Food Chemistry 485 (2025) 144424

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Review

Bioaccessibility and associated concepts: Terminology in the context of *in vitro* food digestion studies

Myriam M.-L. Grundy [a], Amélie Deglaire [b], Steven Le Feunteun [b], Emmanuelle Reboul [c], Paul J. Moughan [d], Pete J. Wilde [e], David Julian McClements [f], Sébastien Marze [g,*]

[a] *INRAE, French National Research Institute for Agriculture, Food and Environment, UMR PEGASE, Institut Agro, 35590, Saint Gilles, France*
[b] *INRAE, L'Institut Agro, STLO, 35042 Rennes, France*
[c] *Aix-Marseille Univ, INRAE, INSERM, C2VN, Marseille, France*
[d] *Riddet Institute, Massey University, Palmerston North 4474, New Zealand*
[e] *Quadram Institute Bioscience, Norwich Research Park, Norfolk, Norwich, NR4 7UQ, United Kingdom*
[f] *Department of Food Science, University of Massachusetts, Amherst, MA 01003, USA*
[g] *INRAE, BIA, F-44316 Nantes, France*

ARTICLE INFO

Keywords:
In vitro digestion
Nutrients
Digestibility
Bioaccessibility
Bioavailability
Definitions

ABSTRACT

In vitro gastrointestinal models are widely used to study food digestion, in combination with analytical methods to determine the physicochemical and biochemical fate of food compounds. The *in vitro* bioaccessibility determined with these models is often used as an indicator of the *in vivo* bioavailability. However, the bioaccessibility concept is not used consistently within the scientific literature, leading to confusion and making it difficult to compare the results from different studies.

The aim of this article is to provide standardized definitions of *in vitro* digestibility and bioaccessibility, detailing the main processes involved, including physical release, solubilization, and biochemical/metabolic reactions. The terminology of complementary cellular, *ex vivo*, and animal/human *in vivo* experiments is also given. Application of the *in vitro* terminology to different nutrients is discussed, including lipids, proteins, carbohydrates, vitamins, and other bioactive compounds. The proposed definitions unify most concepts related to the gastrointestinal fate of ingested food compounds.

1. Introduction

A food compound must be released from its food matrix and/or hydrolyzed by digestive or microbial enzymes before it can be absorbed and reach the bloodstream and targeted organ, tissue, or cell (McClements, Li, & Xiao, 2015). The digestion of foods is a complex and dynamic process involving numerous steps that depend on the nature of the food being consumed, including its composition (*e.g.,* macronutrient and micronutrient content), its physical form (*e.g.,* solid, semi-solid, or fluid), and the structural organization and interactions of its constituents (*e.g.,* food matrix effects) (Goodman, 2010; Lentle & Janssen, 2011). For macronutrients (proteins, starches, and lipids), digestive enzymes must first come into contact with their substrate so that partial or full hydrolysis occurs. The resulting hydrolysis products (*e.g.*, amino acids, peptides, sugars, fatty acids) then move to their sites of absorption at the brush border of the intestinal epithelium cells. Passing through the layer of epithelium cells, they enter the bloodstream (through the portal vein or lymphatic system, depending on their hydrophobicity). Highly lipophilic molecules, such as long chain free fatty acids, monoglycerides, fat-soluble vitamins, and hydrophobic nutraceuticals (such as curcumin and carotenoids) are transported *via* the lymphatic system in the form of colloidal lipoproteins, such as chylomicrons. In contrast, more hydrophilic molecules, such as amino acids, sugars, and short/medium chain free fatty acids are transported through the portal vein (Tso, Bernier-Latmani, Petrova, & Liu, 2025). They may then be distributed to different organs, tissues, and cells within the body. Contrary to macronutrients, most micronutrients (vitamins and minerals) do not require hydrolysis prior to absorption, unless they are esterified or chemically complexed with macronutrients. Nevertheless, they may still be chemically transformed in the gastrointestinal tract prior to absorption. Dietary fibers that interact with gastrointestinal constituents (such as enzymes, bile salts, and mineral ions), or with co-ingested nutrients, can

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[*] Corresponding author.
E-mail address: sebastien.marze@inrae.fr (S. Marze).

https://doi.org/10.1016/j.foodchem.2025.144424
Received 17 February 2025; Received in revised form 15 April 2025; Accepted 18 April 2025

alter the digestion and absorption of macronutrients, micronutrients, and nutraceuticals (Grundy et al., 2016; Zhang et al., 2014). Any compounds not digested and/or absorbed in the upper gastrointestinal tract (GIT) may be metabolized by the gut microbiota residing mainly in the colon, which may convert them into a form that can be absorbed by the body (Capuano & Pellegrini, 2019). It should be noted that microorganisms are found throughout the GIT, but they are particularly numerous and active in the lower small intestine and colon. For instance, some colonic microbes secrete enzymes that can catalyze the hydrolysis of fibers and metabolize the resulting compounds ("fermentation"), leading to the production of short chain fatty acids (SCFAs) and other compounds that can be absorbed.

Several *in vitro* methods can be used to obtain information about the physicochemical processes occurring during the passage of foods through the GIT (Brodkorb et al., 2019; Hur, Lim, Decker, & McClements, 2011; Ji et al., 2022). These methods are often used to complement human or animal studies, as they are usually faster, cheaper, less prone to biological variability, and raise fewer ethical concerns.

Currently, scientists use different terms to describe the same processes occurring during food gastrointestinal digestion (or the same terms to describe different processes), which causes confusion and makes it difficult to compare results between *in vitro* digestion studies. There is therefore a need to define these processes unambiguously and refer to them using a standardized terminology.

A major objective of the current study is to extend and generalize the concepts of *in vitro* digestibility and bioaccessibility introduced in a recent article (Grundy, Moughan, & Wilde, 2024). This previous article mainly focused on the application of these concepts to plant-based foods, focusing on the role of the plant cell walls in limiting the digestibility and bioaccessibility of nutrients. In the current article, we attempt to provide more general definitions that can be applied to the digestion of most foods and the bioaccessibility of most nutrients. The terminology of complementary cellular and *ex vivo* experiments is also given, as well as the main definitions related to animal/human *in vivo* experiments. Throughout the article, specific examples are provided to remove current ambiguities in the literature.

2. Previous digestion schemes

Several classification schemes have been developed to describe the gastrointestinal fate of ingested compounds. In the pharmaceutical industry, the fate of drugs is described by the LADME scheme, which stands for liberation, absorption, distribution, metabolism and excretion of drugs (Ruiz-Garcia, Bermejo, Moss, & Casabo, 2008):

- *Liberation*: Liberation describes the physical release of the drug from the delivery formulation, which must occur before the drug can be absorbed by the body.
- *Absorption:* Absorption describes the uptake of the drug from the gastrointestinal tract into the systemic circulation.
- *Distribution:* Distribution describes the transfer of the drug through the systemic circulation to the different organs, tissues, and cells in the body.
- *Metabolism*: Metabolism describes the transformation of the drug into other compounds (metabolites) in the body due to the action of metabolic enzymes.
- *Excretion*: Excretion describes the removal of the drug or its metabolites from the body, which may occur through the urine, feces, sweat, or breath.

The above classification system is useful for understanding the behavior of drugs in the body, but it is limited for understanding the behavior of nutrients and nutraceuticals. In particular, it does not focus on the various processes that occur within the GIT that affect the bioavailability and bioactivity of ingested food compounds. To take this into account, researchers have developed the Nutraceutical (or Nutrient) Bioavailability Classification Scheme (NuBACS) (McClements et al., 2015):

$$\mathrm{BA} = \mathrm{B}^{*} \times \mathrm{A}^{*} \times \mathrm{T}^{*} \tag{1}$$

For the sake of concision, the term "nutrient" will be used to include macronutrients, micronutrients, and nutraceuticals in this expression. In eq. 1, BA is the effective *bioavailability* of the bioactive agent, which is the fraction of the nutrient in the ingested food that actually enters the body. Moreover, B* is the fraction of the nutrient in the GIT that is bioaccessible, A* is the fraction of the bioaccessible nutrient that is *absorbed* by the epithelial cells, and T* is the fraction of the absorbed nutrient that is present in a metabolically active form (after any chemical transformations within the GIT and liver). It should be noted that other types of chemical transformations may also occur after absorption, which may impact the bioavailability and bioactivity of nutrients, including various metabolic processes, but these are not accounted for in the NuBACs scheme. Each of the parameters in the B*A*T* expression can be further divided into different physicochemical phenomenon. For the bioaccessibility parameter:

$$\mathrm{B}^{*} = \mathrm{F_L} \times \mathrm{F_S} \times \mathrm{F_I} \tag{2}$$

Here, F_L is the fraction of the nutrient liberated from the original food matrix, F_S is the fraction of the liberated nutrient solubilized within the intestinal fluids, and F_I is the fraction of the solubilized nutrient that is not lost due to the formation of insoluble complexes with other compounds in the intestinal fluids. As a specific example (Zhou et al., 2021), vitamin D may be liberated ($\mathrm{F_L}$) from the oil phase of a plant-based milk and then be solubilized within intestinal mixed micelles composed of lipids and bile salts ($\mathrm{F_S}$). However, some of these vitamin D-loaded mixed micelles may precipitate due to fatty acid soap formation in the presence of calcium ions, leaving only a fraction in an absorbable form ($\mathrm{F_I}$). For the absorption parameter:

$$\mathrm{A}^{*} = \mathrm{F_{ML}} \times \mathrm{F_{EL}} \tag{3}$$

Here, F_{ML} is the fraction of nutrient transported across the mucus layer and F_{EL} is the fraction of nutrient that reaches the surfaces of the epithelium cells that is absorbed. This latter factor is influenced by nutrient type and the transport mechanism, such as active or passive transport. For the transformation parameter:

$$\mathrm{T}^{*} = \mathrm{F_C} \times \mathrm{F_M} \tag{4}$$

Here, F_C and F_M are the fractions of nutrient that reach the systemic circulation in a metabolically active form after any chemical ($\mathrm{F_C}$) or metabolic ($\mathrm{F_M}$) transformations in the GIT and/or the liver.

This scheme is unsuitable for probiotics that are not digested and absorbed in the upper GIT. However, it may be adapted for prebiotics and dietary fibers that may be hydrolyzed and fermented within the lower small intestine and colon and produce metabolites that are absorbed by the body (such as SCFAs). Notably, the NuBACs scheme can also be extended to include the distribution, metabolism, and excretion processes that occur after the nutrients enter the bloodstream.

3. Main definitions

The aim of this article is not to develop a new classification scheme identifying the different processes occurring within the GIT, as was done with the NuBACS. Instead, we focus on clearly defining the main terms used to describe food digestion and recommend a common terminology based on the elementary processes of food digestion, so that these terms can be used consistently. We start by defining processes that are measurable *in vitro*, then briefly relate them to cellular, *ex vivo*, and *in vivo* processes. Fig. 1 gives an overview of the terms defined here and how they are related to each other, and Table 1 provides the main definitions.

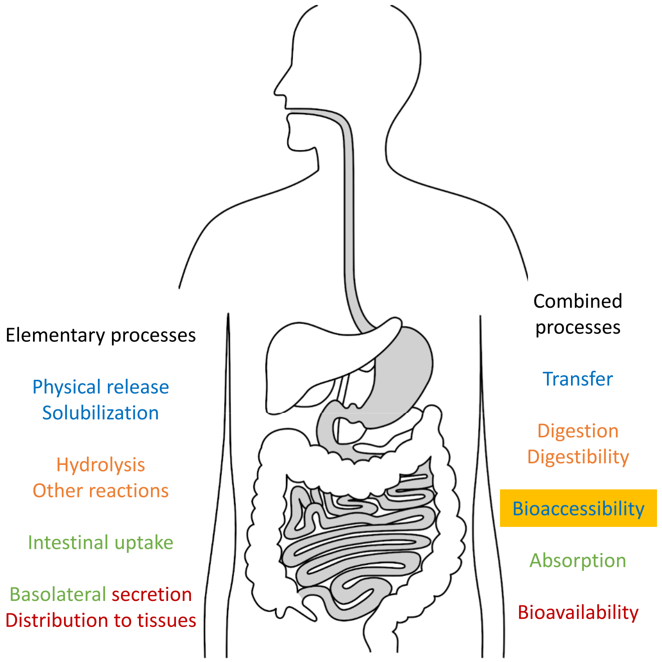

Fig. 1. Overview of the terms defined in the article as used to describe processes occurring to food compounds in the oro-gastrointestinal tract and body. Terms describing elementary processes are on the left. Terms referring to combinations of elementary processes are on the right, using corresponding colors. Bioaccessibility, appearing in two colors, includes both transfer and digestion processes. Basolateral secretion, written in two colors, is included in both absorption and bioavailability.

3.1. *In vitro* experiments

Release: The term "release" is used to describe the physical liberation of a compound (either macronutrient, nutrient, or micronutrient) during food matrix disintegration, anywhere within the oro-gastrointestinal tract (oro-GIT). The term release is used here, rather than liberation (as used in the NuBACs system), because it is more familiar to a broader range of researchers, although both terms have similar meanings. In the literature, release is often used as a synonym for transfer, which describes the movement of a compound from one phase to another. Strictly speaking, release only refers to compounds leaving food-rich phases but using it for transfer is usually acceptable. A counterpart term to release is solubilization, which refers to the dissolution or dispersion of the compounds within the oro-GI fluids (luminal water-rich phases). Note that the term "release" is also sometimes used to describe the liberation of a molecule from a compound due to a biochemical reaction, such as the release of free fatty acid molecules upon triglyceride or phospholipid lipolysis. We recommend not using release in this sense, preferring to refer to the biochemical reaction and its products. When necessary, the detailed terms physical release and biochemical release should be used to avoid ambiguity.

Solubilization: The term "solubilization" refers to the dissolution or dispersion of a compound within the luminal water-rich phases anywhere in the oro-GIT. Polar nutrients may be directly soluble in water, such as the free sugars or amino acids biochemically released from starches or proteins, respectively. In contrast, non-polar nutrients may have to be solubilized within the hydrophobic interiors of intestinal mixed micelles due to their low water solubility. We recommend using the terms *aqueous solubilization* (molecular dissolution) or *micellar solubilization* (colloidal dissolution) to describe the solubilization of polar and non-polar nutrients directly in water or in mixed micelles, respectively.

Transfer measurements: Transfer measurements are used to determine the amounts of a food compound remaining in (retained) or leaving (released) a food-rich phase under oro-GI conditions. These analyses are often carried out by measuring the relative amount (fraction) of a food compound solubilized in the oro-GI fluids after digestion. The amount (or concentration) is usually expressed as mass or mole (per volume unit for concentration), and related to the initial amount (concentration) in the food. In practice, physical release can be measured directly for food

Table 1
Summary of the main definitions given in the article, considering elementary processes, combined processes, and endpoints.

Elementary processes		**Combined processes**		**Endpoints**	
Release	Physical liberation of a compound from food-rich phases	**Transfer**	Movement of a compound from one phase to another	**Bioaccessibility**	Fraction of an ingested compound that is biochemically and/or physically released from the food matrix and solubilized within the gastrointestinal fluids in an absorbable form
Solubilization	Dissolution or dispersion of a compound within the luminal water-rich phases				
Hydrolysis	Reactions catalyzed by human/animal enzymes yielding absorbable derivatives from a parent food compound	**Digestion** **Digestibility**	Luminal transformation of food compounds into absorbable derivatives, focusing mainly on the hydrolyses of nutrients. Digestibility usually refers to the measured rate and extent of hydrolysis of a specified macronutrient		
Other luminal biochemical/ metabolic reactions (oxidation, fermentation)	Other luminal reactions yielding absorbable derivatives from parent food or intermediate compounds, excluding chemically unrelated microbial metabolites				
Intestinal uptake	Amount of a compound crossing the brush border and entering intestinal cells	**Absorption**	Amount of a compound or its derivatives passing from the oro-GIT to the blood or lymph	**Relative bioavailability**	Amount of a specific bioactive metabolite from a food compound that is present in the blood following ingestion
Basolateral secretion (to circulation)	Amount of a compound or its derivatives leaving intestinal cells from the basolateral side				
Distribution to tissues	Amount of a compound or its derivatives delivered to target tissues			**True bioavailability**	Amount of a food compound or its derivatives that reaches the target cells, tissues, or organs in the body

samples that partly remain intact in the GIT since the food can then be easily separated from the oro-GI fluids, or by centrifuging the oro-GI fluids to separate the supernatant from any insoluble matter. In the presence of lipids, centrifugation will separate sample into light (oil), intermediate (soluble), and heavy (insoluble) phases. The solubilized molecules are measured in the intermediate aqueous phase collected after centrifugation.

Biochemical and metabolic reactions: This refers to reactions occurring within the oro-GIT that yield absorbable derivatives from a parent food compound. Enzyme-catalyzed hydrolyses are the main reactions considered during food digestion using *in vitro* models, yet other kinds of biochemical/ metabolic reactions may occur, including oxidation, conjugation, and fermentation. Some food compounds (such as dietary fibers and polyphenols) are not hydrolyzed by mammalian enzymes in the upper GIT but are metabolized by enzymes produced by the gut microbiota, yielding absorbable metabolites. Although not the scope of this article, these processes are increasingly being studied using *in vitro* models that combine upper and lower digestive tract compartments within a single experiment. This involves using a combination of mammalian enzymes and gut microorganisms (Guo et al., 2021; Ji et al., 2022; Moon, Li, Bang, & Han, 2016; Wang et al., 2022). In our definitions, which are focused on conventional *in vitro* digestion experiments, we exclude metabolites produced by microbial fermentation in the GIT and that are not chemically related to the parent food compound, such as the SCFAs produced by fermentation of dietary fibers by colonic microorganisms.

Biochemical reaction measurements: This type of experiment refers to the measurement of a decrease in the concentration of a parent compound as a result of a biochemical reaction occurring within the GIT, or to an increase in the concentration of one or more derivative compounds produced by this reaction. For example, the amount of a hydrolysis product yielded from a given amount of a food compound after a specified time, of which the ratio (or fraction, or proportion) is often referred to as the *hydrolysis degree (or extent)*. For lipids, this could involve measuring the decrease in the concentration of triglyceride molecules present or the increase in the concentration of free fatty acid and monoglyceride molecules present. In general, the type of hydrolysis reaction monitored should be specified, *e.g.*, lipolysis for lipids, proteolysis for proteins, and amylolysis for starch, as will be clarified in section 4. In the case of other types of biochemical/metabolic reaction occurring in the GIT, it is important to specify the nature of the substrate and reaction mechanism involved, such as lipid peroxidation, protein carbonylation, vitamin de-esterification, or amino acid fermentation (deamination, decarboxylation).

Digestion and digestibility: Digestion is a term describing the transformation of food compounds into absorbable derivatives within the oro-GIT, which is most often used to refer to the hydrolysis of macronutrients such as proteins, starches, and lipids. For *in vitro* digestion studies, this term is mainly used to refer to enzymatic reactions occurring within the upper GIT, such as proteolysis, amylolysis, and lipolysis. The term *digestibility* is often used in a broad sense *in vitro* to refer to the susceptibility of a nutrient to enzymatic hydrolysis. For instance, food proteins may have low or high digestibility under gastric pepsinolysis depending on their molecular structure and aggregation state. In animal science and human nutrition studies, however, the term digestibility has a different meaning, referring to the disappearance of an ingested nutrient from the GIT lumen, assumed to equate with the absorption of its different digestion products.

Digestibility measurements: *In vitro* digestibility usually refers to the measured rate and extent of hydrolysis (hydrolysis rate and degree) of a specified macronutrient at a particular location and time within the simulated GIT. Digestibility may also be measured as the rate and extent of one or more absorbable compounds produced by hydrolysis. When a reaction yields intermediate compounds, then the type of reaction and the intermediate products should be identified and ideally quantified. *In vivo* digestibility has been defined as the level of nutrient disappearance at a specific gastrointestinal site, which may be the ileum, caecum, colon, or feces (Schaafsma, 2000). Note that nutrient disappearance measurements have to be corrected for endogenous nutrient flow, particularly when investigating proteins.

Bioaccessibility: The bioaccessibility is defined as the fraction of an ingested compound that is biochemically and/or physically released from the food matrix and solubilized within the gastrointestinal fluids in an absorbable form. These three processes (biochemical reaction, release, solubilization) may occur in any order or simultaneously, bioaccessibility being generally defined as the endpoint of the combined luminal processes.

Bioaccessibility measurements: This type of experiment involves measuring the fraction of an absorbable food compound that is released from the food matrix and solubilized within the aqueous or the micellar phase of a specific GIT site. Typically, this involves centrifuging (and sometimes filtering) the digested sample and then measuring the concentration of the absorbable food compound in the aqueous supernatant, as well as its concentration in the total digested sample, considering all food compounds containing the absorbable derivative. The bioaccessibility is then defined as the percent ratio of these concentrations. In the supernatant, polar compounds may be directly soluble in the water, whereas non-polar ones may be solubilized within the interiors of mixed micelles dispersed in the water. The *in vitro* bioaccessibility is usually different from the *in vitro* digestibility. First, the *in vitro* digestibility is measured in the total digested sample. Then, a food compound may be digested by enzymes, but the reaction products may not be solubilized within the oro-GI fluids and therefore not be considered bioaccessible.

Historically, the term bioaccessibility has not been used to describe the solubilization of absorbable compounds in the mouth or stomach fluids, but only in the small intestine fluids, as it was originally developed as the intestinal lumen counterpart to blood bioavailability (Ruby et al., 1993). However, this definition might be restricting, as it is known that a few compounds can be absorbed from the mouth or the stomach (*e.g.,* medium-chain fatty acids, ethyl alcohol, and some minerals). In these specific cases, this term could therefore be used more broadly, provided the region where it is applied is specified, *i.e.,* oral or gastric bioaccessibility. In any case, bioaccessibility is rarely used to refer to the physical accessibility of enzymes to their substrates, as included in the definitions of Grundy et al. (2024). Using bioaccessibility in this sense would introduce confusion between different processes. We recommend using *enzyme accessibility* for this phenomenon, which is an existing term that is widely used in the literature, especially in the context of starch hydrolysis (He, Liu, & Zhang, 2008).

3.2. Cellular and ex vivo experiments

Several models can be used to evaluate the absorption of bioaccessible compounds: cellular models such as intestinal cultured cells, or *ex vivo* models such as intestine fragments. These models are increasingly being combined with *in vitro* digestion models to provide a more realistic simulation of the gastrointestinal fate of nutrients, including their digestion and absorption (Costa, de Carvalho, de Oliveira, & Madureira, 2024; Kondrashina et al., 2024; Perruchot et al., 2024; Xin, Zhao, Tian, & Li, 2023).

One of the most widely used cellular models is Caco-2 cells. These human cells derive from a colorectal adenocarcinoma and have the ability to spontaneously differentiate into enterocyte-like cells upon confluence (Hevia et al., 2023). As Caco-2 cells do not secrete mucus at their apical surface, they can be used in combination with mucus-secreting HT29-MTX cells to create a more realistic model of the mucus-coated epithelium cells. Cells can be either cultivated on plates or grown on filters using cell culture inserts. The advantage of using filters is that it enables the researcher to have access to the apical chamber that represents the intestinal lumen, as well as the basolateral chamber that represents the blood compartment. It is thus possible to evaluate the uptake, metabolism, and absorption of food compounds or their derivatives through the epithelium cells. The cellular uptake of a food compound represents the amount of the compound that crosses the brush border, and is thus found in the cell, where it undergoes cellular metabolism. The cellular absorption of a food compound or its derivative is typically taken to be the fraction found in the basolateral chamber (relative to the original amount in the apical chamber). Information about the nature of the transport mechanism of a compound can also be obtained using cell culture models, such as paracellular/transcellular or active/facilitated/passive uptake mechanisms. The absorbed molecules may be metabolized within the cells (*e.g.,* re-esterified for fatty acids, cholesterol, vitamin A; sulfated or glucuronidated for phenolic compound), and then be secreted into the basolateral chamber in a free form, associated to proteins, or packed into chylomicron-like particles for fat-soluble compounds.

Regarding *ex vivo* models, intestinal fragments from animal or human origins can be used intact to evaluate the uptake of food compounds or of their derivatives (example in Reboul et al., 2011). In this case, the amounts of a food compound and/or its metabolites are measured in the intestinal tissue after a short incubation period. It is also possible to use Using chamber devices to measure the transport of a given compound through intestine fragments, which reflects the absorption process (example in Margier et al., 2019).

Note that *in vitro* bioavailability is sometimes used to refer to the amounts of derivatives recovered at the basolateral side of cell culture models. This usage is not recommended as this measurement is actually very different from *in vivo* bioavailability, as defined in the next section. We recommend using the term *in vitro* absorption instead.

3.3. *In vivo experiments*

In vivo absorption refers to the passage of a compound from the oro-GIT to the blood or lymph. The term bioavailability refers to the amount of a food compound or its derivatives that reaches the target cells, tissues, or organs in the body, as the endpoint of food ingestion, accounting for distribution, metabolism, and excretion processes (Section 2). In addition to gastrointestinal biochemical and metabolic reactions, a food compound may undergo numerous other chemical modifications after distribution in the body, which means that the final compound at the target site may be very different from the original compound in the ingested food. Quantifying the true bioavailability of a food compound would thus require measuring related metabolites from sampled blood, target tissues, and excreted materials (urine, feces), which is rarely done, except in basic animal research. In practice, the bioavailability is usually taken to be the amount of a specific bioactive metabolite from a food compound that is present in the blood following ingestion (*i.e.,* during the postprandial period). As this is not an absolute measurement of the amount distributed throughout the general circulation, this is considered as a “relative bioavailability”.

Bioavailability measurements: The “relative bioavailability” is often measured as the area under the curve (AUC) of the blood concentration of a specific metabolite from a food compound after ingestion. This curve represents a balance between the amount of absorbed metabolite appearing in the blood and the amount of metabolite disappearing from the blood due to peripheral tissue uptake, metabolism, or excretion processes. The AUC represents the total systemic circulation exposure to the food compound metabolite for a given postprandial period.

4. Application to specific nutrients

The terminology defined in Section 3 is meant to be applicable to most foods and nutrients, yet some terms are not used consistently within the food digestion literature. In this section, we describe the use of these terms for several nutrients in the context of *in vitro* digestion. Table 2 summarizes the main elements discussed here.

4.1. *Lipids*

Most lipids are insoluble in water and may be present in foods in different forms, such as bulk lipids (as in butter or spreads), emulsified lipids (as in milk and dressings), cellular lipids (as adipose tissue in animals or oil bodies in plants), or dispersed lipids (as in baked goods). In the mouth and stomach, lipids are usually released from the disintegrating food matrix and form small oil droplets in the luminal fluids (Marze, 2022). Lipolysis of these lipid droplets in the stomach and small intestine is required to yield free fatty acids (FFA) and other lipolysis products such as monoglycerides (MG) or lysophospholipids (lysoPL). These amphiphilic digestion products then combine with bile salts in the small intestine to form colloidal structures known as mixed micelles, which include micelles, vesicles, and other lamellar structures (El Aoud et al., 2021). For triglycerides, lipid bioaccessibility is defined as the fraction of lipolysis products (FFAs and MGs) solubilized within these mixed micelles. This is typically determined by measuring the concentration of lipid digestion products within the aqueous micellar phase, relative to the initial concentration esterified within the triglycerides (Okuro et al., 2023). Note that some long-chain saturated fatty acids are prone to soap formation in the presence of divalent ions (such as Ca^{2+}), making them insoluble in the aqueous micellar phase and prone to precipitation, thereby reducing their bioaccessibility.

4.2. *Proteins*

Proteins are polymers of amino acids linked together by covalent peptide bonds that need to be hydrolyzed by proteases to yield absorbable molecules, *i.e.*, free amino acids or di−/tri- peptides (Eastwood, 2003). The molecular structure and aggregation state of proteins within the GIT influences the binding of proteases to their specific cleavage sites, thereby altering their digestibility. The acidic pH of the gastric fluids often promotes partial protein denaturation, which can cause polypeptide conformational changes that facilitate access to digestive enzymes. Proteolysis begins in the stomach due to the presence of pepsin, yielding large polypeptides. Pancreatic proteases (including trypsin and chymotrypsin) then complete the hydrolysis of the polypeptides and proteins in the small intestine, producing small peptides and amino acids. Peptidases secreted by the intestinal epithelial cells and released within vesicles at the brush border membrane terminate the proteolysis process. The brush border peptidases are rarely included in *in vitro* digestion experiments, due to their lack of commercial availability. As a consequence, the degree of protein hydrolysis (corresponding to the proportion of peptide bonds cleaved) measured using *in vitro* digestion protocols is typically lower than what would be found *in vivo* or if brush border peptidases were included (Picariello et al., 2015). This leads to an underestimation of the digestibility and bioaccessibility of amino acids. In order to overcome this limitation, it is possible to fractionate the digesta and analyze the fraction below a certain molecular weight, ranging from 1 to 10 kDa, depending on the technique used and the preferences of the researchers, so as to estimate the bioaccessible fraction. For example, Sousa et al. (2023) have proposed a methodology based on the precipitation of the digesta with methanol to separate the bioaccessible supernatant from the non-bioaccessible pellet. Other types of digesta fractionation are also possible, such as acid precipitation (Duijsens et al., 2023), membrane filtration (Ariëns et al., 2021), or size exclusion chromatography (Le Roux et al., 2020; Rieder et al., 2021). The bioaccessible fraction is then analyzed for total nitrogen and/or total amino acids and thus gives an estimate of *in vivo* digestibility, as defined for protein in animal and human nutrition (section 3.1).

4.3. *Starches and sugars*

Starch is a polysaccharide that is primarily used to store energy in plants, and is mainly found in specific organs, such as grains and seeds (*e.g.,* in legumes, nuts, rice, wheat, barley, and oat), and roots (*e.g.,* in

Table 2
Description of the elementary processes occurring to specific food compounds in the oro-gastrointestinal lumen.

Elementary processes / Parent compound	Release (all compounds may require food matrix disintegration)	Solubilization	Hydrolysis	Other luminal biochemical/metabolic reactions (oxidation, fermentation)
Triglycerides	Require lipolysis	Micellar	Monoglycerides, free fatty acids	Peroxidation
Phospholipids	May require lipolysis	Micellar	Lysophospholipids, free fatty acids	Peroxidation
Fat-soluble vitamins	May require lipid hydrolysis	Micellar and binding proteins	De-esterified form of vitamins A and E	Oxidation, possible acid-catalyzed degradation in the stomach
Proteins	No specific requirement	Aqueous	Peptides, amino acids	Fermentation of amino acids
Sugars and starches	No specific requirement	Aqueous	Maltose, maltotriose, α-limit dextrins	Fermentation of resistant starches
Polysaccharides	May require cell wall lysis	Aqueous	None	Fermentation of dietary fibers
Water-soluble vitamins	May require protein hydrolysis	Aqueous and binding proteins	De-esterified form of vitamin C Free form of vitamins B1, B2, and B5 from coenzymes	Oxidation, possible acid-catalyzed degradation in the stomach

potato or yam) (Zeeman, Kossmann, & Smith, 2010). In nature, starches are usually present as small dense granules that have a semi-crystalline structure. Chemically, starches consist of amylose (linear) and amylopectin (highly branched) molecules composed of chains of glucose molecules held together by glycosidic linkages. The starch granules from different sources vary in their sizes, morphologies, and amylose-to-amylopectin ratios, as well as the molecular characteristics of the amylose and amylopectin molecules (Butterworth, Bajka, Edwards, Warren, & Ellis, 2022). When heated in the presence of water, starch granules absorb water, swell, and some of the starch molecules leach out into the surrounding phase. As a consequence, the granules lose their well-organized structure (Schirmer, Jekle, & Becker, 2015), which is known as gelatinization. In general, gelatinized starch is more easily digested than native starch granules. When gelatinized starch is cooled, the starch molecules undergo a coil-to-helix transition, which leads to the formation of crosslinks between the helical regions held together by hydrogen bonds, which is known as retrogradation. In contrast to gelatinization, retrogradation usually decreases the digestibility of starch.

During digestion within the GIT, starch is hydrolyzed by salivary and pancreatic amylases, leading to the production of maltose, maltotriose, and limit dextrins, which are disaccharides, trisaccharides, and oligosaccharides, respectively. These saccharides are water-soluble and can be partially absorbed from the small intestine, thereby constituting the bioaccessible fraction of starch. Most starches require gelatinization before they can be efficiently hydrolyzed by amylases, as this process breaks down the dense crystalline structures within the starch granules, thereby allowing amylases to more easily access the glycosidic bonds (Butterworth et al., 2022). In plant tissue, intact cell walls may inhibit gelatinization of starch granules due to the lack of space to expand (Edwards, Ryden, Mandalari, Butterworth, & Ellis, 2021). Therefore, the rate and extent of starch hydrolysis also depend on the intactness of the cell walls, which is plant/grain/tissue/processing dependent (Butterworth et al., 2022; Wang et al., 2023). Starch granules can also be embedded in a protein network that may also hinder starch hydrolysis by restricting access of the amylases (Wang et al., 2023). Any starch that escapes hydrolysis in the upper GIT and reaches the colon is referred to as resistant starch and corresponds to the non-digestible fraction (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Alvarez, 2010). Nevertheless, this fraction may be fermented by gut microbes and produce SCFAs. As with peptides, the enzymes responsible for the final hydrolysis of oligosaccharides into glucose are not present within the GIT lumen but are located at the enterocyte brush border membrane. Most *in vitro* digestion models currently employed do not contain these brush border enzymes. Consequently, di- or tri- saccharides are usually considered to be the final digestion products of starch digestion in these models.

Sugars are small water-soluble molecules, which are typically either monosaccharides (like glucose or fructose) or disaccharides (like sucrose, maltose, or lactose) (Brady, 2013). These nutrients are important because they contribute to the desirable physicochemical and sensory attributes of foods, but there is concern that overconsumption of some sugars can have adverse health effects. In particular, high levels of glucose in the bloodstream after consumption of some foods have been linked to diabetes and other metabolic disorders. Consequently, it is important to understand and control the release of sugars from food matrices. There has been interest in the use of *in vitro* digestion methods to understand the release of various compounds (vitamins, phenolic compounds, sugars) from fruits and vegetables, as these are considered to be healthy foods (Carbonell-Capella, Buniowska, Barba, Esteve, & Frígola, 2014). These studies have shown that sugars can rapidly diffuse out of plant cells and into the oro-GI fluids due to their small size and high solubility in water. In general, sugars tend to have a relatively high bioaccessibility even in intact plant-based foods such as blackberries (Dou, Chen, Huang, & Fu, 2022). It therefore demonstrates that the cell walls of fruits and vegetables do not hinder the release and digestibility of sugars. This is even more true for free sugars present in processed foods such as beverages, bakery products, confectionery, chocolate, dairy products, and breakfast cereals, where cell walls are likely to be highly damaged during the food manufacturing process (Choi, Kim, Park, Kim, & Shim, 2011; Rauber et al., 2019).

4.4. *Dietary fibers*

Many of the polysaccharides founds in plants are not digestible or absorbable within the upper GIT and are therefore considered to be dietary fibers (McDougall, Morrison, Stewart, & Hillman, 1996). Indeed, the official definition of dietary fibers given by the Codex Alimentarius (2010) is carbohydrate-based plant materials that are neither digested nor absorbed in the upper GIT, *i.e.,* polysaccharides, non-digestible oligosaccharides, and resistant starches. However, lignin, a polymer comprised of phenolic compounds is also classified as a dietary fiber, even though it is not a carbohydrate. Many dietary fibers are found in the cell walls of terrestrial plants (such as cellulose, hemicellulose, and pectin), but may also be found in seaweeds (carrageenan and alginate), in specialized organelles of seeds (*e.g.,* guar gum or locust bean gum), or be secreted by microbes (such as gellan gum and xanthan gum) (Phillips & Williams, 2020).

The presence of dietary fibers in the GIT may inhibit the activity of mammalian enzymes, thereby hindering the hydrolysis of macronutrients. They may do this through various physicochemical mechanisms, including binding of gastrointestinal constituents, encapsulation or coating of macronutrients, and thickening or gelation of gastrointestinal fluids (McClements, 2021). In fruits and vegetables, the rate and extent of macronutrient hydrolysis may be inhibited by the presence of cell walls, especially intact ones, which restrict the ability of the digestive enzymes to access their substrates (Edwards et al., 2021; Grundy, Tang, van Milgen, & Renaudeau, 2022). In this case, it may be important for the cell walls to be physically or chemically disrupted in the mouth, stomach, and/or small intestine before the macronutrients can be released and digested.

It should be noted that dietary fibers can be fermented into oligosaccharides and monosaccharides (fructose and rhamnose) by the gut microbiota in the colon, thereby making their cell walls more permeable. However, these saccharides are intermediate products of gut fermentation, which may be used as substrates to produce SCFAs *via* different metabolic pathways (Guo et al., 2021).

4.5. *Vitamins*

Vitamins are low molecular-weight organic compounds that are critical for human health and wellbeing, but which cannot be synthesized by the human body (at least not in sufficient quantities) (Combs & McClung, 2022). Typically, they are ingested in relatively small levels from foods ($<<$ 1 g/day) and are not therefore considered to be a significant source of energy from the diet. Physiologically, vitamins are essential for the growth, reproduction, and proper functioning of the human body. They must therefore be supplied by the diet. Vitamins may be classified as fat-soluble (vitamins A, D, E and K) or water-soluble (vitamins B and C), depending on their solubility characteristics. Each vitamin has unique biological activities. Typically, water-soluble vitamins have a relatively high bioaccessibility because they readily solubilize within the oro-GI fluids. In contrast, fat-soluble vitamins have to be released from the food matrix and solubilized within intestinal mixed micelles to become bioaccessible before they can be absorbed (Reboul, 2023; Tan & McClements, 2021).

Fat-soluble vitamins reside inside oil droplets within the GIT. The triglycerides in these oil droplets are hydrolyzed by gastric and pancreatic lipases in the stomach and small intestine, while other lipids such as phospholipids are hydrolyzed by various pancreatic lipases. Lipid digestion products combine with bile salts to form mixed micelles, promoting the transfer of vitamins from oil droplets to micelles (Tan & McClements, 2021). Gastric lipase does not hydrolyze vitamin esters. The hydrolysis of such compounds, mainly vitamin A and E esters, begins in the duodenum, the intestinal site of biliary and pancreatic secretions. The pancreatic juice contains several enzymes that can carry out this hydrolysis, including cholesterol ester hydrolase (CEH), pancreatic lipase, and pancreatic lipase-related protein 2 (PLRP2). Under the action of these lipases, all lipids contained within the lipid droplets are progressively hydrolyzed, and the lipid digestion products are incorporated into mixed micelles. These colloidal particles (micelles and vesicles) then transport the lipid digestion products and fat-soluble vitamins to the enterocytes for absorption (Reboul, 2023). Fat-soluble vitamins are also known to be transported by proteins. Indeed, vitamins A and D can bind to milk proteins (such as β-lactoglobulin or caseins) or saliva proteins (such as retinol or vitamin D binding proteins) (Reboul, 2023). In the enterocyte glycocalyx, micelles are isolated from the rest of the intestinal contents in the unstirred water layer. Micellar contents including fat-soluble vitamins can then be transported by various more or less specific carriers for absorption by the enterocyte at low vitamin concentration or absorbed by passive diffusion at high vitamin concentration (Reboul, 2023).

The bioaccessibility of water-soluble vitamins also depends on several GIT processes (Yaman et al., 2021). They first need to be physically released from the food matrix, especially vitamins C, B7, B9, and B12 which may bind to food proteins. Vitamin C may also be found in esterified forms, and vitamins B1, B2, and B5 may be found in coenzyme forms in foods, requiring hydrolysis in the GIT lumen. As with fat-soluble vitamins, they are absorbed by facilitated diffusion (carrier-mediated) at low vitamin concentration and by passive diffusion at high vitamin concentration (Said, 2011). The main difference with fat-soluble vitamins is that they are readily soluble in oro-GI fluids and thus do not require micellar solubilization, instead relying on binding proteins to enhance luminal transport (Said, 2011; Yaman et al., 2021). A very specific case is that of vitamin B12 which requires binding to haptocorrin secreted by salivary glands, and to gastric intrinsic factor secreted by parietal cells of the stomach, so that it is protected from gastric acidic degradation before intestinal absorption (Yaman et al., 2021).

4.6. *Other bioactive compounds*

As well as macronutrients and micronutrients, other types of bioactive compounds found in foods can have health benefits, including nutraceuticals, prebiotics, and probiotics.

Nutraceuticals: Nutraceuticals are bioactive molecules found naturally in foods or intentionally added to foods that have beneficial health effects (Nicolescu et al., 2023). Nutraceuticals may be hydrophobic or hydrophilic molecules that are soluble in oil, water, or neither, depending on their molecular characteristics. In this case bioaccessibility is an appropriate term, as it is related to the fraction of the nutraceutical that is released from the original food matrix and solubilized within the aqueous intestinal fluids. Polar nutraceuticals may be directly solubilized in the water, whereas non-polar ones are solubilized within the hydrophobic interiors of the mixed micelles dispersed throughout the intestinal water phase. Examples of nutraceuticals include polyphenols and carotenoids. The bioaccessibility and bioavailability of these compounds is often relatively low, which may be due to different reasons. For instance, carotenoids tend to have a very low water solubility, which greatly reduces their bioaccessibility (Eroglu, Al'Abri, Kopec, Crook, & Bohn, 2023). In contrast, polyphenols tend to be rapidly metabolized within the GIT, thereby reducing the amount available for absorption (Kawabata, Yoshioka, & Terao, 2019; Ozdal et al., 2016). These effects should therefore be accounted for when investigating nutraceutical bioaccessibility.

Prebiotics: Prebiotics are usually indigestible oligosaccharides that will pass through the upper GIT and be fermented in the lower small intestine and colon (Gibson et al., 2017). In this case, the term bioaccessibility is not used, since these molecules are not directly absorbed. Instead, the term fermentability should be used to describe the fraction of the prebiotics that reach the colon in a form that can be fermented by the colonic microbiota and the subsequent metabolites utilized by the body.

Probiotics: Probiotics are living microorganisms that may have beneficial effects on the colonic microbiota, provided they can reach the colon in a viable form (Latif et al., 2023). In this case, the term bioaccessibility is inappropriate, since these cells are not directly absorbed by the human body. Instead, the term viability should be used to describe the number of probiotic microbes that survive in different regions of the GIT.

Postbiotics: Postbiotics are generally defined as inactivated (*i.e.* dead) probiotics. Recent studies have shown that some inactivated probiotics have a similar health impact to live probiotics (Vinderola, Sanders, & Salminen, 2022). In such a case, the molecular composition of the dead microbe rather than the viability and metabolism of the microbe imparts the health benefit, however the mechanisms are not yet clear. Therefore, for bioaccessibility purposes two separate processes could be considered. Firstly the physical release of nutrients or micronutrients from the inactivated microbe (*e.g.*, vitamins, short chain fatty acids) (Zhao et al., 2024) and secondly, as for prebiotics, the metabolites of fermentation by the gut microbiota of residual materials (*e.g.*, cell wall materials, exopolysaccharides) reaching the colon.

5. Conclusion

Bioaccessibility is a term used in several scientific fields related to the digestion and absorption of nutrients and other bioactive compounds that has not been used consistently. In this article, our goal was to propose a standardized workable definition of bioaccessibility that accounts for the various processes occurring during the gastrointestinal passage of foods, including release from the food matrix, solubilization in oro-GI fluids, and biochemical/metabolic reactions (such as enzymatic hydrolysis or fermentation). Following this work, we propose the following definition of bioaccessibility: the fraction of an ingested compound that is biochemically and/or physically released from the food matrix and solubilized within the gastrointestinal fluids in an absorbable form.

Nevertheless, there are still several questions remaining about the generality of this definition. For instance, some vitamins require transport by binding proteins in the gastrointestinal lumen. Should only the bound form of the compound therefore be considered bioaccessible? Another question is how to define the bioaccessibility of food polymers that are partially hydrolyzed to form multiple oligomers (such as polysaccharides or proteins), or food compounds that undergo major gut microbiota metabolic reactions to yield absorbable derivatives (such as polyphenols, dietary fibers, and some carotenoids). As more studies that couple *in vitro* food digestion with cellular uptake or microbial fermentation are conducted, some of these questions will likely be answered in the near future.

CRediT authorship contribution statement

Myriam M.-L.Grundy: Writing – review & editing, Writing – original draft. **Amélie Deglaire:** Writing – review & editing, Writing – original draft. **Steven Le Feunteun:** Writing – review & editing, Writing – original draft. **Emmanuelle Reboul:** Writing – review & editing, Writing – original draft. **Paul J. Moughan:** Writing – review & editing. **Pete J. Wilde:** Writing – review & editing. **David Julian McClements:** Writing – review & editing, Writing – original draft. **Sébastien Marze:** Writing – review & editing, Writing – original draft, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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