Age-related gastrointestinal alterations of legumes and cereal grains digestibility

Running title: Gastrointestinal alterations with the elderly of pulses and grains

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Abstract

Aging is accompanied by changes in gastrointestinal functions. The impact of the gastrointestinal (GI) conditions of the elderly on the extent of proteolysis and glycolysis as well as calcium bioaccessibility in some cooked legumes (chickpea, lentils, soya bean and white bean) and cereals/pseudocereals (oats, spelt and quinoa) were studied. Samples were digested *in vitro* using three GI models specifically focused on the elderly in which oral, gastric and intestinal conditions were altered (E1: altered oral conditions, E2: altered oral and gastric conditions and E3: altered oral, gastric and intestinal conditions). Samples were also subjected to a standardized GI digestion as a control (C). The extent of proteolysis was only significantly affected with suboptimal intestinal conditions ($p<0.05$). Protein digestibility of cereal grains decreased to a greater extent than for legumes. The release of non-essential amino acids was more affected than that of essential ones, mainly in legumes such as soya bean, lentils and white bean. The extent of glycolysis was much higher in cereal grains than legumes regardless of GI digestion conditions. Glycolysis declined with altered intestinal conditions (E3) compared to the C, in all legumes and spelt. Calcium bioaccessibility was much higher in cereal/pseudocereals than in legumes. However, calcium bioaccessibility seems to be highly limited in elderly people suffering from oral, gastric or intestinal alterations (up to 53% reduction compared to C). Such data might be helpful to develop dietary strategies based on protein-rich vegetal foods, including alternative crops such as oats, quinoa and spelt, specifically used to mitigate sarcopenia and osteoporosis in elderly people.

Keywords: ageing; legumes; grains; digestibility; calcium bioaccessibility

1. INTRODUCTION

The world population has been predicted to exceed 9.7 billion by 2050. In addition, people above age 65 are expected to considerably increase, exceeding the number of children by 2045 (UN, 2019). The nutrition of the elderly is a global concern since health conditions and body composition change with age. Protein intake has an important role as its deficit is associated with muscle mass loss (sarcopenia) or physical weakness (asthenia) amongst other conditions. Moreover, calcium and vitamin D deficiencies have been associated with osteoporosis which increases the risk of fractures (De-la-O et al., 2019; Rémond et al., 2015). Meat, fish and dairy products, which are important sources of high biological value proteins, are often unaffordable for those with low incomes. Therefore, the Food and Agriculture Organization of the United Nations (FAO) has recommended an increase of legume consumption because of their high protein content, their affordability, and their contribution to food security and environmental sustainability (FAO, 2016). Legumes are good sources of vegetable protein and minerals, especially iron, zinc, and calcium as well as relevant quantities of phenolic compounds (Giusti et al., 2019; Ramírez-Ojeda et al., 2018; Roy et al., 2010). Additionally, they also have complex carbohydrates and dietary fiber which makes their glycemic-index low (Esmaillzadeh & Azadbakht, 2012). Studies associate their consumption with a lower prevalence and incidence of illness (obesity, cardiovascular disease, type 2 diabetes, and some types of cancers) (Jeong et al., 2019; Monnet et al., 2019). Thus, to supply the nutritional needs of the elderly, the World Health Organization (WHO) has recommended the intake of healthy legume-based dishes (Stoin et al., 2019). However, some minor grains such as quinoa, oats or spelt, have also gained interest due to their higher content of nutrients not found in relevant amounts in the major cereal crops such as wheat, rice or corn (maize). In addition, they contribute to the diversification of food crops which can help stabilize global food production (Yabe &

Iwata, 2020). Therefore, they should be considered as future alternative sources of protein for the elderly, and for the human population in general. Minor crops such as oats and spelt are also a good source of dietary fiber, vitamin B, and numerous dietary minerals. Additionally, oats contain legume-like protein and their quality is nearly equivalent to soy protein, hence the World Health Organization study has shown they are the closest vegetable proteins to meat, milk, and egg protein (Capurso et al., 2018). Quinoa, considered as a pseudo-cereal in the botanic classification, is characterized by its large amount of essential amino acids (EAA), especially Lys, which is close to the standards set by FAO for human nutrition (Rodríguez et al., 2020). Thus, FAO has recommended quinoa intake due to its well-balanced proteic profile similar to that of milk (Comai et al., 2007). Compared to most cereals, quinoa has higher amounts of vitamins and minerals such as calcium, iron and copper, as well as a lower carbohydrate content (than wheat, barley, corn and rice) (Dakhili et al., 2019). However, these benefits can be limited in the elderly due to poor mastication, reduced digestive enzymes and bile salts secretion, suboptimal pH or longer transit time through the gastrointestinal (GI) tract, amongst others (Satusap et al., 2014). The structural matrix, chemical properties or the interactions among macro- and micronutrients can also modulate digestibility altering hydrolysis with similar digestive conditions. However, studies aiming to elucidate the contribution of food-inherent factors from other crops on digestibility and the different GI alterations in the elderly are limited.

Therefore, this study aimed to analyze the impact of GI alterations, frequently found in the elderly, on protein and carbohydrate digestibility and calcium bioaccessibility in 4 legumes (chickpea, lentils, white bean and soya bean) and three alternative grains (oats, spelt and quinoa) using a static *in vitro* digestion system.

2. MATERIAL AND METHODS

2.1. Chemicals

Pepsin from porcine gastric mucosa (3200–4500 U/mg, P6887), pancreatin (8 x USP, P7545) from porcine pancreas, p-toluene-sulfonyl-L-arginine methyl ester (TAME, T4626), bovine bile (dried, unfractionated, B3883), analytical grade salts (potassium chloride, potassium dihydrogen phosphate, sodium bicarbonate, sodium chloride, magnesium chloride, ammonium carbonate, calcium chloride, potassium sulfate and potassium sodium tartrate tetrahydrate), boric acid, hydrochloric acid (37%), sulfuric acid (95-97%), sodium hydroxide, DNS (3-5′ dinitrosalicylic acid) reagent, D-+-glucose (≥99.5%), ethanol (96%) and invertase from baker´s yeast (Grade VII, ≥300 units/mg solid, I4504) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Nitric acid (70%) and lanthanum (III) chloride heptahydrate (analytical grade) were purchased from Honeywell Fluka (Morris Plains, NJ, USA); petroleum ether (VWR Chemicals, VWR International Pty. Ltd., Murarrie, Queensland, Australia), amyloglucosidase (*Aspergillus niger*) (E-AMGDF, Megazyme, Bray, Ireland) and EZ-Faast amino acid kit (Phenomenex, Torrance, CA, USA) were also used.

Legumes (chickpea (*Cicer arietinum*, Hacendado®, Valencia, Spain), pardina lentils (*Lens culinaris* var. Variabilis, Hacendado®), white bean (*Phaseolus vulgaris*, Hacendado®) and soya bean (*Glycine max,* Biográ®, Barcelona, Spain)) and cereal grains (whole oats (*Avena sativa*, Biográ®), whole spelt (*Triticum spelta*, Biográ®) and quinoa (*Chenopodium quinoa*, Hacendado®)) were purchased previously dried for retail sales at local stores in Valencia (Spain).

2.2. Sample preparation

Legumes and cereal grains were soaked (excepting lentils and quinoa) and boiled before *in vitro* digestion studies. Soaking was overnight with deionized water (Barnstead Mega-Pure deionizer, Thermo-Fisher Scientific, Waltham, MA, USA) at a ratio of 1:3 (w:w) grain:water at 20 ± 1 °C. Subsequently, soaked grains were boiled at 95 ± 5 °C with deionized water with a ratio of 1:3 (w:w) grain:water for 60, 45, 30, 60 and 25 min for soya bean, chickpea, white bean, whole spelt and whole oats, respectively. Pardina lentils and quinoa were directly boiled at the same grain:water ratio for 20 and 10 min, respectively. Cooking time was determined and adjusted for each variety in preliminary analyses considering label recommendation, i.e., until legumes could be crushed with fingers and reached a moisture content of 60 ± 6% (on a wet basis). All cooked samples were drained in a kitchen sieve for 2 min and kept cool at 20 ± 2 °C until they reached this temperature. Cooked samples were then immediately used for composition analysis and *in vitro* digestion.

2.3. Compositional analysis

After cooking and cooling, moisture, ash, fat, fiber and crude protein (using a Kjeldahl factor of 5.70) contents were characterized in the samples according to the AOAC official methods 934.01, 942.05, 920.39, 962.09 and 960.52 (Association of Official Analysis Chemists International (AOAC), 2000), respectively. Initial sugars and total starch content were also determined quantifying glucose using the DNS colorimetric method according to Armellini et al. (2019). Before the measurement of total starch, samples were freeze-dried, mill, gelatinized and digested (using amyloglucosidase). In addition, ash was dissolved in a 20% 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). Air:acetylene (11.5:1.5 L min^{-1})

were used in the flame and samples were measured at 422.7 nm. $CaCO_3$ was used to obtain a calibration curve (from 0 to 10 mg/L of Ca) (Noël et al., 2008).

2.4. Static *in vitro* simulation of GI digestion

The control model (C) corresponded to the standard GI conditions of a healthy adult as often defined in these types of experiments (Minekus et al., 2014). Particularly controversial is the gastric pH. Reports show pH values between 1.5 and 4.0 (Biehler et al., 2011; Oomen et al., 2003; Reboul et al., 2006). The elderly models simulating the accumulative alterations that appear as a consequence of 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)) (Table 1). Specific digestive conditions in the elderly were established according to Shani-Levi et al. (2017), except for the transit time of the gastric and intestinal stages (Denis et al., 2016). Chewing (number of mastication cycles) was standardized (Jalabert-Malbos et al., 2007) and done *in vivo* using a healthy volunteer (male student, 30 years old) with good dentition until reaching a bolus consistency similar to a tomato or mustard paste (Minekus et al., 2014). For the elderly, the number of chewing cycles were reduced to 50% by the same volunteer to mimic one of the most critical oral changes with the elderly, i.e., edentulism, generating a bolus with a larger particle size and more difficult to swallow (Lee et al., 2004; O'Keeffe et al., 2019). Thus, 20 and 10 chewing cycles for a healthy adult and the elderly, respectively, were done for all the cooked foods (except for soya bean). Harder food would generally require more chewing cycles (Chen, 2009), i.e., soya bean, where 30 and 15 chewing cycles were needed.

All materials were digested at least three times using each GI conditions (C, E1, E2 and E3). Table 1 shows the specific conditions of each digestion model. Gastric (SGS) and intestinal (SIS) digestion fluids were prepared fresh daily from stock solutions and the digestive enzymatic activity of the enzymes were tested before each experiment according to Minekus et al. (2014). Briefly, the trypsin activity of pancreatin was measured using a continuous spectrophotometric rate determination (using Helios Zeta UV-VIS Spectrophotometer, Thermo Fisher Scientific) using p-toluene-sulfonyl-L-arginine methyl ester (TAME) as the substrate at different concentrations to obtain the rate at 247 nm. One trypsin unit hydrolyses 1 µmole of TAME/min at 25°C, pH 8.1. Likewise, the enzymatic activity of pepsin was measured at 280 nm using the spectrophotometric stop rate determination using different concentrations of hemoglobin as substrate. One pepsin unit will produce a ΔA_{280} of 0.001/min at pH 2.0 and 37°C, measured as TCA-soluble products.

After digestion, the pH of digests was adjusted to 5 and kept in an ice bath for 10 min to inhibit the enzymatic reactions before fraction separation and analytical determinations. The separation of the liquid fraction from the undigested remaining solids was done using a centrifuge at 4000 x g (5810R, Eppendorf, Hamburg, Germany) for 5 min at 10 °C to obtain the supernatant.

2.5. Analytical determinations

2.5.1. Free amino acids (FAA)

Essential (EAA) and non-essential (NEAA) amino acids from protein digestion were determined using the protocol by Peinado et al. (2016) with some modifications. Briefly, the amine and carboxyl groups of the FAA contained in 100 µL of the bioaccessible fraction were derivatized at room temperature in aqueous solution using the EZ-Faast amino acid kit. Derivatized samples were measured using a GC-MS (Injector 7683B series, Network GC System 6890N series, Inert Mass Selective Detector 5975 series, MSD ChemStation software) (Agilent Technologies, Palo Alto, CA, USA) using norvaline as an internal standard. A calibration of the peak area was prepared for each amino acid using the amino acids standard solution included in the kit. The extent of proteolysis was estimated considering the sum of the FAA in the bioaccessible fraction with respect to the amount of crude protein in undigested cooked food (equation 1).

$$\textbf{\textit{Extent of proteolysis}}\ (\%) = \frac{(\textit{g FAA in bioaccessible fraction})}{(\textit{g crude protein in undigested cooked food})} \times \mathbf{100} \qquad \textbf{(1)}$$

2.5.2. Digestible starch

Reducing sugars released during digestion (monosaccharides) were determined in the bioaccessible fraction with a colorimetric method using dinitrosalicylic acid (DNS) after an invertase and amyloglucosidase secondary digestion (Armellini et al., 2019). An aliquot of 1 mL of the bioaccessible fraction was mixed with 4 mL of absolute ethanol to prepare an extract. The ethanolic extract (50 µL) were added to 250 µL of the enzymatic solution (1% amyloglucosidase + 1% invertase in acetate buffer, pH 5.2) and incubated at 37ºC for 10 min. The DNS mixture (750 µL containing a 1:1:5 mixture of 0.5 mg/mL glucose:4 M NaOH:DNS reagent (10 g/L of 3,5-dinitrosalicylic acid, containing 300 g potassium sodium tartrate and 16 g NaOH)) were added and heated for 15 min at 100ºC. Then, 4 mL of cold deionized water were added and absorbances measured at 530 nm (using a Helios Zeta UV-VIS Spectrophotometer, Thermo Fisher Scientific). Glucose was used to obtain a calibration curve (from 0 to 10 mg/L). The extent of glycolysis was calculated using equation 2:

$$\textbf{\textit{Extent of glycolysis}}\ (\%) = \frac{(\textit{g free glucose Eq. in bioaccessible fraction})}{(\textit{g starch (glucose Eq.) in undigested food})} \times \mathbf{100} \qquad \textbf{(2)}$$

2.5.3. Calcium bioaccessibility

An aliquot of 4 mL of the bioaccessible fraction was used for free calcium determination using flame atomic absorption spectroscopy (FAAS) using the same protocol used to determine the total amount of calcium in undigested samples. The bioaccessibility of calcium was estimated using equation 3:

$$\boldsymbol{Calcium\ bioaccessibility}\ (\%) = \frac{(\boldsymbol{mg\ Ca^{2+} free\ in\ bioaccessible\ fraction})}{(\boldsymbol{mg\ Ca^{2+} total\ in\ undigested\ food})} \times \mathbf{100} \qquad \textbf{(3)}$$

2.6. Statistical analysis

Results were evaluated using an analysis of variance (multivariate ANOVA). In addition, multiple range tests were obtained using the LSD (least significant difference) of the Fisher test to identify homogeneous groups between models and foods. For these analyses, Statgraphics Centurion XVII software (Statgraphics Technologies Inc, The Plains, VA, USA) was used with a confidence level of 95% ($p<0.05$). Principal component analysis (PCA) was also used to determine the relationship among the experimental data (total, EAA and NEAA extents of proteolysis, the extent of glycolysis and calcium bioaccessibility).

3. RESULTS AND DISCUSSION

3.1. Nutritional composition of legumes and cereal/pseudocereal grains

Results from the compositional analysis in terms of the crude protein, total fat, ash, fiber, sugars and starch contents (Table 2) were comparable to those previously reported (Angioloni & Collar, 2011; Iqbal et al., 2006; Longvah, 2017). As expected, legumes showed higher protein content than grains, soya bean being the highest, and oats the lowest. In addition to the nutritional value, soya bean consumption has gained considerable attention given its beneficial effects on cardiovascular health by improving the lipid profile, glycaemia and insulin homeostasis, blood pressure and aiding weight control (Pan et al., 2008). Grains ranged from 1% (spelt and white bean) to 10% (soya bean) of lipids on a dry basis. Moreover, fiber content was higher in legumes than in alternative crops. On the other hand, alternative crops showed greater starch content than legumes. Chickpea and oats were higher in calcium than other legume and grains while lentils and spelt had the lowest content of this mineral. These results were lower than those previously reported (Anitha et al., 2020; Longvah, 2017; Sandberg, 2002; U.S. Department of Agriculture, 2019) for the raw counterparts. Apparently, calcium lixiviation has been reported during soaking and/or cooking in some vegetal materials (Lestienne et al., 2005).

3.2. Protein digestibility of legumes and grains simulating the elderly GI conditions

The biological value of dietary proteins is given by the amino acid profile and its GI digestion. Within the amino acids resulting from the protein enzymatic hydrolysis, the EAA have an important role in muscle protein synthesis (Volpi et al., 2003). Specifically, sarcopenia, the loss of muscle mass as a result of aging, causes functional decline and loss of independence in older adults (Walston, 2012). Figure 1 shows the extent of proteolysis of the EAA and NEAA fractions found in legumes and grains digested with standard (C) and the elderly (E1, E2 and E3) GI conditions. The extent of proteolysis with standardized GI conditions (C) ranged from 56 to 100%, depending on the food matrix. FAA digestibility extents in vegetal foods were similar to those achieved in digested high-protein foods such as meat or egg (60-90 and 40-80%, respectively) (Asensio-Grau et al., 2018; Denis et al., 2016). However, protein in grains was slightly better digested than legumes. Similar results were reported in the literature for proteolysis with values ranging between 80-95% for oats, spelt and quinoa (Abdel-Aal & Hucl, 2002; Sobota et al., 2020; Zarkadas et al., 1995), 70–80% for legumes (Hussain et al., 2020), and 60% for soya bean (Zahir et al., 2020). The extent of proteolysis achieved by the samples, could be even higher than reported because of the extent of proteolysis calculation has been just based on FAA without taking into account the possible short-chain peptides which are also bioabsorbable. Among the legumes, higher proteolysis was obtained with chickpea and white bean compared to lentil and soya bean. The low protein hydrolysis obtained with soya bean could be due, apart from the presence of antinutritional factors, to the low porosity of the matrix. Even if a thermal process was used, remaining intact cells could occur and decrease considerably the cell wall permeability to proteolytic enzymes (Zahir et al., 2020). Soya bean has been associated with low digestibility due that possess a complex matrix mostly composed of protein bodies immersed in a lipid matrix of individual bodies and its cell wall is composed of pectins, being less degradable upon cooking (Zahir et al., 2018).

Only the intestinal alteration mimicked in the E3 model had a significant impact on protein hydrolysis. A significant decrease in proteolysis was observed in these GI conditions compared to values obtained in C. Thus, the extent of proteolysis achieved with E3 conditions ranged from 69 to 40% for white and soya beans, respectively. The decrease depended on the food type, being grain protein (~40% of hydrolysis reduction) the most affected with these alterations than legumes. Reasonably, a decrease in the pancreatic enzyme and bile concentrations lead to maldigestion and malabsorption causing nutritional deficiencies (Rémond et al., 2015). Therefore, protein digestion would only be compromised in people suffering from pancreatic and/or biliar insufficiency.

EAA fraction increased from 30 to 69% in soya beans and from 27 to 41% in chickpeas in C conditions. Moreover, NEAA fraction ranged between 23 and 53% (soya bean being the lowest and chickpea the highest) in C conditions and fell from 13 to 30% (being chickpea/soya bean the lowest, and white bean and grains the highest values) in E3. In like manner, differences in the extent of proteolysis were only observed in suboptimal intestinal conditions (E3) compared to non-altered intestinal conditions (E2). Regarding the EAA:NEAA ratio (Figure 1), a 1:1 ratio was observed for all samples excepting chickpea (3:1 EAA:NEAA ratio) in C conditions. Chickpea protein has been reported as a good source of EAA such as isoleucine, lysine, tryptophan and aromatic amino acids (Alajaji & El-Adawy, 2006). EAA:NEAA ratio increased to 3:1 in soya bean (being 70 and 30% of the extent of proteolysis, respectively for EAA and NEAA) subjected to *in vitro* digestion using altered conditions (E3). Thus, the elderly GI alterations seem to limit to a greater extent the release of NEAA than EAA in this legume.

Tables 3 and 4 gather the EAA and NEAA profiles after GI *in vitro* simulation. These results were consistent with those reported by other authors (Abdel-Aal & Hucl, 2002; Anitha et al., 2020; Koehler & Wieser, 2013; Longvah, 2017). Lys, Leu, Trp and Phe were present as the major amino acids in all cooked grains, whereas Met was determined to be a deficient amino acid. On the other hand, all grains showed low concentrations of Pro while Gln was not found. Pro and Gln are the amino acids present in prolamin and its presence can cause health issues such as celiac disease (Tsopmo, 2015).

In some foods, higher surface area in small particles allows higher enzyme access (Paz-Yépez et al., 2019). However, there were no differences in the results using altered oral stage (E1) compared to standard oral conditions (C) since products from protein hydrolysis were only quantified at the end of the intestinal stage, and therefore, the gastric and intestinal factors (pH, enzymes, surface active materials and other biological components) could mask the effect of differences in particle size. In the same way, the gastric stage did not show differences when E1 was compared to E2, showing that the activity of pancreatic proteases might compensate for the suboptimal conditions in the gastric stage (E2). Therefore, EAA such as Leu, Ile and Val, also known as branched-chain amino acids (BCAA), are EAA that act as important substrates and important regulators in protein synthesis with heavier anabolic effects not just in healthy subjects, but also in the elderly (Engelen et al., 2007).

3.3. Extent of glycolysis of legumes and grains using the GI elderly conditions

Legume and grain starch digestibility was evaluated quantifying the amount of glucose released at the end of standardized (C) and the elderly (E1, E2 and E3) *in vitro* digestion. Figure 2 shows significant differences among legumes and grains in terms of starch hydrolysis, or the extent of glycolysis (%) regardless of the GI digestion conditions. Thus, the extent of glycolysis varied from 22-35% for legumes and from 65 to 90% (average values) for grains when C conditions were simulated. Other studies (Angioloni & Collar, 2011; Bonafaccia et al., 2000; Chung et al., 2008; Goñi et al., 1997; Hoover & Zhou, 2003; Rehman & Shah, 2005; Ruales & Nair, 1994) report a high variability of starch digestibility in legumes and grains, it has been consistent that legume starch is hydrolyzed to a much lesser extent than starch in oats, spelt or quinoa. The starch digestibility increases when subjected to thermal processes (Wang et al., 2003) and depends on the severity of the process, i.e., the damage done to the starch granules could vary (Bao et al., 2018). Hence, the starch thermal behavior differs between legumes and cereals (Liu et al., 2006). The intrinsic characteristics of the plant source could make a difference in terms of starch digestibility. Consequently, the lower digestibility of legume starch, compared to cereal starches, could be attributed to the higher amylose content, existence of intact tissue/cell structures enclosing starch granules, higher content of viscous soluble dietary fiber components, the incidence of a larger number of antinutrients which would affect starch digestion, 'B'-type crystallites and stronger interactions between amylose chains (Wang et al., 2003; Yadav et al., 2009).

On the other hand, the elderly oral alterations (C compared to E1) had a statistically significant ($p<0.05$) negative impact on starch hydrolysis in lentils only; even when a declining trend was observed in other legumes and cereal/pseudocereal grains such as chickpea, soya bean, white bean and spelt. Higher protein content has been associated with strong molecular interactions (Chung et al., 2008), and the decrease in chewing cycles can impact digestion differently depending on the intrinsic properties of each food (particle size, hardness and other physical properties) (Woda et al., 2006). Likewise, the gastric alterations seem to decrease starch digestibility in all legumes and grains, only being statistically significant for lentil and white bean. Proteins can decrease the enzymatic digestion of starch due to the three-dimensional network they form (Chen et al., 2017). Subsequently, if the gastric proteolytic enzyme concentration is reduced (E2), food matrix degradation throughout digestion is expected to fall along with the conversion of starch into sugars. Finally, the elderly intestinal disorders (E3) highly contributed to a remarkable reduction of glycolysis for all legumes and grains, except for soya bean, oats and quinoa in which sugar content resulting from starch hydrolysis was similar to the obtained with healthy GI conditions (C). Carbohydrate digestibility of spelt, chickpea, lentils and white bean was more affected by bile and pancreatic enzyme concentrations than by the time of digestion. On the other hand, it is important to point out that oats and quinoa glycolysis seems to increase when E3 conditions were simulated. Lower fiber and protein contents in cooked oats and quinoa grains promote lower viscosity, leading to easier and more digestible matrices in a shorter time (Chen et al., 2017; Kristensen & Jensen, 2011), especially when they are subjected to the most disadvantageous GI scenery (E3). The legumes could have a greater contribution to hypoglycemia than oats, quinoa and spelt (Wolter et al., 2013).

3.4. Calcium bioaccessibility of legumes and grains using the elderly GI conditions

A diminished digestion of macronutrients, such as proteins and carbohydrates, could lead to a deficient release and solubilization of micronutrients. Results showed that calcium bioaccessibility (%) was much higher in cereals/pseudocereals (from 82 to 103%) than in legumes (from 34 to 65%) in C conditions (Figure 3). There are very few studies on calcium bioaccessibility in legumes and grains and none simulating the elderly GI conditions. Ramírez-Ojeda et al. (2018) reported similar values of calcium bioaccessibility for lentil, chickpea and white bean. Legumes are specially high in antinutrients such as phytates, oxalates and tannins that can form insoluble complexes with calcium (Guéguen & Pointillart, 2000). Phytates, are directly related to fiber (Guéguen & Pointillart, 2000) and protein (Lestienne et al., 2005) contents exerting an adverse effect on calcium absorption. Additionally, some believe that lipids produce insoluble soaps with calcium, lowering its bioavailability (Guéguen & Pointillart, 2000). The higher the protein, fiber and fat contents in legumes, the lower the calcium bioaccessibility. High phytates amounts present in both food groups (Schlemmer et al., 2009) could be affected during processing and cooking. Moreover, phytase is an enzyme found in cereal and legumes which has optimal enzymatic activity in an acidic pH (4.5-5.6) in cereal, and in a neutral or an alkaline pH in legumes (Sandberg, 2002). Consequently, the lower enzymatic activity at gastric pH can affect calcium's GI pathway. Therefore, calcium bioaccessibility was affected with oral alterations for lentil and white bean, gastric alterations for white bean and oats, and intestinal changes for all. Intestinal suboptimal conditions drastically decreased calcium release in all samples except chickpea. A reduction of up to 53% was observed in some cases. Despite the reduction in calcium release from legumes and grains using the elderly conditions, chickpea, soya bean, white bean and oats are still good sources of this mineral in its bioaccessible form. The elderly are recommended to increase calcium intake since bone density tends to decrease with age leading to osteopenia and osteoporosis (McCabe et al., 2004). The latter is a significant health problem that contributes to disability and premature mortality amongst women and older men. Although genetic factors influence maximum bone mass, diet and exercise are modifiable risk factors that can be targeted to prevent osteoporosis (Rémond et al., 2015).

3.5. Descriptive relationship among digestion-end-parameters and the elderly GI conditions

Figure 4 shows the amount of EAA and NEAA (%), and the extents of proteolysis (%), glycolysis (%) and calcium bioaccessibility (%), as well as the scores for the different legumes and grains with the different simulated GI conditions. The first two main components explain 92.1% of the total variance in macronutrients and calcium bioaccessibility percentages in the samples (PC1: 69.9% and PC2: 22.2%). In the score plot, the proximity between samples indicates similar behavior in terms of digestibility. PC1 distinguishes among grains (oats, spelt and quinoa), located at the upper right quadrant of the plot, and legumes (chickpea, lentils, soya bean and white bean) located at the left lower quadrant of the plot. Besides, PCA showed the narrow relationship between the extent of glycolysis, NEAA and calcium bioaccessibility; while PC2 seems to distinguish between chickpea and soya bean from other legumes and cereals in terms of the amount of EAA and the total extent of proteolysis (higher in chickpea and lower in soya bean, than in the other matrices). Finally, PCA showed that as the digestive GI conditions were altered according to the elderly disorders (from the C to E3 models), samples tended to move towards the left side of the graph.

4. CONCLUSIONS

The influence of oral, gastric and/or intestinal alterations appearing with ageing on the luminal digestion of different legumes (chickpea, lentils, soya bean and white bean) and cereal/pseudocereal (oats, spelt and quinoa) grains were analyzed. According to the main results, it can be concluded that oats, spelt and quinoa proteins are more digestible than legumes with healthy GI conditions. Using the elderly GI alterations, and especially when intestinal conditions are suboptimal, proteolysis in grains seems to be, however, more compromised than in legumes. In addition, a preferential release of EAA compared to that of NEAA has been observed when the elderly GI conditions were simulated.

With respect to glycolysis and calcium bioaccessibility, the elderly intestinal alterations reduced the extent of glycolysis in legumes and spelt compared to the hydrolysis of starch achieved with healthy GI conditions. Cereal/pseudocereal grains have been shown to be a greater source of its bioaccessible form than legumes regardless the GI conditions. Although a notable bioaccessibility reduction was found in some foods such as chickpea, oats, soya and white beans as a consequence of the elderly GI alterations, they can still be considered good sources of bioaccessible calcium compared to other vegetal foods.

To conclude, these results support the idea that diet recommendations concerning the consumption of legumes and cereal/pseudocereal grains need to consider the impact of GI conditions of the populations of concern (e.g., the elderly) on their digestibility.

Conflict of interest

There are no conflicts of interest to be declared.

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Tables

Table 1. Specific GI conditions set for the 4 *in vitro* digestion models of this study.

Digestive stage	***In vitro* digestion model**			
	Control (C)	**Elderly 1 (E1)**	**Elderly 2 (E2)**	**Elderly 3 (E3)**
Oral stage	5 g of food sample + human salivary fluid Chewing until a consistency like a tomato or mustard paste is obtained (20 and 30 cycles for other and soya bean, respectively)	5 g of food sample + human salivary fluid **50% of the Control chewing cycles** 55 rpm at 37 °C	5 g of food sample + human salivary fluid **50% of the Control chewing cycles** 55 rpm at 37 °C	5 g of food sample + human salivary fluid **50% of the Control chewing cycles** 55 rpm at 37 °C
Gastric stage	Oral bolus + 10 mL SGF pH 3 Pepsin (2000 U/mL) 2 h	Oral bolus + 10 mL SGF pH 3 Pepsin (2000 U/mL) 2 h 55 rpm at 37 °C	Oral bolus + 10 mL SGF **pH 6** **Pepsin (1500 U/mL)** 2 h 55 rpm at 37 °C	Oral bolus + 10 mL SGF **pH 6** **Pepsin (1500 U/mL)** 2 h 55 rpm at 37 °C
Intestinal stage	Gastric chime + 20 mL SIF pH 7 Bile (10 mM) + Pancreatin (100 U/mL) 2 h	Gastric chime + 20 mL SIF pH 7 Bile (10 mM) + Pancreatin (100 U/mL) 2 h 55 rpm at 37 °C	Gastric chime + 20 mL SIF pH 7 Bile (10 mM) + Pancreatin (100 U/mL) 2 h 55 rpm at 37 °C	Gastric chime + 20 mL SIF pH 7 **Bile (5 mM)** **+ Pancreatin** **(50 U/mL)** **4 h** 55 rpm at 37 °C

Table 2. Total contents of water, crude protein, fat, ash, reducing sugars, fiber, starch and calcium in cooked legumes (chickpea, lentils, soya bean and white bean) and grains (whole oats, whole spelt and quinoa).

Nutrient content /100 g dry basis	**Chickpea**	**Lentils**	**Soya bean**	**White bean**	**Oats**	**Spelt**	**Quinoa**
Moisture (g)	157 ± 0.5^{d}	123 ± 0.2^{a}	175 ± 1^{e}	183 ± 2^{f}	136 ± 3^{b}	145 ± 1^{c}	259 ± 4^{g}
Crude protein (g)	17.8 ± 0.3^{e}	17.1 ± 0.2^{d}	41 ± 1^{f}	18.2 ± 0.5^{e}	11.3 ± 0.2^{a}	14.1 ± 0.1^{c}	12.4 ± 0.1^{b}
Fat (g)	5.7 ± 0.1^{e}	1.7 ± 0.5^{ab}	10 ± 1^{f}	1.1 ± 0.4^{a}	2 ± 0.2^{bc}	0.8 ± 0.1^{a}	2.8 ± 0.4^{d}
Ash (g)	2.2 ± 0.2^{c}	1.91 ± 0.05^{b}	2.9 ± 0.2^{e}	3.17 ± 0.05^{f}	1.62 ± 0.04^{a}	2.1 ± 0.1^{c}	2.61 ± 0.04^{d}
Reducing sugars (g)	0.09 ± 0.02^{a}	0.16 ± 0.01^{b}	0.28 ± 0.02^{c}	0.11 ± 0.002^{a}	0.30 ± 0.01^{c}	0.42 ± 0.04^{d}	0.46 ± 0.05^{d}
Fiber (g)	20 ± 2^{d}	18 ± 2^{d}	17 ± 1^{d}	29 ± 3^{e}	4.0 ± 0.4^{a}	10 ± 1^{c}	7 ± 1^{b}
Starch (g)	55 ± 1^{c}	62 ± 1^{d}	30 ± 3^{a}	48 ± 3^{b}	81 ± 3^{fg}	74 ± 1^{e}	75 ± 4^{ef}
Calcium (mg)	130 ± 20^{e}	13 ± 1^{a}	88 ± 6^{d}	85 ± 4^{d}	120 ± 10^{e}	30 ± 3^{b}	48 ± 5^{c}

Data shown are mean values from triplicates and the standard deviation. [abc] Different lowercase letters indicate significant differences between foods, with a significance level of 95% ($p<0.05$).

Table 3. EAA profile (mg FAA/g protein) of chickpea, lentils, soya bean, white bean, oats, spelt and quinoa after *in vitro* digestion using different elderly digestion models.

Vegetal food	GI conditions	EAA (mg free amino acid/ g protein)								
		Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophan	Valine
Chickpea	C	48 ± 7^{b}	40 ± 4^{b}	90 ± 10^{c}	140 ± 20^{b}	19 ± 2^{c}	67 ± 5^{c}	30 ± 3^{c}	53 ± 5^{c}	51 ± 5^{b}
	E1	46 ± 2^{b}	35 ± 1^{b}	76 ± 1^{b}	130 ± 10^{b}	16.3 ± 0.3^{b}	55 ± 2^{ab}	26 ± 1^{b}	44 ± 2^{b}	44 ± 2^{b}
	E2	48 ± 4^{b}	37 ± 4^{b}	80 ± 10^{b}	130 ± 20^{b}	17 ± 1^{bc}	58 ± 7^{b}	27 ± 2^{bc}	46 ± 3^{b}	46 ± 5^{b}
	E3	36.0 ± 0.4^{a}	26.9 ± 0.1^{a}	59 ± 1^{a}	70 ± 10^{a}	12.8 ± 0.3^{a}	50 ± 1^{a}	16.6 ± 0.3^{a}	35.0 ± 0.4^{a}	33.4 ± 0.4^{a}
Lentils	C	40 ± 3^{b}	36 ± 4^{b}	80 ± 10^{b}	120 ± 20^{b}	13 ± 1^{b}	54 ± 7^{b}	23 ± 2^{b}	39 ± 3^{b}	42 ± 5^{b}
	E1	40 ± 1^{b}	33 ± 2^{b}	70 ± 10^{ab}	110 ± 10^{b}	12 ± 1^{b}	48 ± 3^{ab}	23 ± 1^{b}	37 ± 2^{b}	39 ± 3^{b}
	E2	41 ± 2^{b}	34 ± 1^{b}	73 ± 3^{b}	100 ± 10^{ab}	12.1 ± 0.5^{b}	49 ± 2^{ab}	23 ± 1^{b}	35 ± 1^{b}	40 ± 2^{b}
	E3	32 ± 0.4^{a}	28 ± 1^{a}	61 ± 3^{a}	80 ± 10^{a}	9.2 ± 0.3^{a}	44 ± 1^{a}	15 ± 1^{a}	30 ± 1^{a}	33 ± 2^{a}
Soya bean	C	32 ± 1^{b}	26.3 ± 0.3^{a}	62 ± 1^{a}	80 ± 10^{b}	12.2 ± 0.4^{c}	41 ± 1^{a}	18 ± 1^{b}	34 ± 1^{ab}	31 ± 1^{a}
	E1	32 ± 4^{b}	25 ± 4^{a}	60 ± 10^{a}	90 ± 10^{b}	12 ± 1^{bc}	38 ± 7^{a}	18 ± 2^{b}	35 ± 5^{b}	30 ± 5^{a}
	E2	31 ± 1^{ab}	24 ± 1^{a}	53 ± 1^{a}	80 ± 10^{b}	11 ± 0.1^{b}	35 ± 1^{a}	17.4 ± 0.2^{b}	32 ± 1^{ab}	29 ± 1^{a}
	E3	27 ± 1^{a}	23 ± 1^{a}	51 ± 3^{a}	57 ± 4^{a}	9.4 ± 0.4^{a}	36 ± 2^{a}	13 ± 1^{a}	28 ± 0.1^{a}	26 ± 1^{a}
White bean	C	52 ± 1^{b}	40 ± 2^{b}	100 ± 10^{b}	140 ± 30^{b}	17 ± 1^{c}	64 ± 4^{b}	29 ± 2^{c}	50 ± 1^{c}	48 ± 3^{b}
	E1	49 ± 3^{ab}	37 ± 2^{ab}	84 ± 2^{ab}	130 ± 10^{b}	15.9 ± 0.3^{b}	58 ± 1^{ab}	27 ± 1^{bc}	48 ± 2^{c}	44 ± 2^{ab}
	E2	49 ± 1^{ab}	36 ± 2^{a}	81 ± 5^{a}	140 ± 10^{b}	15.2 ± 0.5^{b}	56 ± 3^{a}	26 ± 1^{b}	44 ± 2^{b}	42 ± 2^{a}
	E3	46 ± 2^{a}	35 ± 2^{a}	80 ± 10^{a}	100 ± 10^{a}	13.3 ± 0.4^{a}	61 ± 6^{ab}	21 ± 1^{a}	41 ± 1^{a}	40 ± 2^{a}
Oats	C	53 ± 2^{b}	39 ± 1^{bc}	80 ± 1^{ab}	120 ± 10^{b}	19.3 ± 0.3^{b}	52 ± 1^{b}	30 ± 2^{b}	59 ± 2^{b}	54 ± 1^{b}
	E1	56 ± 3^{b}	48 ± 7^{c}	100 ± 30^{b}	130 ± 20^{b}	20 ± 1^{b}	55 ± 3^{b}	33 ± 3^{b}	65 ± 1^{b}	58 ± 4^{a}
	E2	53 ± 6^{b}	36 ± 5^{b}	80 ± 10^{b}	100 ± 20^{b}	18 ± 2^{b}	50 ± 6^{b}	29 ± 4^{b}	59 ± 8^{b}	51 ± 8^{b}
	E3	39 ± 2^{a}	26 ± 3^{a}	60 ± 10^{a}	70 ± 10^{a}	14 ± 1^{a}	39 ± 4^{a}	19 ± 2^{a}	46 ± 3^{a}	39 ± 5^{a}
Spelt	C	58 ± 4^{b}	43 ± 1^{b}	87 ± 2^{b}	110 ± 10^{b}	21.3 ± 0.3^{c}	55 ± 2^{b}	32 ± 2^{b}	60 ± 4^{b}	57 ± 2^{b}
	E1	60 ± 1^{b}	43 ± 1^{b}	87 ± 2^{b}	117 ± 3^{b}	21 ± 1^{bc}	56 ± 2^{b}	33 ± 1^{b}	61 ± 1^{b}	58 ± 1^{b}
	E2	60 ± 2^{b}	42 ± 2^{b}	86 ± 4^{b}	100 ± 20^{b}	20 ± 1^{b}	56 ± 2^{b}	31 ± 3^{b}	57 ± 3^{b}	55 ± 3^{b}
	E3	42 ± 1^{a}	30 ± 1^{a}	60 ± 2^{a}	67 ± 4^{a}	15.5 ± 0.2^{a}	42 ± 1^{a}	19 ± 1^{a}	42 ± 2^{a}	39 ± 2^{a}
Quinoa	C	60 ± 1^{b}	43 ± 3^{c}	84 ± 3^{c}	130 ± 10^{b}	22.6 ± 0.5^{c}	54 ± 1^{c}	34 ± 2^{c}	62 ± 1^{c}	58 ± 3^{c}
	E1	57 ± 4^{b}	37 ± 3^{b}	80 ± 10^{b}	120 ± 20^{b}	21 ± 1^{b}	51 ± 3^{bc}	30 ± 2^{b}	61 ± 2^{c}	50 ± 2^{b}

E2	56 ± 3^{b}	35 ± 2^{b}	72 ± 4^{b}	122 ± 14^{b}	20 ± 1^{b}	48 ± 3^{b}	30 ± 2^{b}	56 ± 3^{b}	48 ± 2^{b}
E3	42 ± 2^{a}	25 ± 2^{a}	50 ± 4^{a}	79 ± 10^{a}	16 ± 1^{a}	36 ± 3^{a}	19 ± 2^{a}	43 ± 2^{a}	34 ± 3^{a}

Data shown are mean values from triplicates and the standard deviation. ^{abc} Different lowercase letters indicate significant differences between digestion models, with a significance level of 95% ($p<0.05$).

Table 4. NEAA profile (mg FAA/g protein) of oats, spelt, quinoa, chickpea, lentils, soya bean and white bean after *in vitro* digestion using different elderly digestion models.

Vegetal food	GI conditions	Non-essential amino acids (mg amino acid/ g protein)								
		Alanine	**Asparagine**	**Aspartic acid**	**Cystine**	**Glutamic acid**	**Glycine**	**Proline**	**Serine**	**Tyrosine**
Chickpea	**C**	34 ± 4^{b}	35 ± 4^{a}	22 ± 3^{a}	65 ± 7^{b}	50 ± 10^{b}	18 ± 2^{b}	13 ± 1^{b}	36 ± 4^{b}	120 ± 10^{b}
	E1	29 ± 1^{b}	30 ± 2^{a}	19 ± 1^{a}	59 ± 5^{b}	42 ± 8^{b}	16 ± 1^{b}	11 ± 1^{ab}	32 ± 1^{b}	101 ± 4^{a}
	E2	31 ± 4^{b}	29 ± 6^{a}	22 ± 1^{a}	63 ± 5^{b}	47 ± 9^{b}	17 ± 1^{b}	12 ± 1^{b}	34 ± 4^{b}	100 ± 10^{a}
	E3	18.8 ± 0.3^{a}	-	-	35 ± 1^{a}	16 ± 1^{a}	10.0 ± 0.2^{a}	10.3 ± 0.3^{a}	9.1 ± 0.5^{a}	104 ± 4^{a}
Lentils	**C**	27 ± 2^{b}	31 ± 3^{a}	20 ± 1^{a}	48 ± 3^{b}	41 ± 5^{b}	15 ± 1^{b}	9 ± 1^{b}	27 ± 2^{a}	100 ± 30^{b}
	E1	26 ± 2^{b}	30 ± 2^{a}	20 ± 1^{a}	48 ± 2^{b}	44 ± 5^{b}	14 ± 1^{b}	9.3 ± 0.4^{b}	26 ± 2^{a}	87 ± 2^{ab}
	E2	26 ± 2^{b}	26 ± 3^{a}	20 ± 1^{a}	4.3 ± 0^{a}	40 ± 8^{b}	15 ± 1^{b}	9.9 ± 0.3^{b}	26 ± 2^{a}	83 ± 3^{a}
	E3	18 ± 1^{a}	-	-	-	21 ± 2^{a}	8.9 ± 0.4^{a}	8.2 ± 0.2^{a}	-	99 ± 3^{ab}
Soya bean	**C**	18.9 ± 0.2^{b}	20 ± 1^{a}	12 ± 1^{a}	35 ± 2^{b}	25 ± 3^{b}	9.9 ± 0.2^{b}	6.9 ± 0.3^{ab}	20 ± 1^{a}	82 ± 7^{b}
	E1	19 ± 2^{b}	20 ± 3^{a}	13 ± 2^{a}	36 ± 5^{b}	30 ± 3^{c}	10 ± 1^{b}	7 ± 1^{ab}	20 ± 2^{a}	66 ± 9^{a}
	E2	19 ± 1^{b}	18.8 ± 0.4^{a}	12.2 ± 0.5^{a}	35.9 ± 0.3^{b}	30 ± 2^{bc}	10.2 ± 0.2^{b}	7.5 ± 0.1^{b}	20.3 ± 0.3^{a}	59 ± 2^{a}
	E3	13 ± 1^{a}	-	-	20.6 ± 0.02^{a}	12 ± 2^{a}	6.3 ± 0.2^{a}	6.4 ± 0.5^{a}	-	63.8 ± 0.2^{a}
White bean	**C**	31 ± 2^{c}	31 ± 4^{b}	20 ± 1^{b}	55 ± 2^{b}	47 ± 2^{b}	16.5 ± 0.5^{c}	10.2 ± 0.1^{b}	34 ± 3^{b}	130 ± 10^{c}
	E1	28 ± 1^{bc}	29 ± 2^{b}	19 ± 1^{b}	53 ± 3^{b}	45 ± 5^{b}	15.5 ± 0.4^{bc}	10 ± 0.4^{b}	31 ± 1^{b}	100 ± 10^{a}
	E2	27 ± 2^{b}	27 ± 2^{b}	19 ± 1^{b}	52 ± 2^{b}	48 ± 7^{b}	15 ± 1^{b}	10.1 ± 0.3^{b}	31 ± 2^{b}	109 ± 2^{ab}
	E3	21 ± 1^{a}	8 ± 2^{a}	2 ± 0.4^{a}	34 ± 2^{a}	27 ± 2^{a}	10.4 ± 0.3^{a}	8.6 ± 0.03^{a}	13 ± 3^{a}	130 ± 10^{bc}
Oats	**C**	35 ± 2^{b}	30 ± 4^{a}	23 ± 1^{b}	82 ± 3^{b}	55 ± 1^{b}	21 ± 1^{b}	16 ± 1^{ab}	37 ± 3^{b}	160 ± 10^{b}
	E1	38 ± 4^{a}	34 ± 5^{a}	27 ± 3^{b}	90 ± 6^{b}	58 ± 4^{b}	24 ± 2^{b}	18 ± 1^{b}	40 ± 5^{b}	170 ± 20^{b}
	E2	33 ± 5^{b}	27 ± 3^{a}	24 ± 3^{b}	86 ± 11^{b}	50 ± 10^{b}	22 ± 3^{b}	17 ± 2^{b}	36 ± 5^{b}	160 ± 20^{b}
	E3	22 ± 3 ^{a}	-	6 ± 5^{a}	56 ± 3^{a}	18 ± 5^{a}	14 ± 1^{a}	14 ± 1^{a}	17 ± 4^{a}	120 ± 10^{a}
Spelt	**C**	35 ± 1^{b}	28 ± 4^{ab}	23 ± 3^{a}	97 ± 7^{b}	50 ± 10^{b}	20 ± 1^{b}	19 ± 1^{a}	40 ± 4^{a}	160 ± 10^{b}
	E1	34.9 ± 0.5^{b}	30 ± 2^{b}	25 ± 1^{a}	102 ± 2^{b}	63 ± 6^{b}	21.0 ± 0.5^{b}	19.7 ± 0.5^{ab}	41 ± 2^{a}	150 ± 10^{b}
	E2	34 ± 3^{b}	22 ± 5^{a}	23 ± 3^{a}	99 ± 5^{b}	60 ± 10^{b}	21 ± 1^{b}	21 ± 1^{b}	39 ± 4^{a}	152 ± 4^{b}
	E3	22 ± 1^{a}	-	-	64 ± 1^{a}	30 ± 2^{a}	12 ± 1^{a}	20 ± 1^{ab}	-	122 ± 3^{a}
Quinoa	**C**	38 ± 2^{c}	32 ± 3^{b}	27 ± 3^{a}	92 ± 5^{b}	61 ± 6^{c}	22 ± 1^{c}	16 ± 1^{b}	39 ± 3^{b}	154 ± 4^{b}
	E1	33 ± 2^{b}	28 ± 2^{ab}	23 ± 2^{a}	89 ± 5^{b}	47 ± 7^{b}	20 ± 1^{bc}	15 ± 1^{b}	34 ± 2^{a}	150 ± 10^{b}

E2	32 ± 1^b	25 ± 2^a	25 ± 2^a	83 ± 5^b	49 ± 2^b	20 ± 1^b	15 ± 1^b	34 ± 1^a	150 ± 10^b
E3	20 ± 2^a	-	-	55 ± 5^a	19 ± 5^a	13 ± 1^a	12 ± 1^a	-	110 ± 10^a

Data shown are mean values from triplicates and the standard deviation. ^{abc} Different lowercase letters indicate significant differences between digestion models in each grain, with a significance level of 95% ($p<0.05$).

Figure legends

Figure 1. Extent of proteolysis (%) of the EAA and NEAA fractions of A: Legumes (chickpea, lentils, soya bean and white bean) and B: Grains (oats, spelt and quinoa) obtained with different *in vitro* digestion models (C, E1, E2 and E3). [abc] Different lowercase letters indicate significant differences of EAA and the total extent of proteolysis between digestion models in each legume/grain ($p<0.05$).

Figure 2. Extent of glycolysis (%) in cooked legumes (chickpea, lentil, soya bean and white bean) and grains (oats, spelt and quinoa) obtained with different *in vitro* digestion models (C, E1, E2 and E3). Data presented as g of free glucose E/100 g initial starch. [abc] Different lowercase letters indicate significant differences among digestion models with a significance level of 95% ($p<0.05$).

Figure 3. Calcium bioaccessibility (%) of cooked legumes (chickpea, lentils, soya bean and white bean) and grains (oats, spelt, quinoa) digested with different *in vitro* digestion models (C, E1, E2 and E3). [abc] Different lowercase letters indicate significant differences among digestion models with a significance level of 95% ($p<0.05$).

Figure 4. Biplot of the different end-products resulting from digestion and their relationship with the legume/grain samples (chickpea, lentils, soya bean, white bean, oats, spelt and quinoa) and the GI conditions (C, E1, E2 and E3) using principal components analysis (PCA).

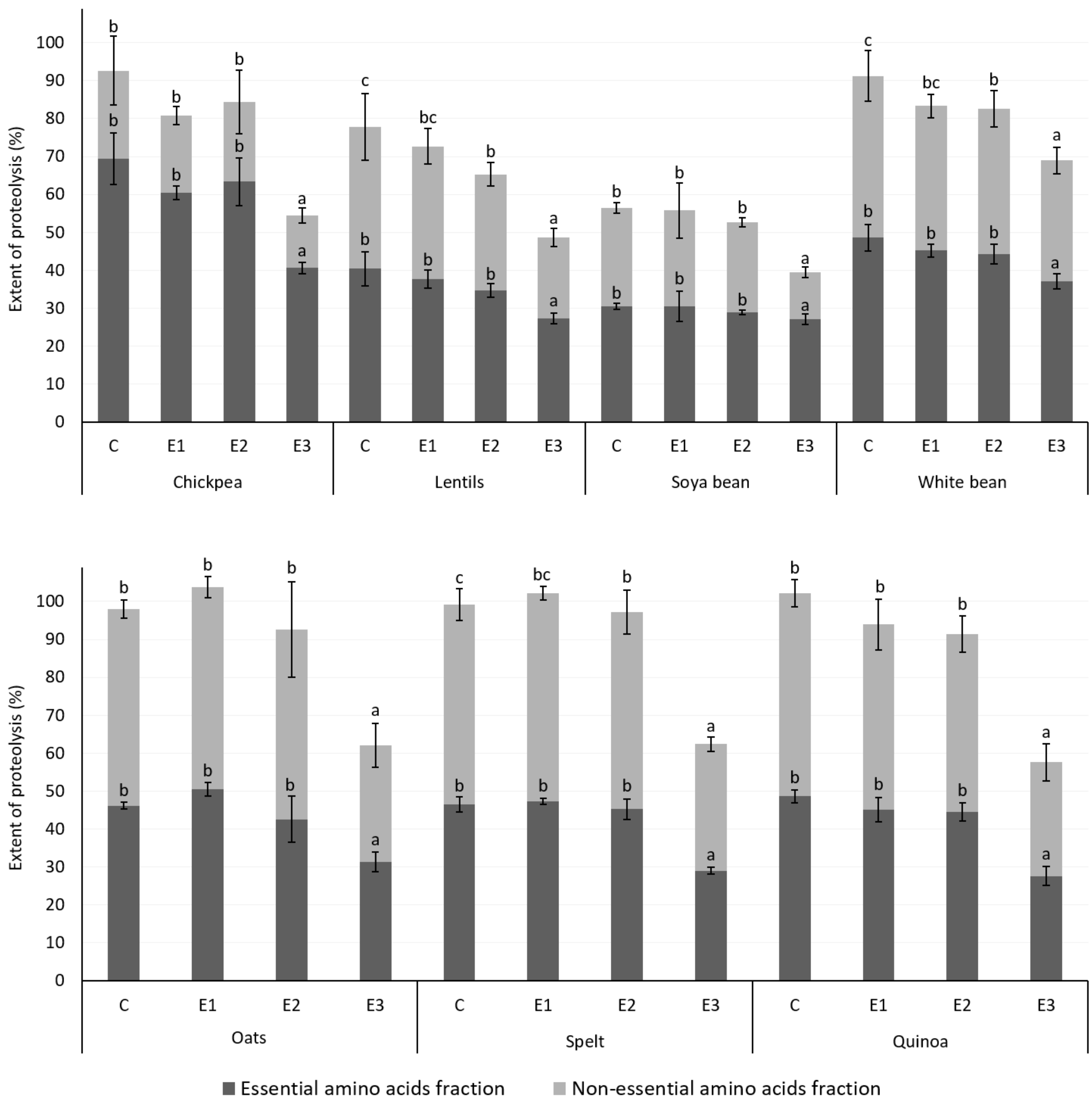
Extent of proteolysis (%)
C
E1
E2
E3
Chickpea
Lentils
Soya bean
White bean
Oats
Spelt
Quinoa
Essential amino acids fraction
Non-essential amino acids fraction

Figure 1.

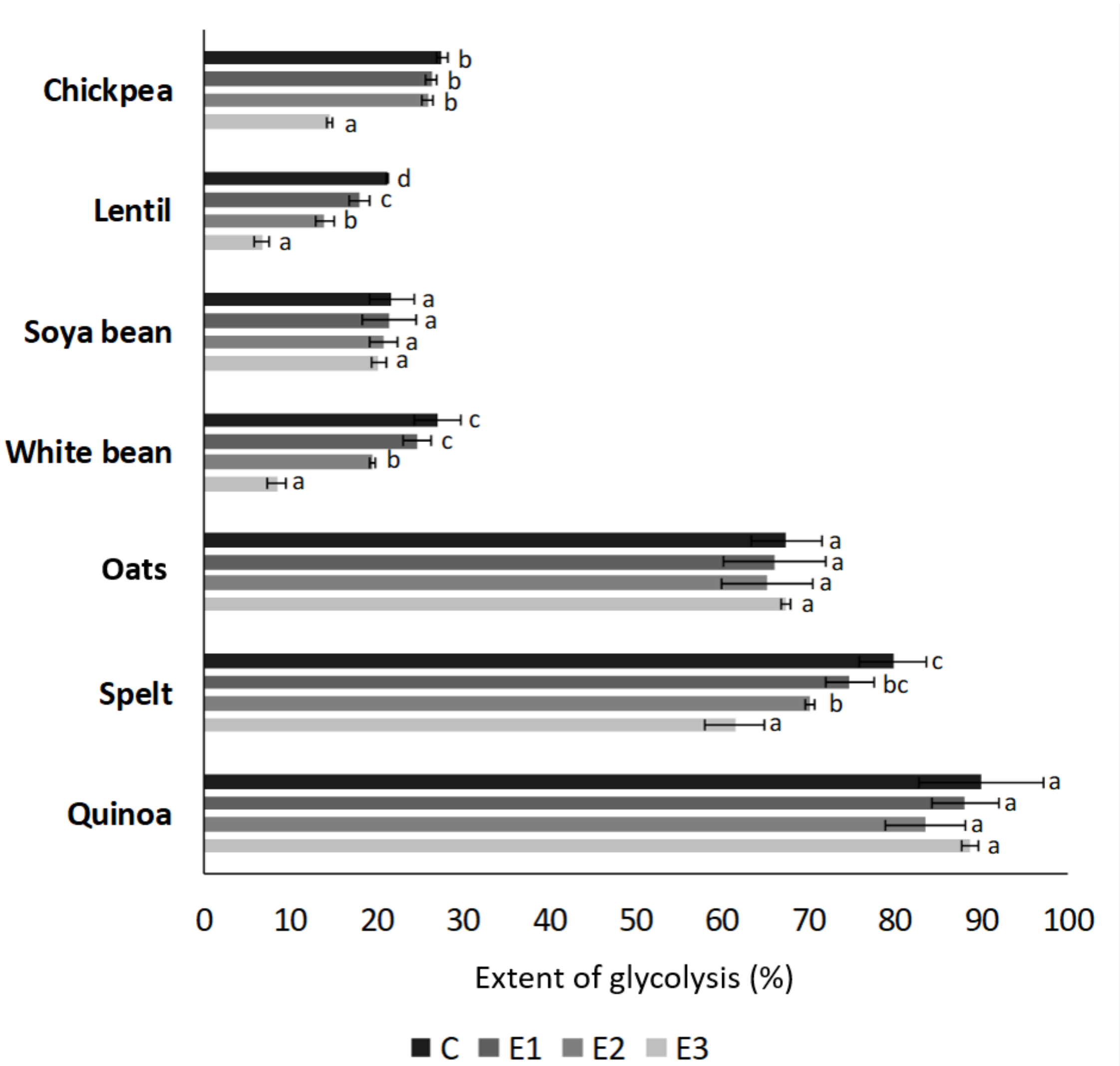
Chickpea
Lentil
Soya bean
White bean
Oats
Spelt
Quinoa
0
10
20
30
40
50
60
70
80
90
100
Extent of glycolysis (%)
C
E1
E2
E3

Figure 2.

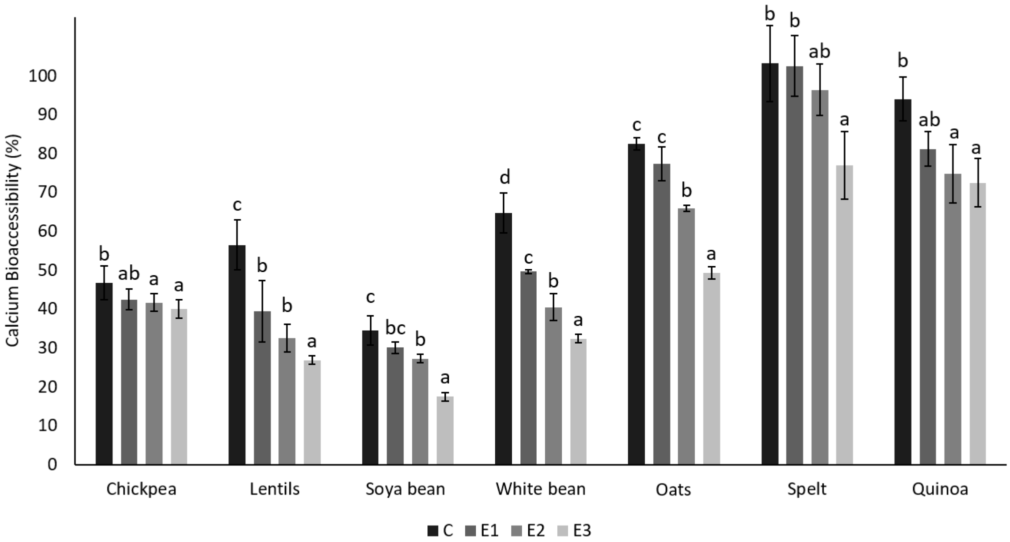
Calcium Bioaccessibility (%)
0
10
20
30
40
50
60
70
80
90
100
b
ab
a
a
c
b
b
a
c
bc
b
a
d
c
b
a
c
c
b
a
b
b
ab
a
b
ab
a
a
Chickpea
Lentils
Soya bean
White bean
Oats
Spelt
Quinoa
C
E1
E2
E3

Figure 3.

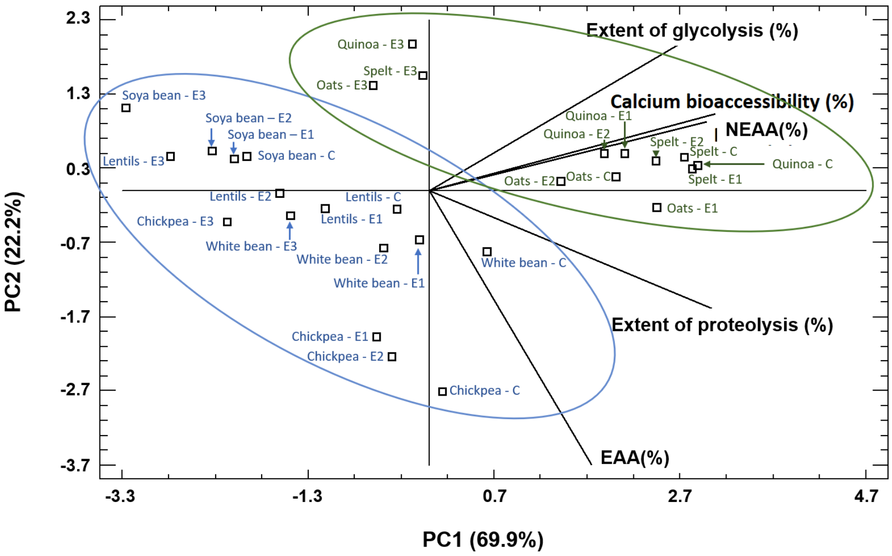
Extent of glycolysis (%)
Calcium bioaccessibility (%)
NEAA(%)
Extent of proteolysis (%)
EAA(%)
Quinoa - E3
Spelt - E3
Oats - E3
Soya bean - E3
Soya bean – E2
Soya bean – E1
Soya bean - C
Lentils - E3
Lentils - E2
Lentils - C
Lentils - E1
Chickpea - E3
White bean - E3
White bean - E2
White bean - E1
White bean - C
Chickpea - E1
Chickpea - E2
Chickpea - C
Quinoa - E1
Quinoa - E2
Spelt - E2
Spelt - C
Quinoa - C
Spelt - E1
Oats - E2
Oats - C
Oats - E1
PC2 (22.2%)
PC1 (69.9%)
2.3
1.3
0.3
-0.7
-1.7
-2.7
-3.7
-3.3
-1.3
0.7
2.7
4.7

Figure 4.