^{ab}	10.0 ± 0.8^{b}	0.96 ± 0.02^{d}	6.36 ± 0.15^{d}	1.85 ± 0.06^{b}	69.8 ± 0.6^{b}
	E1	12 ± 3^{ab}	8.2 ± 0.7^{b}	0.30 ± 0.10^{b}	6.9 ± 0.6^{c}	2.1 ± 0.3^{b}	71 ± 3^{b}
	E2	13.3 ± 0.9^{b}	8.3 ± 1.5^{b}	0.10 ± 0.19^{a}	6.7 ± 0.2^{c}	2.1 ± 0.4^{b}	69.7 ± 0.7^{b}
	E3	8.40 ± 0.10^{a}	8.65 ± 0.16^{b}	0.62 ± 0.04^{b}	4.29 ± 0.05^{b}	1.84 ± 0.01^{b}	76.2 ± 0.3^{c}
Aged cheese	Non digested	98.1 ± 0.7^{c}	1.3 ± 0.4^{a}	1.15 ± 0.12^{b}	0 ± 0^{a}	0 ± 0^{a}	0.5 ± 0.2^{a}
	C	18 ± 4^{b}	8 ± 2^{b}	0.3 ± 0.3^{a}	7.8 ± 0.9^{d}	3.1 ± 0.3^{b}	62.8 ± 0.5^{b}
	E1	10 ± 2^{a}	8 ± 3^{b}	0.4 ± 0.7^{ab}	9.8 ± 0.4^{e}	3.5 ± 0.9^{b}	67.99 ± 0.19^{c}
	E2	6.7 ± 1.4^{a}	6.9 ± 0.9^{b}	1.1 ± 0.5^{ab}	6.2 ± 0.3^{c}	2.8 ± 0.7^{b}	76 ± 4^{d}
	E3	7.06 ± 0.19^{a}	5.3 ± 0.2^{b}	1.14 ± 0.04^{ab}	3.35 ± 0.13^{b}	2.43 ± 0.16^{b}	80.7 ± 0.8^{e}

Data shown are mean values from triplicates and the standard deviation. *AG: acyl groups. $^{a\text{-}d}$ different lowercase letters means significant difference between models ($p<0.05$).