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Understanding the influence of simulated elderly gastrointestinal conditions on nutrient digestibility and functional properties

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ABSTRACT

Background: The increase in the senior population has enhanced the interest in evaluating the food digestibility in that age group, using simulated digestion methods.

Scope and approach: The effects of gastrointestinal changes on food digestion in the elderly were reviewed. Articles with an elderly-adapted simulated gastrointestinal digestion were selected (28 of 136 screened).

Key findings and conclusions: The changes in oral physiology affect food disintegration, with consequences in perception and food digestion. Under elderly conditions (*vs.* adult), proteolysis tends to be diminished or delayed, but the lack of harmonization of methodology does not allow conclusive results to be drawn. An increase in lipid digestibility was observed, although the contribution of gastric lipase remains unknown. A lower lactose release and changes in starch hydrolysis dependent on the food matrix and system model (static or dynamic) were related. Oral phase adaptation and the inclusion of non-glycolytic enzymes should be considered in future studies. The micronutrient bioaccessibility (Ca, vitamins A, D and E) was reduced under elderly conditions. These conditions may modify the bioactivity of digesta release (enzymatic inhibitory properties and, antioxidant capacity), depending on the bioactive compound. Besides, there are scarce studies carried out on the bioaccessibility of bioactive compounds (capsaicin, plant sterols, polyphenols). All these findings could shed additional light on the development of foods adapted to the requirements of older people, reducing the incidence of malnutrition, and be useful in the design of dietary recommendations in this population group, although they need to be confirmed by further *in vivo* studies.

1. Introduction

According to the latest available data, elderly people (>65 years old) represent a fifth of the total population (20.6%) in the European Union, almost three times more than 10 years ago (2020 *vs.* 2010) (Eurostat, 2021). Older people suffer from a physiological decline, with gastrointestinal alterations derived from the ageing process. In this sense, relevant changes in oral physiology occur, such as dentition worsening and salivation reduction, accompanied by an increase in the ion and salivary amylase concentration (Khan et al., 2014; Nagler & Hershkovich, 2005). On the other hand, the ageing process slows down gastric emptying, reduces the secretion of gastric lipase and pepsin, and alkalizes the gastric environment (Holt, 2007; Moreau et al., 1988; Rémond et al., 2015; Salles, 2007). At small intestinal level, a decline in peristaltic activity and a reduction in the secretion of pancreatic enzymes and bile salts is produced (Holt & Balint, 1993; Laugier et al., 1991; Soenen et al., 2016). On the other hand, colonic health and microbiota alter as we age. In this sense, a reduction in colonic motility occurs, that predisposes to constipation (Salles, 2007). Likewise, there are changes in the colonic microbiota, among which the decrease in microbial diversity and in the ratio of Firmicutes/Bacteroidetes, reduction in *Bifidobacterium* and *Lactobacillus*, as well as of butyrate-producing species (i.e., *Eubacterium*), the increase in potentially pathogenic species (i.e., *Escherichia*), and changes in microbiome involved in the metabolism of cobalamin and folate stand out (Lee et al., 2021b; Mangiola et al., 2018; Yatsunenko et al., 2012). These gastrointestinal changes play an etiological role in age-related malnutrition (de Groot et al., 2000), which is associated with serious consequences (increased hospital stay, decreased motor capacity, worsening of quality of life or increased mortality risk) (Agarwal et al., 2013).

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This fact highlights the importance of evaluating the effect of elderly gastrointestinal conditions on food digestibility using simulated gastrointestinal digestion models. These are the methods of choice since they are non-invasive and have a lower cost than *in vivo* studies. Due to the diversity of factors included in *in vitro* digestions, the COST Action INFOGEST network has developed a standardized static (Minekus et al., 2014) and semi-dynamic (Mulet-Cabero et al., 2020) method, in order to harmonize gastrointestinal conditions in healthy adults and allow comparison between studies. The static method was updated by incorporating gastric lipase and establishing the oral phase as mandatory, among other parameters and procedures, such as the determination of enzyme activity assays (Brodkorb et al., 2019).

Although changes in gastrointestinal physiology in older adults have been well studied, their impact on the digestibility of food components remains unknown and it would be necessary to adapt the conditions of the harmonized INFOGEST method to those of the senior population. For this purpose, changes are made in parameters such as enzymatic activity, bile salt concentration, saline concentration of the digestive fluids, pH, and duration and frequency of incubation, based on the gastrointestinal modifications that occur during ageing (Rémond et al., 2015; Shani-Levi et al., 2017); however, these adaptations have not been standardized (Mackie et al., 2020).

The main objective is to carry out a critical review of these studies in order to understand the effects of changes in elderly gastrointestinal conditions on the structural characteristics of the oral bolus, food digestion, including macro- (protein, lipid and carbohydrates) and micronutrients (fat- and water-soluble vitamins and minerals). In addition, as a secondary aim, it has been collected information on the bioactivity of food after digestion, as well as the bioaccessibility of non-nutrient compounds. Furthermore, the shortcomings, the limitations of the studies and future aspects will also be addressed.

2. Methodology

2.1. Search strategy

The review was conducted in compliance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA 2020) (Page et al., 2021). We searched in 3 databases (PubMed, Web of Science and SCOPUS) initially on December 20, 2021, using the following search strategy: ("elderly" OR "senior" OR "elder") AND ("*in vitro*" OR "simulated") AND ("digestion" OR "gastrointestinal digestion") searched by title, abstract or keywords. There was no restriction on language. The search was updated on May 17, 2022 to capture the latest publications prior to manuscript submission. Articles of all types regardless of the publication date were included at this stage.

2.2. Inclusion and exclusion criteria, data extraction, and bias assessment

Original studies were selected based on the population, intervention, comparison, outcomes, and study design (PICOS) framework. Our target population was elderly and healthy adults. Regarding the intervention,

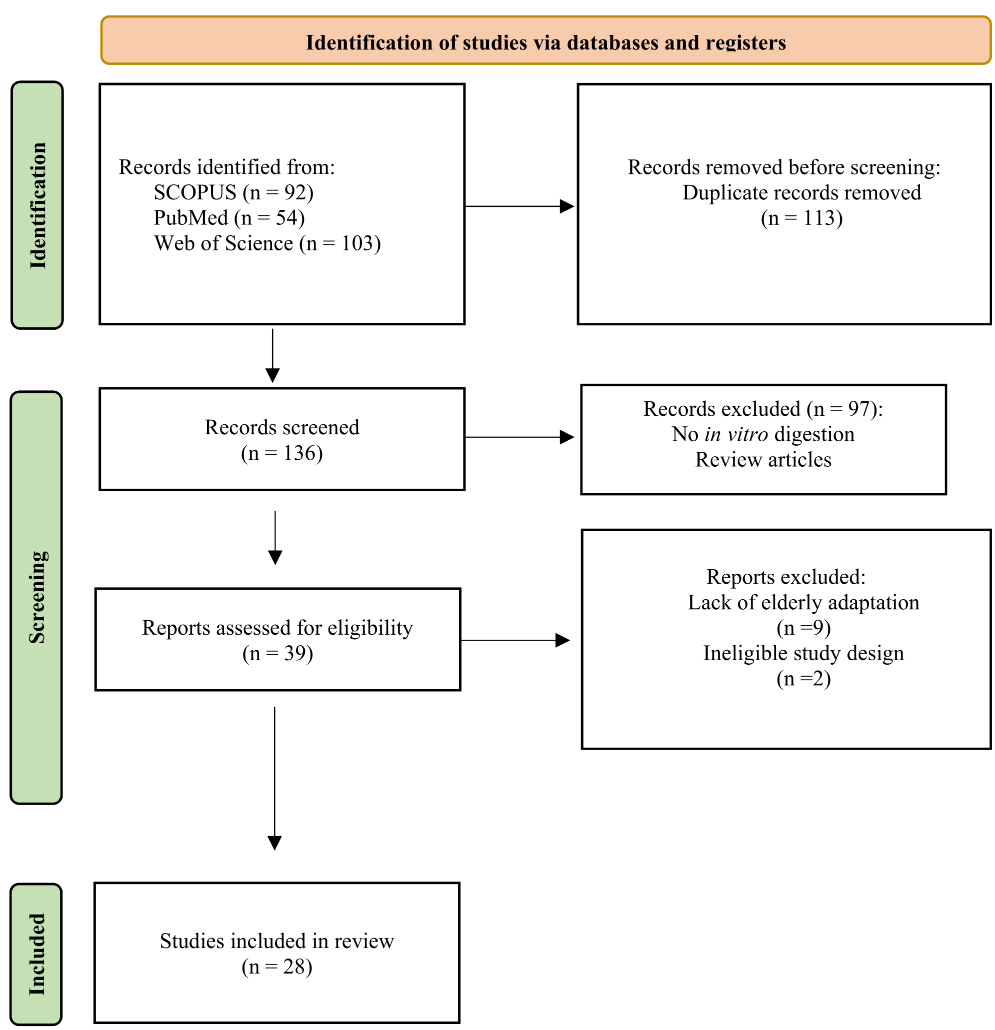

Fig. 1. Literature search flowchart with database and record searches.

studies that carried out *in vitro* digestion were included, and the review articles and articles that did not perform an *in vitro* digestion were excluded. Papers were included regardless of the outcome. In terms of study design, all *in vitro* assays were included, whether they performed static, semi-dynamic, or dynamic digestion. However, those studies that applied an *in vitro* digestion without adapting the conditions to the elderly were excluded (lack of elderly adaptation), as well as those in which it was not clear if the adaptation had been carried out (ineligible study design). A flow diagram detailing the study selection process is shown in Fig. 1. A total of 28 articles were selected from 136 screened.

3. Results and discussion

The simulated gastrointestinal digestion methods, whether static or dynamic, integrate the main parameters of digestion (duration, pH, temperature, agitation and concentration of digestive fluids and enzymes) and encompass oral, gastric, and intestinal stages, and some of them, colonic fermentation. Static studies have a reduced economic and time cost, allowing the analysis of several parameters in different matrices. Differences in the methodology of published static studies are due to the performance or not of the intestinal phase, differences in pH or in the duration of digestion (see Table 1). This makes it difficult to compare them and draw conclusive results. On the other hand, dynamic digestions allow reproducing the physiological pH gradient, the sequential release of digestive fluids and peristaltic movements. In this case, the adaptations to the physiology of older adults are more homogeneous. However, the matrices used are simpler and there is less variety of foods (see Table 2). In addition, the main limitations identified regarding the adaptations to the physiology of the elderly can be observed in Table 3. Furthermore, an overview of the main physiological gastrointestinal changes that occur in the elderly population, and impact of the elderly gastrointestinal digestion on food digestibility and functional properties are summarized in Fig. 2.

3.1. Structural characteristics of the oral bolus

The food structure disintegration, that starts in the mouth, is a relevant factor in nutrient digestion (Gao et al., 2021), likely to explain the differences in digestion between adult and elderly conditions (Levi et al., 2017). In addition, the different disintegration of food by the chewing of an older adult generates changes in oral comfort and food perception. For all these reasons, different studies have evaluated the structural characteristics of the oral bolus, and its influence on food digestion (Assad-Bustillos et al., 2019, 2020a, 2020b; Levi et al., 2017; Peyron et al., 2021).

The structural characterization of an enteral nutrition formula and its oral bolus obtained with simulated adult and elderly saliva showed emulsion instability. Flocculation was observed in the adult oral bolus, while coalescence was reported in the elderly one. The structural differences were attributed to the salivary composition, which presents a higher concentration of potassium and chloride in the elderly (19.2 *vs.* 27.8 and 19.7 *vs.* 29.5 mmol L^{-1}, respectively), together with an increased α-amylase activity (748 *vs.* 1476 U L^{-1}) (Khan et al., 2014; Nagler & Hershkovich, 2005). The authors suggested that these microstructural differences could affect sensory properties and nutrient digestion (Levi et al., 2017).

Oral bolus formation from pulse proteins enriched-sponge cake and brioche was studied in elderly volunteers (Assad-Bustillos et al., 2020a, b). Volunteers chewed samples in three stages (total chewing duration, i.e., before swallowing point, and 1/2 or 1/3 of total chewing duration). Oral comfort was assessed with a questionnaire, and properties of the oral bolus (hydration, particle size distribution, and apparent viscosity) were measured. The authors observed that chewing duration depended on the food, dental status and salivary flow rate. Longer chewing duration was reported in subjects with a poor dental status for fortified brioche, and a shorter duration in individuals with a higher salivary flow rate for fortified sponge cake, as this latter food is easier to fragment. Furthermore, the protein fortification did not challenge the oral comfort perception. In this sense, the interest in the design of texture-modified foods to adapt the diet to the oral conditions of the older people is on the rise, in which novel techniques and ingredients are being used (Gallego et al., 2022).

3.2. Proteolysis

In the oral phase, depending on the texture of the food, the number of chewing cycles and the density of the saliva, a more compact or more disintegrated bolus will be formed, subsequently influencing the rest of the digestion. Then, protein digestion continues in the gastric phase with pepsin, modulated by gastric pH and influenced by the time the bolus remains in the stomach. Finally, in the duodenum, pancreatic proteases produce the final part of protein degradation for intestinal absorption. Any modification in the enzymatic activity, in the duration of digestion or in the different structure in the oral bolus, is likely to produce changes in protein digestibility in the elderly when compared to adults.

Sarcopenia is defined as a progressive loss of muscle mass associated with a loss of functionality in skeletal muscle. This is one of the main health problems associated with malnutrition in the senior population. It is mainly associated with oropharyngeal dysphagia (Khan et al., 2014), loss of appetite, decreased physical activity or other diseases that can cause it secondarily, such as cancer or neurodegenerative diseases (Muscaritoli et al., 2010). Sarcopenia produces various comorbidities in the elderly population, such as an increased risk of falling, worsening of digestive function and, in general, greater frailty. Therefore, this fact not only worsens the quality of life of older adults, but also increases mortality (Landi et al., 2013).

Some nutritional factors influence this health problem, whether they are caloric intake or some micronutrients (like zinc or group B vitamins), non-nutritional factors such as physical activity, and psychosocial factors such as economic capacity or loneliness, among others (Muscaritoli et al., 2010). An insufficient intake of protein will inevitably lead to sarcopenia in the elderly with respect to young adults. Thus, it is important to know if such intake is equally effective in terms of protein digestibility. Based on this possible change, not only the daily intake recommendations but also the formulation of some foods could be adapted to the needs of the elderly.

As far as we are concerned, a recent review was carried out regarding the effect of elderly gastrointestinal conditions on proteolysis (Lee et al., 2021a). In the present review, we have compiled several studies that applied static (see Table 1), or dynamic (see Table 2) digestion methods adapted to the conditions of the elderly to evaluate protein digestibility in various food matrices. Most of them compare this parameter with a control group with healthy adult conditions for the purpose of observing differences between them.

The determination of protein digestibility varies between the different works, from quantifying the proportion of large and small peptides, the proteolysis extent (expressed as proportion of free amino acids in the bioaccessible fraction *vs.* initial undigested protein content), the degradation of specific peptides or the degradation rate of them, to the ratio of essential/non-essential amino acids (EAA/NEAA). This fact complicates the comparison of studies to establish ranges or means of proteolysis. Due to the abundance of assays evaluating this parameter, it was decided to separate static and dynamic trials, with the aim of comparing similar studies that favor understanding.

3.2.1. Static in vitro digestion trials

When analyzing the partial adaptations (oral and oral plus gastric) of the gastrointestinal digestion to the elderly conditions, a significant reduction ($p < 0.05$) compared with adult was observed solely in dairy products and fish (Table 4). Data is only available on dairy products, producing a significant reduction ($p < 0.05$) in the proteolysis extent of between 8 and 27% when adapting the oral and gastric phase, and 16%

Table 1
Features of the static digestion assays adapted to the elderly physiology.

Sample	Oral phase	Gastric phase	Intestinal phase	Evaluated parameter	Reference
Pea protein-fortified sponge cake, pea protein isolates	Sample:SSF 1:1 α-Amylase 75 U mL^{-1} 2 min Bolus pools: low and high viscosity obtained by subjects (65–80 years old) with satisfactory/poor dentition	Pepsin 2000 U mL^{-1} pH 3 2 h/shaking incubator	Pancreatin (trypsin activity 100 U mL^{-1}) pH 7 2 h/shaking incubator	Proteolysis and structural characteristics of the oral bolus	Assad-Bustillos et al., (2019)
Pea grass flour	Sample:SSF: 1:1 *vs.*1:2 α-Amylase 150 *vs.* 300 U mL^{-1} Agitation 170 *vs.* 65 rpm	Pepsin 2000 *vs.* 1500 U mL^{-1} Agitation 170 *vs.* 85 rpm pH 2.5 1 h	Not applied	Proteolysis	Romano et al., (2019)
Protein sources: meat, cheese, milk fractionation proteins, legumes, and seeds	Not applied	Pepsin 2000 *vs.* 1200U mL^{-1} pH 2 *vs.* 3 Digestion time 2 *vs.* 1 h	Trypsin 100 *vs.* 67 U mL^{-1} Chymotrypsin 25 *vs.* 16.75 U mL^{-1} Pancreatic lipase 2000 *vs.* 400U mL^{-1} pH and digestion time not indicated	Proteolysis	Duval et al., (2020)
Fish	Bolus: adult subject with normal dentition. Mastication cycles 20 *vs.* 10 (30 *vs.* 15 in soy)	Pepsin 2000 *vs.* 1500 U mL^{-1} pH 3 *vs.* 6 2 h	Pancreatin 100 *vs.* 50 U mL^{-1} Bile salts 10 *vs.* 5 mM pH 7 Digestion time 2 *vs.* 4 h	Proteolysis, lipolysis and calcium and vitamin A and D BA	Hernández-Olivas et al., (2020a)
Milk and dairy products				Proteolysis, lipolysis, glycolysis and calcium, vitamin A and D BA	Hernández-Olivas et al., (2020b)
Legumes and cereals				Proteolysis, glycolysis, and calcium BA	Hernández-Olivas et al., (2021a)
Eggs				Proteolysis, lipolysis and vitamin A and D BA	Hernández-Olivas et al., (2021b)
Meat				Proteolysis and antioxidant and ACE inhibitory activity	Hernández-Olivas et al., (2022)
Milk	Sample:SSF 1:1 without α-amylase Agitation 200 rpm/2 min	Pepsin 2000 *vs.* 1400U mL^{-1} Agitation 200 *vs.* 100 rpm pH 6 2 h	Not applied	Proteolysis	Alaei et al., 2021a,b
Cheese products	Sample:SSF 1:1 without salivary amylase 2 min/shaking incubator	Pepsin 1000 *vs.* 750 U mL^{-1} pH 3 2 h/shaking incubator	Pancreatin 100 U mL^{-1} (trypsin activity) Bile salt (modification not indicated) pH 7 *vs.* 6.5 2 h/shaking incubator	Antioxidant and α-glucosidase and lipase inhibitory activity	Plante et al., (2020)
Cheese-fruit combination				Antioxidant, α-glucosidase inhibitory, antimicrobial and immunomodulatory activity	Plante et al., (2021)
Non-emulsified vitamin E; Oil-in-water emulsions with 10% (w/w) oil (olive oil, tallow, or lard)	α-Amylase 0.15 *vs.*0.07 mg mL^{-1} pH not given 5 min 150 *vs.* 100 rpm	Pepsin 1.25 *vs.* 0.63 mg mL^{-1} pH not given 2 h 150 *vs.* 100 rpm	Pancreatin 2.6 *vs.* 1.3 mg mL^{-1} Bile 4.3 *vs.* 2.15 mg mL^{-1} 2 h pH not given 150 *vs.* 100 rpm *Escherichia coli*: *Lactobacillus* spp 60:40 *vs.* 40:60 6 h	Vitamin E BA	Lee et al., 2021c
Aqueous solution of vitamins C, B_6 and B_{12}	Saliva was halved in elderly *vs.* adult 5 min 150 *vs.* 100 rpm	Gastric juice was halved in elderly *vs.* adult pH not given 2 h 150 *vs.* 100 rpm	Small intestinal juice was halved in elderly *vs.* adult pH not given 2 h 150 *vs.* 100 rpm Proportion of *Lactobacillus casei* and *Escherichia coli* was reversed 4 h	Vitamin BA and antioxidant activity	Lee et al., 2022
Plant sterol- enriched milk-based fruit beverage	Sample:SSF 1:1 α-Amylase activity 75 U mL^{-1} 2 min/95 rpm	Pepsin 2000 *vs.*1500 U mL^{-1} Gastric lipase 60 *vs.* 9 U mL^{-1} pH 3 *vs.* 6 Digestion time 2 *vs.* 3 h Agitation 95 *vs.* 50 rpm	Pancreatin 100 *vs.* 50 U mL^{-1} (trypsin activity) Bile salt 10 *vs.* 5 mM pH 7 Digestion time 2 *vs.* 3 h Agitation 95 *vs.* 50 rpm	Sterol BA	Miedes et al., (2022)
Ground beef	Sample:SSF 1:1 without salivary amylase 2 min/150 rpm	Pepsin 2000 *vs.*1500 U mL^{-1} pH 3 2 h/150 rpm	Pancreatin 100 *vs.* 45.6 U mL^{-1} (trypsin activity) Bile salt 10 *vs.* 6.7 mM pH 7 2 h/150 rpm	Polychlorinated biphenyl BA	Planche et al., (2022)
Apple	Sample:SSF 20:4 (w/v) α-Amylase activity 150 *vs.* 225 U mL^{-1}	Pepsin 4000 *vs.* 3000 U mL^{-1} pH	Pancreatin 200 *vs.* 92 U mL^{-1} Bile salt 20 mM pH not	Polyphenol BA and antioxidant activity	Shang et al., (2022)

(continued on next page)

Table 1 (*continued*)

Sample	Oral phase	Gastric phase	Intestinal phase	Evaluated parameter	Reference
Meat and soy protein powder	1 min/oscillation 9 *vs.* 6 stages Not applied	not given 4 h Pepsin 2000 *vs.* 1500 U mL^{-1} pH 3 *vs.* 6 2 h	given 4 h Pancreatin 100 *vs.* 50 U mL^{-1} Bile salt 10 *vs.* 5 mM pH 7 2 *vs.* 4 h	Proteolysis and peptide analysis	Wang et al., (2022)

Data shown points to the differences between (adult vs. elderly) in the different static trials. Adult gastric and intestinal conditions, and SSF (Simulated salivary fluid), SGF (simulated gastric fluid) and SIF (simulated intestinal fluid) composition, and enzyme ratios followed Minekus et al. (2014), except in the studies by Lee et al., 2021b, 2022. ACE: angiotensin converting enzyme; BA: bioaccessibility.

when adapting only the oral phase (in cheese) (Hernández-Olivas et al., 2020b). In fish, it was observed a larger reduction ($p < 0.05$) of the proteolysis extent (data not given by the authors) in fattier fish like salmon and sea bass (14.0 and 6.7 g fat 100 g^{-1}, respectively), compared with leaner species as sardine and hake (3.6 and 0.34 g fat 100 g^{-1}, respectively) (Hernández-Olivas et al., 2020a). On the other hand, a significant and higher reduction ($p < 0.05$) in the proteolysis extent (between 32 and 49%) was estimated in different matrices after the complete adaptation of the gastrointestinal digestion to the elderly conditions (Hernández-Olivas et al., 2020a, b, 2021a, b). Substantial differences between specific food matrices and culinary techniques, except for poached egg, in which a reduction was not shown (Hernández-Olivas et al., 2021b). These authors explained that it could be attributed to its semisolid texture, which makes it mix and digest better.

A lower proteolysis ($p < 0.05$), measured as protein solubility after precipitation with trichloroacetic acid (TCA) (15, 26, 26 and 28% decrease in pork, chicken, turkey, and beef, respectively) has also been observed in meats when comparing completely adapted conditions of the elderly with adult conditions (Hernández-Olivas et al., 2022). However, oral adaptation did not produce significant changes and oral and gastric adaptation even slightly increased the degree of proteolysis. The authors claim that with a pH of 6 (used in those adaptations), proteins are negatively charged, since that pH is far from the isoelectric point. As a result, the solubility of proteins is improved, and as a consequence the access of enzymes is enhanced, and increased proteolysis occurs. Similar findings were reported concerning another study with different meats and soy protein powders (Wang et al., 2022). TCA protein solubility was measured comparing elderly gastric and intestinal conditions, showing a marked reduction ($p < 0.05$) in the release of TCA soluble peptides after 2 h of gastric and 2 h of intestinal digestion in the elderly model compared to the adult model (44, 36, 37 and 62% decrease in chicken, beef, pork and soy powder, respectively).

No decrease in the degree of protein hydrolysis, calculated from the release of free amino groups (NH_2), was observed between high-viscosity boluses and low-viscosity boluses obtained from pea protein-fortified biscuits (Assad-Bustillos et al., 2019). The samples underwent oral digestion by healthy elderly subjects and were subsequently digested with the INFOGEST method for adults (without digestive adaptation to the elderly regarding gastric and intestinal phases). In that case, it is, therefore, not possible to compare the proteolysis with that of an adult and evaluate the possible differences.

Besides, a lower protein digestibility ($p < 0.05$), measured by the release of NH_2 after digestion, was observed in legumes and seeds extracts than in meat and dairy and milk fractionation products, under elderly conditions (Duval et al., 2020). These results could suggest a greater suitability of meat or dairy protein consumption for this age group, although a standard adult digestion was not included.

In addition, it may be important to elucidate the relationship between EAA and NEAA, since the former are much more relevant for protein synthesis and, ultimately, for the prevention and treatment of sarcopenia in the elderly. The results of the EAA/NEAA ratio were positive, since in all the matrices evaluated (eggs, meat, legumes, and cereals), except for cereals, this ratio increased ($p < 0.05$) compared to adults only when all digestion conditions were adapted to the elderly. This could mean that the intestinal digestive conditions that impair the protein digestibility are likely to affect the NEAA more strongly. It has been observed that pancreatic enzymes have greater affinity for some specific peptide bonds, such as lysine and arginine (Aderinola et al., 2018). Since pancreatic enzymes are reduced in the elderly model, this could explain why the NEAA release is hindered to a greater extent than that of EAA. The above-mentioned exception, cereals, could be of relevance since amino-acid lysine is limiting and, therefore, its content could mark protein synthesis. But it was shown that the reduction in its content was around 40% (the same than general protein content), meaning a potential protein synthesis defect (Hernández-Olivas et al., 2021a; b, 2022).

Relative abundance of larger or smaller peptides also determines the degree of proteolysis in a sample, since a lower proportion of smaller peptides at the end of the digestion will indicate worse protein degradation. In this sense, it has been observed that in meat and soy protein powders, the proportion of short peptides (3–8 amino acids) after complete adaptation of the gastrointestinal digestion to elderly conditions was lower (14, 7.9, 10.4, 7.1 *vs.* 18.7, 10, 13.7, 7.5% of total peptides in chicken, beef, pork and soy, respectively) than after complete adult digestion ($p < 0.05$), showing a decrease in protein breakdown in the elderly (Wang et al., 2022). Following the same trend, in raw milk, the proportion of larger peptides (21–30 amino acids) was higher in gastric elderly adapted conditions (intestinal phase was not adapted in this assay) *vs.* adults (47 *vs.* 37%, $p < 0.05$). In accordance with this, smaller peptides (11–15 amino acids) were generated in a lower proportion (12 *vs.* 22%, $p < 0.05$), thus showing deficient protein degradation in the elderly model. However, in high pressure pasteurized milk (400 MPa/15 min), no differences were observed in terms of proportion of peptides between the elderly and adult model. The authors attribute this result to the fact that this process previously hydrolyzes the milk peptides, making them more accessible for digestion by the elderly (Alaei et al., 2021a, b). These results agree with those obtained in pea grass flour, where a complete peptide digestion was observed at 60 min of gastric digestion in both elderly and adult models. Nevertheless, the speed of degradation of this protein band was lower in the elderly (35 and 24% of initial peptide remained at 20 min of gastric digestion, in the elderly and adult model, respectively) (Romano et al., 2019).

The formation of bioactive peptides has also been evaluated under elderly conditions in chicken, beef, pork and soy protein powders. Compared with adult gastrointestinal conditions, elderly complete digestion adaptation has shown a lower percentage ($p < 0.05$) of potentially bioactive peptides in all matrices (mean of 8.3 *vs.* 9.5% in elderly and adult, respectively). The decrease in proteolysis affects not only digestibility, but also the potential non-nutritional benefits from protein sources (Wang et al., 2022).

3.2.2. Dynamic in vitro digestion trials

Dynamic tests and their specific methodology and elderly adaptations are summarized in Table 2. In cooked beef, elderly conditions did not decrease neither digestibility (96%, expressed as proportion of nitrogen in the ileal effluents *vs.* nitrogen in the meat) nor bioaccessibility (60%, expressed as proportion of nitrogen dialyzed in the jejunum and ileum *vs.* nitrogen in the meat) of proteins compared with adult

Table 2
Features of the dynamic digestion assays adapted to the elderly physiology.

Sample	Oral phase	Gastric phase	Intestinal phase	Evaluated parameter	Reference
Lactoferrin, β-lactoglobulin, α-lactalbumin	Not applied	Pepsin 1000 *vs.* 750 U mL^{-1} pH gradient 4.5–1.2 *vs.* 6.2–2 Agitation 200 *vs.* 100 rpm Gastric emptying [f = 2(t/80.5)$^{0.7}$ *vs.* f = 2(t/80.5)$^{0.4}$] Digestion time 2 *vs.* 3 h	Trypsin 100 *vs.* 46 U mL^{-1} α-chymotrypsin 50 *vs.* 23 U mL^{-1} Bile salts 4 *vs.* 2.67 mM (3 *vs.* 2 mL) pH 6.1 *vs.* 6.5 Digestion time 2 *vs.* 3 h	Proteolysis	Levi & Lesmes, (2014)
Cooked semi-membranous muscle from beef	Homogenized with water	Pepsin 520 *vs.* 312 U min^{-1} Gastric lipase 24 *vs.* 4.8 U min^{-1} pH gradient 6–1.8 (slower descent in elderly model) 5 h Half emptying 85 min	Pancreatic juice 89 *vs.* 59 mg min^{-1} Bile 20 *vs.* 13.4 mg min^{-1} (first 30 min) 10 *vs.* 6.7 mg min^{-1} (after first 30 min) pH at 6.8 and 7.2 in jejunal and ileal compartments, respectively 5 h	Proteolysis	Denis et al., (2016)
Enteral nutrition formula lactoferrin, β-lactoglobulin, α-lactalbumin, Whey protein isolate	For whey and purified proteins: not applied For enteral formula: SSF:formula (1:1) KCl 5 *vs.* 10 mL (stock) α-Amylase 150 *vs.* 300 U mL^{-1}	Pepsin 1000 *vs.* 750 U mL^{-1} Gastric lipase 1500 *vs.* 255 U mL^{-1} pH gradient 4.5–1.2 *vs.* 6.2–2 Agitation 200 *vs.* 100 rpm Gastric emptying [f = 2(t/80.5)$^{0.7}$ *vs.* f = 2(t/80.5)$^{0.4}$] Digestion time 2 *vs.* 3 h Gastric lipase 1500 *vs.* 255 U mL^{-1}	Trypsin 100 *vs.* 46 U mL^{-1} α-chymotrypsin 50 *vs.* 23 U mL^{-1} Pancreatic lipase 2000 *vs.* 1400 U mL^{-1} α-Amylase 200 *vs.* 100 U mL^{-1} Bile salts 4 *vs.* 2.67 mM (3 *vs.* 2 mL) pH 6.1 *vs.* 6.5 Digestion time 2 *vs.* 3 h	Proteolysis and structural characteristics of the oral bolus	Levi et al., (2017)
Silk moth pupae flour	Suspensions (5% w/v) of flour with water were mixed with SSF (1:1)	Pepsin 1000 *vs.* 750 U mL^{-1} pH gradient 4.5–1.2 *vs.* 6.2–2 Agitation 200 *vs.* 100 rpm Gastric emptying [f = 2(t/80.5)$^{0.7}$ *vs.* f = 2(t/80.5)$^{0.4}$] Digestion time 2 *vs.* 3 h	Trypsin 100 *vs.* 46 U mL^{-1} α-chymotrypsin 50 *vs.* 23 U mL^{-1} Bile salts 4 *vs.* 2.67 mM (3 *vs.* 2 mL) pH 6.1 *vs.* 6.5 Digestion time 2 *vs.* 3 h	Proteolysis	David-Birman et al., (2019)
Whey protein isolate with/without food-grade carrageenans	Not applied	Pepsin 1000 *vs.* 750 U mL^{-1} pH gradient 4.5–1.2 *vs.* 6.2–2 Agitation 200 *vs.* 100 rpm Gastric emptying [f = 2(t/80.5)$^{0.7}$ *vs.* f = 2(t/80.5)$^{0.4}$] Digestion time 2 *vs.* 3 h	Trypsin 100 *vs.* 46 U mL^{-1} α-chymotrypsin 50 *vs.* 23 U mL^{-1} Bile salts 4 *vs.* 2.67 mM (3 *vs.* 2 mL) pH 6.1 *vs.* 6.5 Digestion time 1 *vs.* 1.5 h	Proteolysis	David et al., (2020)
High-amylose corn starch with capsaicin	α-Amylase 75 *vs.* 150 U mL^{-1}	Pepsin 1000 *vs.* 750 U mL^{-1} pH gradient 4.5–1.2 *vs.* 6.2–2 Agitation 200 *vs.* 100 rpm Gastric emptying [f = 2(t/80.5)$^{0.7}$ *vs.* f = 2(t/80.5)$^{0.4}$] Digestion time 1 *vs.* 1.5 h	Trypsin 100 *vs.* 46 U mL^{-1} α-chymotrypsin 50 *vs.* 23 U mL^{-1} Bile salts 4 *vs.* 2.67 mM (3 *vs.* 2 mL) pH 6.5 Digestion time 3 h	Capsaicin bioaccessibility	Isaschar-Ovdat et al., (2021)
Pork frankfurters	Artificial mastication 27 *vs.* 9 cycles (Normal *vs.* deficient mastication)	Pepsin 520 *vs.* 312 U min^{-1} Gastric lipase 24 *vs.* 4.8 U min^{-1} pH gradient 4–2 *vs.* 4–3 5 h Half emptying 95 min	Not applied	Proteolysis and lipolysis	Peyron et al., 2021
***Tsampa*: roasted barley flour and butter tea**	α-Amylase 150 *vs.* 225 U mL^{-1}	Pepsin 4000 *vs.* 3000 U mL^{-1} Peristaltic frequency 3 *vs.* 2 times min^{-1} pH gradient 5–1.8 (slower descent in elderly model) 3 h	Not applied	Starch hydrolysis	Li et al., (2022)
Apple	α-Amylase activity 150 *vs.* 225 U mL^{-1}	Pepsin 4000 *vs.* 3000 U mL^{-1} Fluid velocity 1–2.2 mL/min pH 6–2 (more gradual reduction in the elderly) 2 min/3 *vs.* 2 times/min 4 h	Pancreatin 200 *vs.* 92 U mL^{-1} Bile salt 20 mM pH not indicated 4 h/100 mm/min	Polyphenol bioaccessibility and antioxidant activity	Shang et al., (2022)

Data shown point to the differences between (adult *vs.* elderly) in the different dynamic trials. SSF: Simulated Salivary Fluid.

conditions (Denis et al., 2016). Despite of this, hydrolysis in the duodenum was lower in elderly conditions, using for the first time the TIM model. The main problem is the absence of simulation of the oral phase, which could make a difference if it was adapted to the elderly and ultimately could cause worse protein digestibility. In this case, instead of an oral phase, the meat was homogenized. In addition, the use of undercooked meat affects protein denaturation and subsequent digestibility in the elderly, as was observed in an *in vivo* trial with elderly volunteers (n = 10; 70–80 years old). The intake of rare meat (55 °C, 5 min) produced a lower protein synthesis (40 *vs.* 56% of leucine intake, p

Table 3
Limitations of the adaptations to the elderly physiology in the compiled studies.

Stage of digestion assay	Parameters	References
Oral phase	No modification of activity of salivary amylase in carbohydrate-containing products	Duval et al., (2020)
	Lack of elderly volunteer	Hernández-Olivas et al., 2020a, b, 2021a, b, 2022
Gastric phase	No use of gastric lipase in lipid-containing products	Hernández-Olivas et al., 2020a, b, 2021, b, 2022
Intestinal phase	No modification of pancreatin activity	Assad-Bustillos et al., 2019; Planche et al., 2022
	Absence of application of intestinal phase	Romano et al., 2019; Alaei et al., 2021a,b; Peyron et al., 2021; Li et al., 2022

< 0.05) than the intake of well-done meat (90 °C, 30 min), as well as a lower plasma concentration of amino acids and leucine (Buffière et al., 2017). However, in a simulated gastric digestion trial, a lower release of peptides ($p < 0.05$) was observed at the end of gastric digestion under the conditions of the elderly coupled with poor chewing (13.55 mg mL^{-1}), when compared with adult and with good chewing (18.45 mg mL^{-1}) (Peyron et al., 2021). Furthermore, even without considering chewing, the peptide release kinetics was significantly slower ($p < 0.05$) in the elderly model when compared to adults.

A reduction in lactoferrin and α-lactalbumin degradation, and a slower rate of peptide degradation were observed by SDS-PAGE in the elderly model compared to adults ($p < 0.05$) (Levi & Lesmes, 2014). However, in the case of β-lactoglobulin, the opposite effect occurred, thus, no conclusion can be drawn as to whether there was a decrease or not in proteolysis of whey protein The same research group re-evaluated this same protein but also a whey isolate and an enteral nutrition formula (Levi et al., 2017). They found that under the digestive conditions of the elderly, the digesta after both gastric and intestinal phases presented greater flocculation compared to adults, and a lower destabilization of the fat particles, which could decrease the capacity of lipases to degrade the droplets. This leads to a worse protein degradation in the elderly model, observing for example that, after 3 h of gastric phase, some peptides continue without degradation, while in only 2 h in the adult model they have already been degraded.

Protein digestibility was studied with an *in vitro* dynamic system and *in vivo* postprandial amino acid serum response with 20 healthy subjects (65-year-old, considered as an example of the elderly) from two different isocaloric protein supplements (one leucine-enriched whey protein and another with casein, both formulated to provide 150 or 320 kcal) (Luiking et al., 2016). A 2-fold higher *in vivo* leucine peak was observed ($p < 0.05$) with whey than with casein supplements, and with lower calorie content (regardless of whether whey or casein). Leucine is key to the stimulation of protein synthesis and prevents the loss of muscle mass of the elderly. *In vitro*, a greater release of small peptides was observed in casein compared to whey supplements ($p < 0.05$); however, after 30 min of intestinal digestion, this trend was no longer observed, and at the end of digestion, a greater release of amino acids and small peptides was observed in supplements with low caloric density, as in the *in vivo* assay. The authors attribute the higher amino acid peak in the low-calorie supplements to carbohydrate interference in the case of the high-calorie supplements. This could be a key to recommending food intake or supplements to older people, with the aim of optimizing their protein synthesis. Nevertheless, no comparison between adults and the elderly was performed.

Subsequently, it was investigated how carrageenans could affect whey protein digestion, differentiating between adults, toddlers, and elders, since it has been observed that these could negatively affect proteolysis at gastric level (David et al., 2020). In this sense, similar

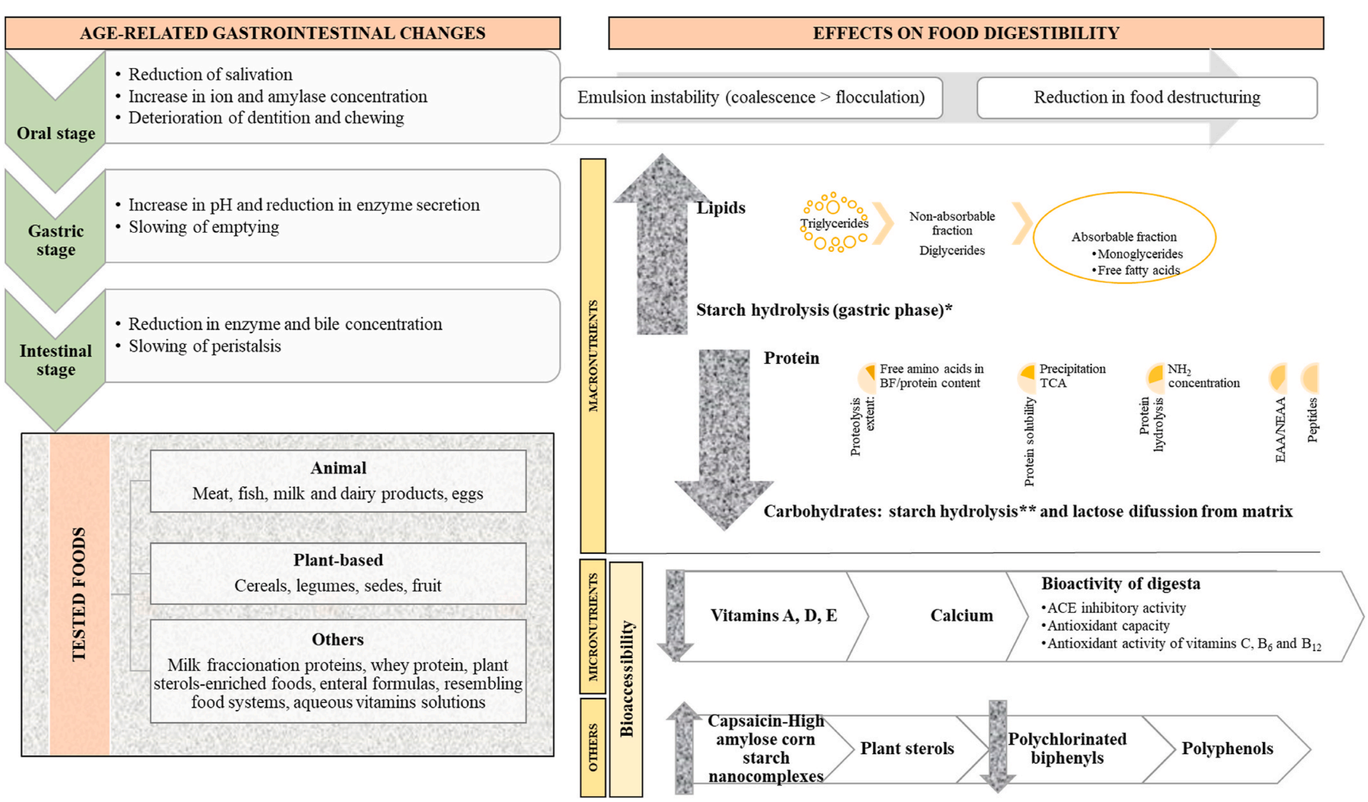

Fig. 2. Overview of changes on food digestibility from elderly physiological conditions in the tested foods. ACE: angiotensin converting enzyme; BF: bioaccessible fractions; EAA/NEAA: essential/non-essential amino acids; TCA: trichloroacetic acid; * Effects obtained from dynamic model with oral and gastric phases; ** Effects obtained from static model with oral, gastric and intestinal phases.

Table 4
Relative reduction (%) in proteolysis extent in elderly *vs.* young adult models in the assessed matrices.

Matrix	Oral modification	Oral and gastric modification	Oral, gastric and intestinal modification	References
Salmon, sea bass, sardine	Reduction (No data)	Reduction (No data)	Reduction (No data)	Hernández-Olivas et al., (2020a)
Hake	No reduction	No reduction	Reduction (No data)	
Milk	Not done	27	49	Hernández-Olivas et al., (2020b)
Yogurt	Not done	8	34	
Fresh cheese	16	25	35	
Aged cheese	16	24	40	
Cereals	No reduction	No reduction	≈40	Hernández-Olivas et al., (2021a)
Legumes	No reduction	No reduction	≈33	
Poached eggs	No reduction	No reduction	No reduction	Hernández-Olivas et al., (2021b)
Omelet	No reduction	No reduction	32	
Hard-boiled egg	No reduction	No reduction	38	

"No reduction" indicates that differences are not statistically significant.

protein digestion to that obtained by Levi et al. (2017), without the addition of carrageenan was reported, with the elderly model showing a lower protein degradation of α-lactalbumin compared to the adult model. Carrageenans (κ, ι, λ) were added to the whey protein solution at a 1:10 ratio (common concentration in commercial products). λ-Carrageenan and ι-carrageenan hindered protein digestion of lactoferrin both in the elderly and in adults, while in the case of β-lactoglobulin, these carrageenans only had a negative impact on protein degradation in the elderly model ($p < 0.05$). These two carrageenans are the ones that had the greatest effect in delaying the protein breakdown of both lactoferrin and α-lactalbumin. This indicates that the elderly seems to be more influenced by the negative effect of lower proteolysis when ingesting whey protein with carrageenan and, therefore, special attention should be paid to the addition of these to foods, formulas or supplements intended for the older adults.

3.3. Lipolysis

Studies evaluating fat digestibility by *in vitro* gastrointestinal digestion adapted to elderly physiological conditions are scarce and have been carried out on fresh and aged cheese (Hernández-Olivas et al., 2020b), fish (Hernández-Olivas et al., 2020a) and eggs with different culinary treatments (Hernández-Olivas et al., 2021b), using the INFOGEST harmonized method (Minekus et al., 2014). In all of them, the effect of oral, gastric and intestinal modifications from adult to elderly conditions (see Table 1) regarding lipid digestion were evaluated, determining the lipid profiles of different glyceryl structures: triglycerides, non-absorbable fraction (diglycerides 1,2-DG and 1,3-DG) and absorbable fraction (monoglycerides (1-MG and 2-MG) and free fatty acids (FFA)).

In dairy products, namely fresh and aged cheese, containing 20.2–28.8 g fat 100 g^{-1} with a similar amount of triglycerides (around 98%), the digestion under elderly conditions evoked a significantly higher triglyceride hydrolysis (91.6–93 *vs.* 82–89%, $p < 0.05$) and an increase of the absorbable fraction of lipids (82.3–86.5 *vs.* 73.7–78%, $p < 0.05$) compared to adult conditions (Hernández-Olivas et al., 2020b). Similar results were obtained for triglyceride hydrolysis (97.7 *vs.* 95.6%, $p < 0.05$) and absorbable fraction (87.9 *vs.* 83.5%, $p < 0.05$) in poached eggs. However, the opposite effect was observed in the triglyceride hydrolysis (95.2 *vs.* 99.7%, $p < 0.05$) in hard-boiled eggs due to a decrease in the non-absorbable fraction (9.2 *vs.* 13.9%, $p < 0.05$). No changes in these parameters were reported in omelet (Hernández-Olivas et al., 2021b). On the other hand, considering high-fat fish (salmon (14 g fat 100 g^{-1}) and sea bass (6.7 g fat 100 g^{-1}) with 99% of triglycerides) the same degree of hydrolysis of triglycerides in both types of fish (82–83%) was reported, with no effect of elderly digestion conditions on fat digestion. In addition, the influence of the food matrix was indicated, attributable to the different composition of moisture and fat in the two types of fish, a fact that is reflected in a higher percentage of FFA in salmon than in sea bass when using elderly digestion conditions compared to adults (Hernández-Olivas et al., 2020a).

It is worth mentioning that gastric lipase (one of the enzymes responsible for the hydrolysis of triglycerides with optimal activity at pH between 3 and 6) was not added in any assay. It has been suggested to reduce its activity to 15% in digestions using conditions of the elderly versus the adult ones (Shani-Levi et al., 2017). Several authors found that in healthy humans, gastric lipolysis leads to the hydrolysis of 10–30% of the ingested triacylglycerols, generating FFA, DG y MG (Bauer et al., 2005). However, in the intestinal phase, pancreatin and bile salts were reduced, and digestion time was increased (Table 1). These modifications did not cause a decrease in lipolysis in the aforementioned foods, even considering that the longer intestinal digestion time is expected to produce an increase in it, despite the decrease in pancreatin lipase and bile salts, all of them determinant factors on the extent of lipid digestion.

One of the doubts that arises in these studies is whether the lipid digestion values would be underestimated with respect to *in vivo* adult or elderly conditions by not adding gastric lipase, but also other enzymes such as colipase, phospholipase A2 or cholesterol esterase can contribute to this purpose. In this sense, since elderly people have a decrease in pancreatic secretion, gastric lipase plays an efficient role in the duodenum helping in lipid digestion, where it benefits from favorable conditions (Favé et al., 2008). Likewise, the absence of the other lipidic enzymes during simulated digestion could be relevant since if lipase and colipase are not present, bile salts cannot be displaced from the interphase and consequently triglyceride hydrolysis could be reduced. Similarly, the lack of colipase (hydrolyzes triglycerides at sn-2 position giving FFA and lysophospholipids) and cholesterol esterase (acts on triglycerides producing glycerol and FFA) may affect lipid digestion (Bauer et al., 2005). Moreover, lipid digestion and absorption are highly efficient processes, with more than 95% of the lipids consumed being absorbed (Lamothe et al., 2012). However, as indicated by Rémond et al. (2015), it is not clear whether lipid digestion in the small intestine is negatively affected by the slight decrease in lipase secretion noted in the elderly since there is no data available in the literature in this regard. Nevertheless, and more specifically, it is not fully elucidated if the bioavailability of essential FFA such as linoleic acid (LA, 18:2 n-6) and α-linolenic acid (ALA, 18:3 n-3) is affected with advancing age. In this sense, one study quoted the same bioavailability of ALA in young subjects and in 45–69 years old subjects. Accordingly, it has been reported that the age of the subject (25–69 years old) is not an important modulatory factor in influencing circulating levels of ALA (Patenaude et al., 2009).

As far as we are aware, only one study evaluates, in pork sausages, the kinetics of the digestion of lipids (30–150 min at intervals of 30 min). No differences were observed in the amount of lipids released during gastric digestion between adult and elderly conditions. Deficient mastication simulated by a masticator apparatus did not produce differences in the total amount of lipids released during gastric digestion, although a delay (at 60 and 90 min) in the release of lipids was observed (Peyron et al., 2021).

3.4. Carbohydrate digestibility

One of the most notable age-related changes in the macronutrient intake is the increase in the percentage of dietary energy from carbohydrates in old age (de Groot et al., 2000). In addition, the carbohydrate metabolism is affected by the ageing process, with a decrease in glucose tolerance (Elahi & Muller, 2000). For these reasons, the interest in assessing carbohydrate digestibility under elderly gastrointestinal conditions has increased. However, only few studies have evaluated the impact of changes in the gastrointestinal physiology of elderly people on carbohydrate digestibility (Hernández-Olivas et al., 2020b, 2021a; Li et al., 2022).

In dairy products (milk, yogurt, and aged and fresh cheese), lactose solubility (diffusion from the food matrix) was evaluated by adapting the INFOGEST method to the gastrointestinal conditions of the elderly (see Table 1) (Hernández-Olivas et al., 2020b). Lactose solubility was reduced ($p < 0.05$) in milk (23–28 *vs.* 35%) after the adaptation of gastric and both gastric and intestinal phases, and in aged cheese (39 *vs.* 50%) after the adaptation of all phases. It was suggested that the lactose solubility is mainly affected in high-lactose food (57 mg glucose eq. g milk^{-1}). However, this factor does not seem relevant in aged cheese, a low-lactose food (4 mg glucose eq. g cheese^{-1}).

Glycolysis was affected by elderly gastrointestinal conditions in legumes and cereal/pseudo-cereals in a matrix-dependent manner (Hernández-Olivas et al., 2021a). A reduction ($p < 0.05$) in starch digestion in lentils (approximately 25%) was observed in the adapted oral phase, possibly due to lower enzyme accessibility by halving chewing cycles. In addition, a decrease ($p < 0.05$) in starch hydrolysis was observed in the adaptation of the oral-gastric (lentils, white bean and spelt, approximately 12–50%) or all phases (all samples, except soybeans, oats and quinoa, approximately 15–75%). The lower proteolysis in elderly gastric conditions (see section "3.2. Proteolysis"), which would reduce the access of the glycolytic enzymes on their substrate, and the intestinal enzymatic activity decreased could explain these observations. Despite these results, some limitations in this study were observed (Table 3), highlighting the lack of gastric lipase that could reduce the reproducibility of the conditions given the importance of lipids in starch digestibility (Dona et al., 2010).

Starch hydrolysis, although mainly produced in the oral and intestinal phases, may occur due to residual action of salivary amylase until the pH drops below 4 (Fried et al., 1987). In this sense, the difference in starch hydrolysis in the gastric phase between adult and elderly conditions was evaluated in a traditional Tibetan food (*Tsampa*) made from roasted whole-grain flour and salted butter tea (Li et al., 2022). Increased starch hydrolysis in elderly conditions (13.7 *vs.* 9.7% at the end of digestion, $p < 0.05$) was shown, which could be attributed to the higher concentration of salivary amylase, higher gastric pH, and longer digestion time. This finding does not imply a greater intestinal glucose absorption due to the differences between adult and elderly.

3.5. Micronutrients

The implementation of vitamins and minerals in the diet in order to guarantee the nutritional needs of the older adults has become important, focusing dietetic recommendation of foods rich in vitamin D, folate, calcium, and magnesium or even by their association with age-related diseases (Berendsen et al., 2019; Hoffman, 2017). However, beyond the content in foods, it is important to evaluate the fraction of the nutrient available for intestinal absorption (i.e., bioaccessibility). For this purpose, *in vitro* digestion methods are adequate and employed mainly for this objective (Wu & Chen, 2021); besides, they are effective in observing the trend of bioavailability. However, they are not suitable for predicting the magnitude of bioavailability, since it depends on factors that are not considered in these methods (transport, distribution, and excretion, among others) (Bohn et al., 2018). In this sense, numerous studies have analyzed the micronutrient bioaccessibility under elderly gastrointestinal conditions (Hernández-Olivas et al., 2020a, b, 2021a, b; Lee et al., 2021c, 2022).

Ageing is related to an increased risk of different disorders, including osteoporosis. The inadequate intake of calcium is a modifiable risk factor for osteoporosis (Srivastava & Deal, 2002), and it has been demonstrated that calcium supplementation (750 mg day^{-1}) reduces the bone loss in elderly people (405 subject were ≥60 years of age) with deficient intake (<450 mg day^{-1}) (McCabe et al., 2004). Calcium bioaccessibility in fish (Hernández-Olivas et al., 2020a), dairy products (Hernández-Olivas et al., 2020b), and legumes and cereals (Hernández-Olivas et al., 2021a) under adult and elderly gastrointestinal conditions have been evaluated (see conditions in Table 1). A reduction ($p < 0.05$) in calcium bioaccessibility was observed following the adaptation of oral (lentils and white bean) (approximately 35 and 25%, respectively), oral-gastric (salmon, sardine, chickpea, lentils, soya bean, white bean, oats, and quinoa) (21–61% in fish and approximately 12–45% in legumes and cereals), or all phases (sea bass, salmon, sardine and all legumes and cereals) (30–61%in fish and approximately 10–60% in legumes and cereals). However, in dairy products, calcium bioaccessibility was reduced ($p < 0.05$) only in ripened cheese due to oral (31%), oral-gastric (69%) and oral-gastrointestinal adaptation (50%). In general, it has been shown that elderly conditions reduce calcium bioaccessibility, and this could relate with lower protein (Hernández-Olivas et al., 2020b) or lipid digestion (Hernández-Olivas et al., 2020a) and a reduction in phytase activity (hydrolase of divalent cationic chelating called phytate) in the case of legumes and cereals (foods with significant contents of phytate) (Hernández-Olivas et al., 2021a). However, matrices more prone to structural disintegration (milk, yogurt and fresh cheeses) do not seem to be affected (Hernández-Olivas et al., 2020a).

The reduction in the concentration of bile salts and enzyme activities in the intestinal lumen could negatively affect the absorption of fat-soluble vitamins, although there is discrepancy (Kasper, 1999). Different studies have evaluated the effect of elderly gastrointestinal conditions on the bioaccessibility of vitamins A and D. In fatty fish (sea bass, salmon and sardine) (Hernández-Olivas et al., 2020a), a reduction on bioaccessibility of fat-soluble vitamins was observed after adaptation of all digestion phases (by 35–40%), with exceptions in sea bass (vitamin D) and sardine (vitamin A). In eggs (Hernández-Olivas et al., 2021b), only the bioaccessibility of vitamin D is lower under elderly conditions (approximately 10–30%), and dairy products (Hernández-Olivas et al., 2020b) were the least affected, observing a reduction on vitamin A bioaccessibility only in fresh cheese (by 35%) and ripened cheese (by 33%). On the other hand, a study evaluated vitamin E diazability in a water dissolution or different fat emulsions (olive oil, tallow or lard emulsion) applying the *in vitro* gastrointestinal model with adult and elderly conditions (Lee et al., 2021c). In the elderly conditions, a slight reduction ($p < 0.05$) of the diazability of vitamin E in lard emulsion was observed (by 9.8%). This suggests that elderly conditions do not have a great effect on vitamin E diazability in solution or emulsion, probably due to structural characteristics, in contrast to food matrices. The reduction in bioaccessibility/diazability could mainly be due to a lower bile salt concentration, that contribute to the solubility of lipophilic compounds (Hernández-Olivas et al., 2021a; Lee et al., 2021c). In addition, the reduction in lipid digestion due to lower lipase activity could reduce the formation of emulsions derived from triglycerides, and consequently the solubility of fat-soluble vitamins (McClements & Li, 2010). This would explain why Lee et al. (2021c) did not observe differences in fat-soluble vitamin bioaccessibility between the elderly and adult models.

The effect of *in vitro* digestion with gut microbiota under elderly gastrointestinal conditions on diazability of water-soluble vitamin in aqueous solutions (0.5 mg mL^{-1}) showed that diazability of vitamins C and B_6 was not affected by the elderly model. However, vitamin B_{12} diazability was higher under elderly conditions (45 *vs.* 36%, $p < 0.05$), which the authors attributed to a greater presence of *Escherichia coli* in the elderly, a bacterium with the ability to biosynthesize such vitamins (Lee et al., 2022).

3.6. Bioactivity of foods following gastrointestinal digestion

The physiological changes and risk of non-communicable diseases in elderly people make them a target population for functional foods. Their bioactive compounds could help to reduce risk factors of diseases, restore physiological functions and improve their quality of life (Jędrusek-Golińska et al., 2020; Martirosyan et al., 2021).

After the gastrointestinal digestion, a bioaccessible fraction is formed in which: (i) macronutrients can be modified giving rise to bioactive compounds (i.e., bioactive peptides from proteins), and (ii) digestion conditions may modify the native structure of the bioactive compounds (i.e., polyphenols) or their biological activity (i.e., antioxidant capacity).

Bioactive peptides have shown a wide range of biological effects (Jia et al., 2021). Some studies have focused their attention on evaluating the formation of bioactive peptides under elderly gastrointestinal conditions, as well as their biological activity (Hernández-Olivas et al., 2022). However, it should be noted that the observed biological effects must be confirmed in more complex models in order to verify their bioefficiency. In this regard, compared with adult, elderly gastrointestinal conditions have shown lower percentage of potentially bioactive peptides in different foods such as chicken, beef, pork, and soy protein powders (mean of 8.3 *vs.* 9.5%, $p < 0.05$) (Wang et al., 2022).

Silk moth pupae flour is a high-protein food (40.4 g 100 g flour^{-1}) (David-Birman et al., 2019). A delayed protein digestion breakdown was observed in the elderly *vs.* adults (data not shown). However, up to 14 peptides with >80% probability of carrying bioactivity (using a predictive software tool) were found in the elderly model, but not in the adult model. The authors suggested that predicted bioactivity could help combat pathogenic bacteria or confer opioid-like effects due to their antimicrobial and acyl-Co-A binding capacity. On the other hand, in a previous study by the same group using the adult model, there were more bioactive peptides from β-lactoglobulin and α-lactalbumin compared to the elderly (Levi et al., 2017).

Since a lower release of bioactive peptides during digestion is generally observed in elderly conditions, it is of interest to evaluate the biological effects in digesta of protein-rich foods. In this regard, using elderly gastrointestinal conditions in different meats (chicken, turkey, pork and beef), a reduction ($p < 0.05$) in angiotensin converting enzyme (ACE) inhibition of gastric and intestinal digesta (approximately 20–50 and 5–20%, respectively) was observed, in addition to the antioxidant activity (DPPH) in the intestinal phase (approximately 7–23%) (Hernández-Olivas et al., 2022). These results could be ascribed to the reduction of pepsin and pancreatic protease activity, suggesting that the reduction in protein digestibility translates into a lower biological activity.

The biological activity of eleven different cheeses after simulated digestion was evaluated under adult and elderly conditions (see conditions in Table 1) (Plante et al., 2020), observing lower ($p < 0.05$) DPPH inhibition in six cheeses in the elderly model (approximately 10–75%). A similar trend was observed in other antioxidant parameters. Ferric reducing antioxidant power (FRAP) and total phenolic content (TPC) were reduced (12–20% in all cheeses and 10–90% in five cheeses, respectively). In addition, the inhibitory properties on α-glucosidase and pancreatic lipase were evaluated, observing in the elderly model a lower inhibitory activity (5–90% in nine cheeses and 25–75% in four cheeses). In summary, the lower bioactivity, probably due to a reduction in release of bioactive compounds (not evaluated in this study), indicates that the elderly might benefit less from the bioactive properties of cheese. Some limitations of the study must be highlighted such as the not using of gastric lipase, an enzyme that affects the solubilization of bioactive compounds of lipophilic nature (Iddir et al., 2021), nor the frequency of agitation, neither the modification of the incubation time.

In order to choose the best combination of foods oriented to the elderly population, Plante et al. (2021) evaluated the effect of combining fruit with cheese on bioactive properties following simulated elderly gastrointestinal digestion. That study showed that the combination of cheese with fruit increase ($p < 0.05$) the FRAP (1.4–3.5-fold higher, in all cheeses), α-glucosidase inhibitory (3.4–4.5-fold higher, only in cheddar), antimicrobial (5-8-fold higher, only in feta) and immunomodulatory properties (TNF-α) (48–59%, only in feta) of the cheese. However, few digestion parameters were adapted to the elderly conditions (see Table 1), which could reduce the reliability of the results.

Shang et al. (2022) evaluated the polyphenol bioaccessibility and the antioxidant activity of apple digesta with static and dynamic digestion models under adult and elderly conditions (see Tables 1 and 2), observing overall a higher ABTS and ·OH radical scavenging (by 10–40%) under elderly conditions, despite presenting a lower polyphenol bioaccessibility (see section "3.7 Others"). This suggests that the evaluation of the bioactivity complements the bioaccessibility assessment of bioactive compounds, thus enabling the attainment of more complete results.

With less complex matrices using standard compounds, it has been shown that the antioxidant activity of vitamin C, Lee et al. (2022) was not different between adult and elderly. However, the antioxidant activity (ABTS) of vitamin B_6 was higher in gastric phase under elderly conditions (71 *vs.* 25%, $p < 0.05$). On the contrary, vitamin B_{12} showed a lower activity in the small intestinal phase in elderly (78 *vs.* 66%, $p < 0.05$). The authors indicate that a lower gastric pH in adult conditions could affect the bioactivity of these vitamins.

3.7. Others

Other studies focused on the evaluation of the bioaccessibility of bioactive compounds such as capsaicin (CAP) (Isaschar-Ovdat et al., 2021), plant sterols (PS) (Miedes et al., 2022) and polyphenols (Shang et al., 2022), or toxic compounds such as polychlorinated biphenyls (PCBs) (Planche et al., 2022).

CAP has shown beneficial health effects, but due to its high pungency and instability in gastric conditions it requires a suitable vehicle. In this sense, amylose can form a V-type crystalline structure with a hydrophobic cavity, capable of stabilizing and releasing lipophilic compounds whilst being in the gastrointestinal tract. These properties of amylose have allowed the development of complexes with bioactive compounds, such as the complex between CAP with high-amylose corn starch. From this fact arises the interest of evaluating the CAP bioaccessibility in CAP-high-amylose corn starch nanocomplexes applying a semi-dynamic *in vitro* digestion model (see conditions in Table 2) (Isaschar-Ovdat et al., 2021). CAP bioaccessibility in elderly models was higher than in adults (intestinal phase 60 *vs.* 40%, $p < 0.05$). The greater salivary amylase concentration in elderly conditions and the higher gastric pH increases starch hydrolysis and consequently CAP release. Despite the lipophilic properties of CAP, it was observed that starch digestibility is key in its bioaccessibility, above other aspects of lipid digestion such as the bile salt concentration. This indicates that the effect of elderly conditions on the bioaccessibility of food compounds depends on the matrix composition or nature.

Another group of bioactive compounds of interest to the elderly population are PS due to their numerous health benefits, including a serum cholesterol-lowering effect (Poli et al., 2021). In a PS-enriched milk-based fruit beverage, an increase ($p < 0.05$) in the bioaccessibility of total PS (by an 88%) and cholesterol (194%) was observed in elderly conditions *vs.* adult (see conditions in Table 1) (Miedes et al., 2022). This finding is attributed to changes in gastric conditions, specifically, the lower pepsin and lipase activity and longer incubation time. The changes in the intestinal conditions also contributed, although to a lesser extent, to the improvement of sterol bioaccessibility (longer incubation time and lower cholesterol provided from bile and pancreatin). These results support the recommendation of the PS-enriched beverage to reduce the risk of cardiovascular disease in elderly people. It should be noted that this study is the only one included in this review that has used gastric lipase in the INFOGEST method, which could improve the *in vivo* reproducibility of physiological conditions as suggested by Makran et al. (2022).

Apple polyphenol bioaccessibility was evaluated under adult and elderly conditions using a static and a dynamic digestion system (see Tables 1 and 2) (Shang et al., 2022). In both models the release of total polyphenol compounds was halved (20–62 *vs*. 10–33 μg 100 g fresh apple^{-1}) in elderly conditions. These results could be due to the reduction in the activity of pepsin (the enzyme that improves the polyphenol release through hydrolysis of the chemical bond between polyphenols and proteins) and the reduction in the intestinal stage of agitation and enzyme activity. This suggests that the changes in the bioactive compound bioaccessibility after elderly adaptation depends on the type of interaction between nutrients and bioactive compound, since proteins seem to improve bioaccessibility in PS (Miedes et al., 2022), unlike polyphenols (Shang et al., 2022).

In relation to toxic compounds, PCB bioaccessibility in ground beef (11% fat) with different extent of cooking exposed to PCBs was evaluated after adult and elderly gastrointestinal digestions (Planche et al., 2022). PCB bioaccessibility was lower under elderly conditions (17 *vs.* 25%, $p < 0.05$) probably due to the reduction in the bile salt concentration and the lower digestion of proteins.

4. Conclusions

Age-related changes in chewing and saliva composition modify the food disintegration, which could lead to changes in food perception and digestion of nutrients in both gastric and intestinal stages. Protein digestibility and bioaccessibility of micronutrients (Ca and vitamins A, D and E) is decreased under elderly gastrointestinal conditions that could lead to a deterioration of the quality of life due to a decrease in muscle mass and essential function in the organism. The increase in the lipid digestibility in older adults could worsen the risk of cardiovascular disease and obesity but could also increase the effects of beneficious lipidic compounds. Carbohydrate digestibility does not show a clear trend which is dependent on the applied digestion methods (static or dynamic) and on the food matrix.

The main identified gaps in these studies show differences in proteolysis analysis methods, scarce knowledge about the influence of gastric lipase, and the lack of uniformity in gastrointestinal adaptations to the elderly that make difficult to obtain conclusive results. In addition, while some nutrients are widely studied (e.g., protein), there is a lack of assessment of critical micronutrients for older adults, like iron, magnesium, or folate, among others and scarce research in the bioactivity of food after digestion. Also, there is few research available on such important aspects as antinutrients, cooking and food processing, among others. Moreover, since only two studies included the colonic phase, there is a clear lack of research in the elderly that includes the entire digestion, from mouth to anus.

Considering future perspectives of research, findings of the present review show the need to perform elderly-adapted *in vitro* digestion methods with harmonized methodology, not only to standardize the digestive conditions of the elderly, but also the evaluation methods. Once harmonized, next step would be the performance of *in vivo* studies that evaluate the digestibility of food compounds in older people, in order to correlate the data obtained. Finally, another aspect to bear in mind would be the tailor-made adaptation of texture and nutritional composition of foods oriented to the elderly target population.

Author contributions

Mussa Makran: Investigation, Formal analysis, Writing – original draft. Diego Miedes: Investigation, Formal analysis, Writing – original draft. Antonio Cilla: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing –review & editing. Reyes Barberá: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing –review & editing. Guadalupe Garcia-Llatas: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing. Amparo Alegría: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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Declaration of competing interest

The authors declare no conflict of interest, financial or otherwise.

Data availability

The authors are unable or have chosen not to specify which data has been used.

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