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In vitro bioaccessibility assessment as a prediction tool of nutritional efficiency

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Abstract

The term "bioaccessibility" is a key concept to ascertain nutritional efficiency of food and food formula developed with the aim of improving human health. In this review, working definitions of bioavailability, bioaccessibility, and bioactivity are examined, taking into account the complete sequence of events that take place during the digestive transformation of food into material that can be assimilated by the body, the absorption/assimilation into the cells of the intestinal epithelium, the presystemic metabolism, and, lastly, the development of biologic actions. Comparison among in vivo and in vitro techniques to assess bioaccessibility is accomplished, considering the strengths and limitations of each experimental approach, with a complete description of in vitro procedures applied to determine bioaccessibility of carotenoids. Although a great development has been achieved on the in vitro approaches, these are especially intended for initial screening and should be complemented with in vivo studies, which will remain as the criterion standard for bioaccessibility of nutrients and bioactive compounds at specific target populations. Application of bioaccessibility assessment in foods claiming a health benefit because of their nutrients or bioactive compounds content is described. Measurement of bioaccessibility provides valuable information to select the appropriate dosage and source of food matrices to ensure nutritional efficacy of food products. In addition, in vitro bioactivity measurements to support health benefits of bioactive compounds should be accomplished with estimation of their bioaccessibility, to adequately give nutritional significance to health claims.

Keywords: Bioaccessibility; Bioavailability; Food processing; Fortified food; Nutrient content claims; Nutritional efficiency
Abbreviations: EC, european community; RDI, recommended daily intake.

1. Introduction

New eating habits, diet recommendations from public health organizations, and the presence of bioactive compounds in food, all aimed at extending and improving our health, have given rise to new and considerable challenges for nutrition and food sciences and the food industry. Although natural food is considered to be functional food, it is standard practice to extract bioactive compounds from appropriate sources, thereby making them more pure and more concentrated, and then adding them to a consumable food matrix. This simple processing sequence requires integration of diverse aspects, such as identifying the bioactive compound, performing a toxicology assessment, using extraction and separation techniques, and making stability and bioaccessibility measurements, which must be considered, evaluated, and brought together to create the product [1].

Not all of the aforementioned aspects receive the same amount of attention in functional food design. The essential aspects are toxicology assessment, to ensure that the new product does not pose any health risks, and the choice of technology, to optimize resources and minimize the physicochemical impact on the compound/food [2,3]. Bioaccessibility has not been a priority goal at the initial

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development of functional foods for 2 reasons. First, there were experimental models for obtaining data on bioavailability providing an overall picture of the effectiveness of the process, but these models did not allow us to distinguish effectiveness from bioaccessibility and assimilation. Moreover, different experimental conditions have been applied to establish bioaccessibility efficiency, even to the same group of nutrients or bioactive compounds. Second, there is no consensus in US/European legislation on functional foods to take into account the importance of this parameter. The initial lack of defined experimental models for studying bioaccessibility has not prevented the acquisition of relevant information on the bioavailability of essential nutrients and bioactive compounds. Rather, not all of the acquired information can be directly applied to the design of food and formulas because the control criterion in this case is the effectiveness of the bioaccessibility parameter. This parameter is directly influenced by the composition of the food matrix and by the synergies and antagonisms that may be established between the different components, permitting a potentially digested material to be available to the body.

The idea of bioavailability is foremost in all different definitions of functional food, such that when a claim is made (both in terms of nutritional content and of health properties), it must be shown that the nutrient or component that provides this benefit is, first, efficiently digested and assimilated and then once absorbed, performs a positive function in the body. Consequently, the concept of bioavailability includes bioaccessibility (Fig. 1). The scientific support of claims of what a food contains (nutritional content or comparison to other foods) is provided by bioaccessibility without the need of performing bioactivity studies.

For those bioactive compounds that perform their action in the stomach or gut, some but not all the events included in bioaccessibility should apply. This is the case of phytochemicals that interact with reactive oxygen species, developing an antioxidant action in the intestinal lumen [4,5], or are preabsorbed by gut microflora, which metabolizes phytochemicals leading to metabolites that once excreted by microflora could be already absorbed or develop an activity in the gut [6]. One example where only the term "bioactivity" applies is based on nondigestible polysaccharides and oligosaccharides, which produce several health benefits although they are not absorbed [7]. Substantiation of claims of health benefits of food is based on bioaccessibility and bioactivity measurements.

In this review, a working definition of the term "bioaccessibility" is outlined, describing some situations where application of the concept of bioaccessibility would be used, such as functional food design, addition and fortification of food formula with bioactive compounds, and the effect of food processing on nutritional quality.

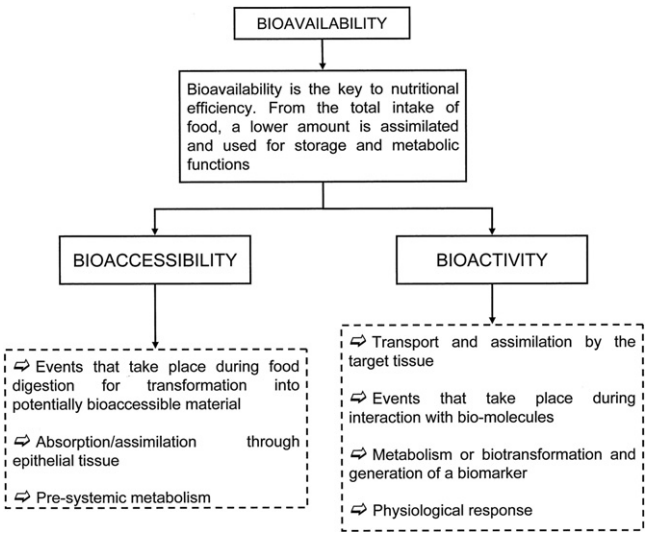

Fig. 1. Definition of bioavailability as a sum of bioaccessibility and bioactivity. Physiochemical events involved on each stage.

2. Bioavailability, bioaccessibility, and bioactivity

The term "bioavailability" has several working definitions, depending on the research area it applies to. From the nutritional point of view, bioavailability refers to the fraction of the nutrient or bioactive compound ingested that is available for use in physiologic functions or to be stored [8]. Benito and Miller [9] define bioavailability as the proportion of a given nutrient in a given food or diet that the body can actually use. Bioavailability is a key concept for nutritional effectiveness [10], irrespective of the type of food being considered (functional or not). Only certain amounts of all the nutrients and bioactive components in food will be used effectively by the organism. From a pharmacologic point of view, the Food and Drug Administration defines bioavailability as the rate and extent to which the therapeutic moiety is absorbed and becomes available at the site of drug action. The term "bioavailability" includes availability for absorption, absorption, metabolism, tissue distribution, and bioactivity. However, there are practical and ethical difficulties in the measurement of delivery and bioactivity of food/drug components on specific organ sites of biologic activity, so that the term "bioavailability" is usually defined as the fraction of an oral dose of a parent compound or active metabolite from a particular preparation that reaches the systemic circulation [11], a definition in which bioactivity is skipped, and more related to the term "bioaccessibility" (see definition below). Although bioavailability and bioaccessibility are often used indistinctly, it is important to note that bioavailability includes bioactivity.

Bioaccessibility has been defined as the fraction of a compound that is released from its matrix in the gastrointestinal tract and thus becomes available for intestinal absorption (ie, enters the blood stream) [9]. Bioaccessibility includes the entire sequence of events that take place during the digestive transformation of food into material that can be

assimilated by the body, the absorption/assimilation into the cells of the intestinal epithelium, and lastly, the presystemic metabolism (both intestinal and hepatic). Bioaccessibility analyses can be approached using general experimental techniques (there are systematic techniques common to all types of foods) that can be adapted to all types of claims regarding nutritional content.

The concept of bioactivity includes events linked to how the bioactive compound is transported and reaches the target tissue, how it interacts with biomolecules, the metabolism or biotransformation that it may undergo, and the generation of a biomarker and the physiologic response it causes (Fig. 1). Bioactivity measurements are mainly based on the events that take place during the time the bioactive component interacts with biomolecules. This interaction gives rise to a metabolite, a signal, or a response that will continue to modulate and amplify until the systemic physiologic response is produced, that is, the health benefit. Claims of what a food can do (healthy properties or reduced risk of disease) are based on bioactivity studies. There are in vivo, ex vivo, and in vitro experimental models used to study bioactivity that involves individual and specific approaches for each health benefit claim. The experimental procedures used to measure bioactivity are very specific and need to be adjusted to every health benefit claim, and therefore, there is no common system due to the definition of the concept of bioactivity itself and to the environment in which it takes place [12]. Research on biologic actions of food components has become a real specialization and, as mentioned earlier, one of the challenges of functional food. This challenge can also be posed in reverse, that is, starting from an effect or specific activity and establishing a method to calculate it, to look for a (bioactive) compound that adequately modulates the said effect or activity. The discovery of bioactive components, either in pure form or present in plant extracts, constitutes a golden opportunity for the food (or pharmaceutical) industry because these components could be used in a food on an exclusive basis if their use is suitably protected by means of a patent [13]. Thus, in vitro methods have been developed to determine bioactivity, including the screening of diverse activities (antioxidant, anti-inflammatory, antitumor, regulating xenobiotic enzymes, etc). This provides a real test bench that, together with the development of robotics and high-performance bioinformatics tools, has given an enormous boost to the practical and economic potential of bioactivity measurements.

3. Methodologies applied to measure bioaccessibility of nutrients and bioactive compounds

Different analytical approaches can be applied to measure bioaccessibility of nutrients and bioactive compounds: in vivo and in vitro studies both present strengths and drawbacks, which are summarized in Table 1. Within in vivo studies, balance studies and tissue concentration are 2 strategies that allow determination of the absorbed amount of nutrients, bioactive compounds, or their metabolites. Balance studies determine the absorbed amount by measuring the difference between the fed and excreted amounts of the nutrient or bioactive compound. Tissue concentration consists of monitoring the increase in plasma/serum concentration of the nutrient or bioactive compound. These approaches have been applied either with experimental animal or human subjects to determine absorption of carbohydrates, minerals, vitamins, phytochemicals, and others. [9-22]. In vivo human studies are the criterion standard approach to determine bioaccessibility of food nutrients or bioactive compounds, although some experimental approaches are ethically and technically unaffordable. Digestion and absorption involve several different steps, and each one could cause an effect on the nutrient or bioactive compound so that a detailed picture is not obtained with the balance and bioassay studies.

Table 1
Strengths and drawbacks of in vivo and in vitro approaches to assess bioaccessibility of nutrients and bioactive compounds

Strengths	Drawbacks
In vivo	
In vivo situation	Lower throughput
Allow enough sampling to perform pharmacokinetic studies	Overall contribution of factors involved on bioaccessibility
Selection of individuals for specific target population of intended use	Doubts of relevance for human situation when animal model is used
	Lack of certified reference standards to compare data among studies/laboratories
	Cost and ethical considerations
In vitro	
High-throughput screening tools	Validation against in vivo bioavailability data
Provide information about efficiency of each digestion/absorption step and transport mechanisms	Dynamic environment of intestine is not fully reproduced with biochemical and cell culture models
Validation and standardization with reference material	Effect of intestinal microflora and hepatic metabolism is not considered
Cost, automatization, and miniaturization	

In vitro studies have been developed to simulate the physiologic conditions and the sequence of events that occur during digestion in the human gastrointestinal tract. In a first step, an in vitro gastrointestinal method is applied to the food, mirroring the physiochemical conditions that take place during human digestion, considering the 3 areas of the human digestive system (mouth, stomach, and intestine). The main features of the in vitro gastrointestinal methods are temperature, shaking or agitation, and the chemical and enzymatic composition of saliva, gastric juice, duodenal juice, and bile juice [23]. When physical processes that occur in vivo are not reproduced (shear, mixing, hydration, changes in conditions over time, peristalsis), the in vitro gastrointestinal model is defined as a static or biochemical

model. The dynamic models mimic the in vivo physical processes so that they take into account new variables, such as changes on viscosity of the digesta, particle size reduction, diffusion, and partitioning of nutrients. Several examples of in vitro gastrointestinal static and dynamic models have been described [24-28]. During the application of the in vitro

Table 2
Experimental conditions of different in vitro digestion models applied to assess digestion efficiency of carotenoids from fruits and vegetables, test meals, and oils

Sample matrix	Composition of digestive fluids	In vitro digestion conditions	Aim of the study	Reference
Baby food, containing vegetables and chicken	Gastric juice: porcine pepsin (40 mg/mL in 0.1 mol/L HCl) Intestinal juice: mixture of 2 mg/mL pancreatin and 12 mg/mL bile extract in 100 mmol/L sodium bicarbonate solution	Gastric phase: sample was acidified to pH 2, and 2 mL of gastric juice was added. The homogenate is incubated at 37°C in a water bath shaking at 95 rpm for 1 h. Intestinal phase: pH was adjusted to 5.3, and 9 mL of intestinal juice was added and incubated at pH 7.5 in a shaking water bath (95 rpm) at 37°C for 2 h.	Carotenoid transfer to micelles isolated as described by Hernell et al [29] and subsequent measurement of in vitro assimilation efficiency with Caco-2 cell culture–based model	[26]
Fruits and vegetables homogenized with a blender (15-s intervals) to simulated mastication	Saliva solution: organic and inorganic components and α-amylase (145 mg)[a] Gastric juice: organic and inorganic solutions, mucin (1 g), bovine serum albumin (1 g), and pepsin (1 g) from porcine stomach Intestinal juice: duodenal juice, containing organic plus inorganic solutions and porcine pancreatin (3 g); bile solution, containing bovine bile, (0.6 g) human pancreatic lipase (1 U); enzyme cocktail of colipase (12.5 μg), cholesterol esterase (5 U), phospholipase A2 (50 μL) and taurocholate salts (19.9 mg)	Gastric phase: sample (10 g) was incubated in a shaking water bath (95 rpm, 37°C, 5 min) at pH 6.5 with 9 mL of saliva solution. Gastric juice (13.5 mL) was added and pH adjusted to 1.1 and the mixture incubated for 1 h. Intestinal phase: pH was adjusted to 7.8 and 25 mL duodenal juice, 9 mL of bile solution and enzyme cocktail were added, and the sample incubated for 2 h.	Stability, isomerization, hydrolysis, and transfer efficiency of carotenoids and tocopherols to aqueous phase, analyzed at the digesta after decantation (16 h) and centrifugation at 5000 rpm for 20 min	[38]
Raw and cooked carrots	Gastric juice: 0.5% pepsin solution and salts solution at physiologic concentrations[b] Intestinal juice: mixture of porcine pancreatin (4 g/L) and porcine bile salt (25 g/L) dissolved in a 0.1 mol/L $NaHCO_3$ solution stabilized with dl-α-tocopherol (1% wt/vol)	Gastric phase: 5 mL of gastric juice is added to sample at pH 2. Mixture was incubated in a water bath with orbital shaking (13-mm diameter, 250 rpm, 1 h) at 37 °C. Intestinal phase: pH was adjusted to 5, and 3 mL of intestinal juice was added. The pH was increased to 7.5 and the mixture incubated for 30 min.	Carotenoid accessibility measured at the digesta once it was centrifuged at 5000 g for 20 min	[39]
Papaya, loquat, paprika, and marigold oleoresins	Intestinal juice: phosphate buffer 0.1 mol/L, bile salts and sodium chloride/calcium chloride solution. Enzyme solution was a mixture of lipase in calcium chloride solution (5 mmol/L, 50-60 U/assay for lipase from porcine pancreas or 9 U/assay for lipase from human pancreas).	Intestinal phase: sample is preincubated for 30 min at 37°C with 10 mL of phosphate buffer. Bile salts (30 mg) and 250 μL of salts solution were added and the mixture incubated for different time intervals (1-4 h).	Efficiency of enzymatic hydrolysis of carotenoid esters analyzed at the digesta without further operations.	[40]
Green leafy vegetables	Gastric juice: 300 mg/mL pepsin solution in 0.2 mol/L HCl-KCl buffer Intestinal juice: solutions in 0.1 mol/L phosphate buffer of pancreatin (5 mg/mL), lipase (7 mg/mL), porcine bile extract (17.5 mg/mL); solution in 0.1 mol/L Tris-maleate buffer of α-amylase	Gastric phase: sample was incubated with 0.2 mL of gastric juice at pH 1.5, 40°C for 1 h. Intestinal phase: sample is incubated in successive steps at pH 7.5, 37 °C, 6 h with pancreatin solution (1 mL), lipase solution (2 mL), porcine bile extract (2 mL), and α-amylase solution (1 mL at pH 6.9, 37 °C for 16 h).	β-Carotene and lutein availability analyzed at the digesta once it was centrifuged at 3000 g for 15 min. This procedure included an in vitro colonic fermentation process to assess possible absorption at the colon.	[41]

[a] Organic and inorganic components of saliva solution and gastric and intestinal juices are described by Oomen et al [42].
[b] Physiologic concentration and composition of salts solution are described by Diem and Lentner [43].

gastrointestinal method, food nutrients or bioactive compounds can be monitored to determine whether they are affected by digestion conditions (pH, enzymes) or if interactions with other food components (fiber, sucrose polyester, fat replacers) take place, which could affect efficiency of digestion. The final processed material of the experimental procedure is a digesta or intestinal preparation. To analyze the lipophilic content that has been effectively incorporated to mixed micelles, the micellar fraction can be isolated from that processed material by the application of an ultracentrifugation protocol [29].

The second step consists of determining the amount of nutrient or bioactive compound that is assimilated from the digesta by the intestinal mucosa. At this step, different experimental options are available for that purpose, including in vivo methods (intestinal perfusion), ex vivo techniques, and cell culture–based models. Cell culture–based models were first described in the 1990s to test the passage of drugs through the intestinal mucosa [30]. A monolayer of cells is grown on a filter separating 2 stacked well plates. The upper well is filled with donor solution (containing a fraction of the digesta), and the bottom is filled with acceptor solution, where the amount of nutrient or bioactive compound is measured to determine the absorption efficiency. Different cell lines are available to mimic the intestinal mucosa with particular characteristics on expression level of transporters, suitability for transfection, time, and maintenance of culture. The Caco-2 cell model is often used with applications to the study of active transport of bile acids, vitamins, amino acids, minerals, and phytochemicals [26,31-34]. Cell culture–based models present some drawbacks and limitations, mainly from the analytical point of view. The applied analytical technique must be sensitive and avoid interference with the chemical content of the culture and transport media [35]. With the aim of improving the use of cell culture and to increase its application, some conditions are tested, such as reducing culture time, inclusion of pH gradients, and modification of the buffers and media used [35,36]. Despite the advantages of in vitro methods, the data obtained from this approach should be complemented with additional in vivo direct studies, especially to test the efficacy of the food formulation on the specific target population of intended use.

Carotenoids are a group of bioactive compounds that have the attracted interest of the food industry and academia because of their positive impact on human health. Mammals rely on diet to incorporate these compounds, which develop different biologic actions such as pro–vitamin A activity, antioxidant capacity, and enhancers of the immune system [37]. These biologic actions, their relevant role and potential positive impact on human health, and the interest of the food industry in adding these compounds to food products have promoted the attention on bioaccessibility/bioavailability of carotenoids. In vitro digestion procedures have been applied to carotenoid-rich meals or formulations with different experimental conditions as summarized in Table 2. Garret et al [26] developed a complete in vitro digestion procedure in combination with the Caco-2 cell-culture model to assess the bioaccessibility of carotenoids from meals. The effect of various factors on incorporation of carotenoids to mixed micelles was studied (impact of gastric phase, presence or absence of bile extract and pancreatin, and lipid composition). Any source of interaction at the micellization process will reduce efficiency of the digestion step and affects final bioaccessibility. Carotenoids as lipophilic compounds need to be incorporated to mixed micelles to reach epithelial cells so that the carotenoid amount incorporated to the micellar fraction gives an estimation of the efficiency of the digestion step, and it is usually expressed in terms of percentage with respect to the total initial carotenoid amount. The method of Garret et al [26] has been applied to assess bioaccessibility of different test meals and supplements in other studies [44,45]. Other procedures simply apply the in vitro digestion model and make use of the assimilation stage with a cell culture–based model. The experimental approach developed by Granado-Lorencio et al [38] is an optimization of the validated method of Oomen et al [42] and includes a complete in vitro digestion procedure (simulation of mastication and saliva solution, gastric and intestinal phases), with the addition of human pancreatic lipase and other specific enzymes (cholesterol esterase, phospholipase A2) to achieve more physiologic conditions. The method applied by Hedrén et al [39] applies an in vitro digestion method to estimate the maximum amount of carotenoids released from food matrix without isolation of micellar fraction and determination of cellular uptake. The latter studies and others [40,41,46,47] have been performed with the aim of establishing in detail the factors involved on the micellization process and its efficiency, including the effect of composition of the lipid environment and the activity of pancreatic lipases on hydrolysis of xanthophylls esters.

The strength of the in vitro protocols arises from their consistency of results obtained from in vivo studies. Thus, most of the carotenoid bioaccessibility values obtained through in vitro assessment and predictions of changes in bioaccessibility due to dietary factors or physiologic modifications, outlined from those procedures, are similar to the in vivo observations. However, it must be stressed that an in vitro versus in vivo validation process should be carried out to delineate the reliability of the in vitro models.

4. Foundations for application of bioaccessibility measurements through in vitro studies

In vitro studies constitute an analytical method that can be used to establish the importance and scope of various factors in the effectiveness of bioactive component bioaccessibility and to provide in-depth analysis of the influence of the composition of the food matrix on the digestive process. Measuring bioaccessibility is a key factor in the design of foods or formulas that claim a health benefit due to containing 1 or several bioactive compounds. It might be

assumed that adding fat-soluble bioactive compounds to formulas with higher fat content, such as oily extracts, would be the best way to optimize bioaccessibility. However, transformation of an essentially fatty food into material that can be assimilated by the intestinal epithelium involves the lipid content undergoing a series of dispersion and emulsion processes, which may not optimize the amount of bioactive compound made available to the organism. Formulas in which the lipid content is finely dispersed in a hydrophilic matrix could potentially increase the bioaccessibility effectiveness of these compounds, thus introducing new variables (the qualitative and quantitative composition of the emulsifying agents) into the formula design [47-49]. Thus, in vitro models provide analytic support for measuring the bioaccessibility of bioactive compounds and can be useful in the design of emulsifying agents, discriminating between ingredients that optimize bioaccessibility and those that minimize it.

Applying in vitro models serves to perfect the food design process, with the term "bioaccessibility" being not just an attribute of quality but also a necessity that forms part of the concept of functional food. When a food label includes a nutritional claim like "source of [names of vitamins] and/or [names of minerals]" or "high [names of vitamins] and/or [names of minerals] content" according to the appendix to Regulation EC (European Community) 1924/2006 [50] on nutrition and health claims, it is not sufficient to certify that the food contains the amount of the nutrient or bioactive component specified on the label; rather, it has to be shown that the nutrient of bioactive component is digested and effectively made available to the body. In other words, a bioaccessibility study must be performed. Thus, the aforementioned regulation states the following in its initial considerations (specifically, in section 15): "In order to ensure that the claims made are truthful, it is necessary that the substance that is the subject of the claim is present in the final product in quantities that are sufficient, or that the substance is absent or present in suitably reduced quantities, to produce the nutritional or physiological effect claimed. The substance should also be available to be used by the body. In addition, and where appropriate, a significant amount of the substance producing the claimed nutritional or physiological effect should be provided by a quantity of the food that can reasonably be expected to be consumed."

The meaning of the previous paragraph can be visualized with the aid of an example. Claims have been made regarding the phytochemicals present in tea and the health benefit they provide by strengthening the pool of antioxidants in our bodies [51]. These claims are based on several scientific studies that have shown that tea naturally contains compounds that help to protect us by reducing the risk of developing degenerative diseases [52,53]. These claims deal with the content (tea contains antioxidants); however, verifying the claims means not only certifying that tea contains those compounds (it is not a matter of compositional analysis) but rather whether the antioxidants in tea are effective for the proposed purpose. To certify these claims, as is the case with any other type of claim or statement, bioaccessibility is the concept that gives them scientific foundation, which is mentioned indirectly in section 15 of the abovementioned regulation.

The chemical constituents of food acquire their vital significance when they are ingested, absorbed, and metabolized in our bodies. The possible modifications a compound could undergo during digestion (gastric and intestinal), and the routes toward the liver and presystemic metabolism, known as the first-pass effect, should be taken into consideration when measuring the potential benefit of such a compound. Returning to the example of the antioxidant compounds in tea and their effect on human health, to make the nutritional and health claim in the advertisement, it is not enough to simply establish that tea contains these antioxidant compounds. Rather, it has to be shown that these compounds are ingested, digested, and effectively assimilated, passing the presystemic metabolism to reach the target tissue where they carry out their function, 2 stages that can be verified through a bioaccessibility study.

5. Involving bioaccessibility in the design of functional foods

Functional foods have made use of innovations in food technology. Indeed, they would not be successful without it, considering the properties that define a functional food (as long as it is not conceived as a natural food). However, it is necessary to begin considering that the design and application of technology to food development must rely on the concept of bioaccessibility and its measurement to complement design and technology and guarantee their effectiveness (ie, that the technological criteria do not lose sight of the end goal of a food product, namely, to provide nutrients that can be absorbed by the organism). Thus, 2 situations can be defined where the concept of bioaccessibility must be used: food design and technological application. Critical factors for the former are dosage and source of the food matrix, whereas the latter includes critical factors relating to the processing parameters. In all of the described cases, it must be considered that the positive modulation that a process may exert over bioaccessibility entails a revaluation of both the product and the technology as it manages to improve a relevant property of the food. When the effect is negative, there is sufficient information to modify, when possible, the food formula design or the control parameters of the process and to minimize the negative effect on bioaccessibility.

5.1. Food design: fortification programs and addition of bioactive compounds to food matrices

Fortification programs are developed to replace nutrients that are removed during food processing, to insert nutrients

into alternative foods, or to correct deficiencies in specific groups of the population. Food fortification has enabled consumers to have multiple foods at their disposal that can provide them with essential nutrients, without these foods necessarily being ones that have provided these nutrients in the past. This eliminates the restricted consumption of specific foods to avoid nutritional deficiencies, and the food supply chain becomes steered by other properties such as hedonism (sensory pleasure), convenience, and healthy attributes. However, fortification promoted by functional food design is not well articulated as critical factors are not assessed, such as the appropriate dosage of the fortified nutrient and the influence that the matrix has on how it is absorbed, both being factors linked to the concept of bioaccessibility [54]. The effectiveness of a fortified food is subsequently calculated without taking the factors mentioned in its design into consideration, often resorting to the use of excessive doses (better to have too much than not enough) or generalizing the effect of the matrix (if a matrix works for one case, why would it not work for all cases?).

Measuring bioaccessibility is necessary to ascertain whether the fortified amount is insufficient, sufficient, or excessive and whether the selected matrix is the most suitable. When the level is insufficient, the fortified food does not fulfill the objective it was designed for, requiring a redesign of the food and modification of the matrix to select one that is an ideal carrier, not only in terms of its sensory and convenience qualities but also so that the fortified component is absorbed effectively. When focusing on the bioaccessibility measurement of fortified nutrients in foods, this type of error is discovered. Recently, in the United States, it was discovered that wheat flour fortified with iron is not effective because the iron it contains is not bioaccessible [55]. Measuring bioaccessibility before implementation of a fortification program is therefore unavoidable. In other circumstances where the concept of bioaccessibility has played a part in the design of fortified food products, positive results have been achieved [56,57].

There are foods to which bioactive compounds (not nutrients) are added that are the source of the content claim. This is an appropriate strategy if the aim is to incorporate into the diet bioactive compounds that are found in unusual foods or foods without sensory properties that might promote consumption or if the bioactive compound is not present in the appropriate concentration. When the aim is for the bioactive compound to perform a function in a specific segment of the population, selective addition to the most ideal food matrices is also used. For all of these scenarios, the solution is to isolate the bioactive component and to add it to the matrix best suited to ensuring its bioaccessibility. As with fortification, dosage and the properties of the matrix are the key design factors in achieving an effective product that is characterized initially by means of bioaccessibility measurement [58].

However, unlike fortification, the dosage of most bioactive compounds is not regulated by a set of daily dosage intake. The dose is dictated by what is required to obtain the health claim of the functional food (the dosage needed to obtain the benefit). In this case, bioaccessibility measurements can fulfill a double role: first, in specifying the amount of the bioactive compound that can be acquired and, therefore, the starting level for obtaining health effectiveness, and second, in establishing recommended intake levels (the bioaccessible amount estimated as a minimum). With this estimation and a toxicology report on the bioactive compound, a recommended daily intake can be established [59].

Some studies have dealt with bioaccessibility measurements to check the suitability of the selected food matrix to ensure absorption of the added bioactive compound. de Pascual-Teresa et al [60] analyzed the effect of food matrix characteristics in the bioaccessibility of soy isoflavones through enriching different food matrices (fruit juice, chocolate bars, and cookies). The study showed that genistein and daidzein bioaccessibility was affected in a different fashion depending on the matrix they were incorporated. Bioaccessibility of wine-related polyphenols added to different liquid matrices (vegetable juice, white wine, and white grape juice) was not associated with nature of the matrix [61]. In other cases, studies concerning bioaccessibility of bioactive compounds have been scarcely reported, although the food product has been successfully marketed [62-65].

5.2. Effect of processing on bioaccessibility

To determine the impact of food processing on the nutritional quality of food, physicochemical changes on key nutrients content are determined. However, the overall content of nutrients or bioactive compounds in processed foods is not the full indicator of their nutritional quality. Measurement of bioaccessibility gives a more accurate picture of nutritional quality, as processing may affect bioaccessibility. Even in cases where the main objective of the technological application is to increase bioaccessibility, this must be assessed in some way.

Thus, when modification of the intramolecular and/or intermolecular matrix of a nutrient/bioactive compound is the root of the design, or of the technological intervention used in creating a food product, a situation is established where bioaccessibility could be another control criterion for nutritional effectiveness.

Functional foods based on structural lipids, bioactive compounds in capsule form liposomes or emulsifying agents, and foods where the matrix has been technologically modified to control parameters such as stability, sensory properties, solubility, and energy content, and others, exemplify this case [66-69]. In addition to those parameters, modification of matrix may enhance bioaccessibility of the bioactive compound in the final formulation, so that knowledge of this effect would increase value of the product from the nutritional point of view. Thus, lipophilic

vitamins are often encapsulated to prevent degradation in the food formula where they are incorporated. However, encapsulation also modifies their bioaccessibility, usually achieving an increase in absorption. The encapsulation provides the lipophilic compounds a higher solubility in the gut, increasing their permeation/transport through the intestinal mucosa. Ingestion of β-carotene hydrosols significantly increased bioaccessibility of the pro–vitamin A [70]. Other delivery approaches, such as self-emulsifying delivery systems and microparticles, have shown an increase in bioaccessibility of coenzyme Q10 and soybean isoflavones [71,72].

Food products created exclusively on the basis of components/food ingredients (manufactured food) represent another example of a case in which bioaccessibility is the control criterion for effectiveness. Measurement of bioaccessibility of a food-based formulation of lycopene was the quality criteria in the design of the formulation, with the aim of providing similar and even higher lycopene bioaccessibility in comparison with natural lycopene matrices [48].

In all of the above examples, bioaccessibility measurements introduce a new quality control for the process that provides information to improve design and to redefine the applied technological parameters, incorporating the corrective measures where the processing technique has a negative impact on the bioaccessibility of the nutrient/bioactive compound.

Indirectly, there are other cases where bioaccessibility, although not the ultimate goal of a specific processing technique, can nevertheless provide benefits, as shown in the examples below. First, heat treatment and homogenization of vegetable food products are technologically straightforward and widely used processes that have a positive impact on the bioaccessibility of minerals and phytochemical compounds. Lee and Clydesdale [73] showed that thermal processing increased iron bioaccessibility from spinach. Gärtner et al [74] demonstrated that lycopene is more bioaccessible from tomato paste than from fresh tomatoes, as processing facilitates transfer from the food matrix and solubilization in the lumen.

Fermentation also has a positive effect on bioaccessibility through various mechanisms, for example, by reducing the content of antinutritional factors that interfere with iron bioaccessibility [75] or increasing the digestibility of proteins [76]. Processing emerging technologies also positively modulates the bioaccessibility of key nutrients/bioactive components of food. Applying nonthermal processing techniques as an alternative to the traditional heat process (eg, using pulsating electrical fields or high pressure) can improve bioaccessibility, as long as the applied process parameters are carefully considered. In these cases, there is a lack of knowledge about which processing conditions are the most ideal when considering criteria such as the retention and stability of nutrients. As explained above, bioaccessibility may be affected to some degree; therefore, active feedback needs to be imposed, and bioaccessibility measurements need to be applied as the control criterion [3,77-79].

6. Future research and conclusions

To ensure the predictive value of the in vitro approaches, further research is needed to establish reliable in vitro methods complemented with validation studies, which should be carried out to test the agreement between results from in vitro bioaccessibility protocols and those obtained from in vivo procedures.

The inclusion of information concerning nutritional content of foodstuff in the food labeling offers valuable information from which the consumer can adapt the selection of food items to cover requirements for key nutrients and to maintain health. The consideration of Recommended Daily Intake values theoretically should promote an increase in nutritional quality of food because manufacturers have to analyze in detail the nutritional composition of their products, assuring that key nutrients are present in the levels indicated on the label and controlling the effect of processing on those compounds to restore adequate levels if necessary. Similarly, introducing the term “bioaccessibility” would theoretically promote further changes. Studies concerning health benefits based on functionality of nutrient or bioactive compounds would be significant if the required amount to achieve the in vitro bioactivity is compared with the bioaccessible amount that can be reached from some natural sources or food formula where the bioactive compound is present. To avoid performing an irrelevant bioactivity study, it is convenient to perform some bioaccessibility measurements to test whether the parent compound is absorbed as it is, or if it is metabolized. Thus, appropriate selection of the chemical form, in which the bioactive compound is absorbed, could be made to subsequently carry out the bioactivity test.

Efforts made during processing to minimize the impact of applied technology on key components of food would be more valuable if nutritional quality is considered not only as a stability issue but also as a bioaccessibility one. Some situations exist in which the bioaccessibility of nutrients in natural food can be increased through processing, or in which different product processes or formulations can provide increased bioaccessibility, making the final product more nutritious.

Companies performing the necessary scientific tests to obtain “nutrient bioaccessibility indices” would achieve 3 main objectives. First, they would offer information about the nutritional efficiency of food that is more practical and valuable to the consumer, mainly to ensure nutrient bioavailability index values. Second, they would obtain data on and achieve control of applied processes and nourishing formulations. Finally, as a consequence of the previous points, the companies would be able to offer a distinguishing innovative image compared with their

competitors and, perhaps most importantly, gain in credibility when offering information on nutritional efficiency, a necessary step on the path toward tailored food.

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