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Trends in Food Science & Technology 41 (2015) 5–23

TRENDS IN FOOD SCIENCE & TECHNOLOGY

Review

Bioaccessibility assessment methodologies and their consequences for the risk–benefit evaluation of food

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The *in vitro* techniques provide deeper insight into the phenomena underlying bioaccessibility. The *in vivo* approach has some analytical as well as ethical constraints, it is time demanding, and requires large resources for an adequate experimental control. Nevertheless, whenever possible, *in vivo* studies should be used for the validation of *in vitro* models. On the basis of the results provided by bioaccessibility estimation, a more reliable assessment of the risks and benefits associated to food consumption may usher in a new era in the field of public health, by forcing a rethinking of the recommended nutrient intakes and contaminant thresholds.

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http://dx.doi.org/10.1016/j.tifs.2014.08.008

Introduction

Health and nutrition are intimately linked. Food provides nutrients, but also antinutritional components and contaminants. This raises the issue of quantifying and balancing the risks and benefits associated to a given food. Such an analysis requires knowledge of the consumption frequency levels in a population or subgroups of it, because, as Paracelsus once stated, 'it is only the dose which makes a thing poison'. Scenarios can also be constructed on the basis of hypothetical consumption frequencies. A reliable and in-depth quantitative evaluation of the risk–benefit binomial is a fundamental requirement. Moreover, such evaluation must take into account that foods are typically subjected to further culinary treatment before ingestion (Greffeuille *et al.*, 2011). Besides, the level of a nutrient or contaminant in a portion of food that is eaten may be quite different from the bioaccessible level, that is, the component concentration that is released from the food matrix into the intestinal lumen after digestion, and, according to some other definitions, after absorption across the intestinal wall and the pre-systemic metabolism. This content may also differ from the content found in the systemic circulation, available to be absorbed by the various cells in any tissue of the human organism, stored and/or used in metabolic functions—bioavailable content (Fernández-García, Carvajal-Lérida, & Pérez-Gálvez, 2009). The bioaccessible content is always equal or higher than the bioavailable content and, whenever considered as a proxy for the latter, it generates a worst case scenario regarding contaminants. Therefore, the different approaches to assess bioaccessibility are the focus of a thorough analysis and comparison. There are two main methodological classes: *in vitro* and *in vivo* studies (Moreda-Piñeiro *et al.*, 2011). The *in vivo* approach is very time demanding, requires a painstaking planning and specific resources for an adequate experimental control, and has some analytical and ethical constraints. On the other hand, the *in vitro* methods in their huge variety offer a wider experimental scope, which may be useful in providing deeper insight into the phenomena underlying bioaccessibility.

Many developments in this scientific field are recent and have aimed at overcoming difficulties in defining and measuring the bioaccessibility factor. This factor is affected by the biochemical composition of the food matrix and by the synergies and antagonisms that may be established between the different components (Fernández-García *et al.*,

2009). Previous studies were based on experimental animal models for obtaining data on bioavailability offering an overall picture of the effectiveness of the process, but these models were not able to differentiate between bioaccessibility effectiveness and assimilation. This difficulty was compounded by the absence of an agreed set of experimental conditions for the measurement of bioaccessibility efficiency, even for the same group of food constituents (Fernández-García *et al.*, 2009; Moreda-Piñeiro *et al.*, 2011).

Alternative definitions

The ambiguous application of the concepts in this novel scientific field requires clear working definitions of each scientific term. From the nutritional point of view, bioavailability refers to the fraction of the food nutrient/contaminant ingested that is available for use in physiologic functions or to be stored in body (Fairweather-Tait, 1993). Hence, a food substance bioavailability encompasses the release of the food matrix and concomitant availability for absorption, absorption itself, metabolism, tissue distribution, and bioactivity. However, there are practical and ethical difficulties in the measurement of delivery and bioactivity of food nutrients/contaminants on specific organs, main biologic activity sites (Fernández-García *et al.*, 2009). Therefore, bioavailability is usually defined as the fraction of an oral dose of a substance that reaches the systemic circulation (Schumann *et al.*, 1997), a definition that does not take into account bioactivity and becomes very similar to one of the definitions of bioaccessibility (see below). Despite the practical difficulty in quantifying delivery of food components to specific sites of biological activity, many nutritional scientists have considered the delivery of food components to specific sites to be so relevant that they have retained it within the bioavailability concept (Stahl *et al.*, 2002). In this latter paper it was proposed that once a compound is absorbed it is inevitably bioactive, irrespective of whether or not it is chemically inert *in vivo*. Thus, the concept of bioavailability is not separate from but includes bioactivity (Stahl *et al.*, 2002). This is the source of much ambiguity, leading to the undifferentiated use of both scientific terms bioavailability and bioaccessibility. Some authors mention sometimes a bioavailability at the intestinal level (Ekmekcioglu, 2002; Fu & Cui, 2013), which is at odds with the definition given here and must be viewed as bioaccessibility measurement.

Bioactivity stands for a set of phenomena that occur after a nutrient/contaminant has reached systemic circulation, namely, transport to relevant tissues, interaction with biomolecules, metabolism in these tissues, and all the cascade of physiological effects it generates. Bioactivity may be assessed according to *in vivo*, *ex vivo*, and *in vitro* experimental models. The used methodologies are very specific and have to take into account the particular health benefit or risk being claimed (Fernández-García *et al.*, 2009).

Given this background, the authors opted to present two alternative definitions. A more stringent and much less used definition as presented by Fernández-García *et al.* (2009) (Fig. 1) and the most used definition. In accordance to the former, bioaccessibility encompasses all steps of digestive transformation up to the release from the food matrix into the intestinal lumen, absorption across the intestinal wall, and the presystemic metabolism (including the hepatic tissue metabolic transformations). For the latter definition, bioaccessibility of a substance is defined as the fraction that is soluble in the gastrointestinal (GI) environment and is available for absorption (Paustenbach, 2000) (Fig. 1). Many studies (Dhuique-Mayer *et al.*, 2007; Hu *et al.*, 2013; Salovaara, Sandberg, & Andlid, 2002; Thakkar, Maziya-Dixon, Dixon, & Failla, 2007) have used this second definition. Likewise, for Versantvoort, Oomen, Van de Kamp, Rompelberg, and Sips (2005), bioaccessibility is defined as the fraction of external dose released from its matrix in the GI tract. Hence, this definition does not encompass absorption across the intestinal wall or any metabolic processing at the presystemic level. Depending on the definition, it may be considered that many works only address phenomena that contribute to bioaccessibility (Degrou, Georgé, Renard, & Page, 2013; Lei *et al.*, 2013). As with the elaboration of a standard common set of experimental conditions for the simulation of digestion, a working definition of bioaccessibility must be established. The authors of this review have abstained from promoting a definition of bioaccessibility over the other. Whenever not stated, it should be easy to understand from the context and original papers if a study only addresses the intestinal availability for uptake or it aims at the strictest meaning of bioaccessibility.

Moreover, the oral bioaccessibility must be distinguished from other bioaccessibility concepts whose route to the systemic circulation involves other physiological barriers and tissues, such as, the skin or the lungs. The largest area of concern is the oral/ingestion pathway followed by the dermal and respiratory exposure routes (Paustenbach, 2000). In this review paper, oral bioaccessibility and its counterpart, oral bioavailability, will be the main working concepts subject to analysis.

Bioaccessibility assessment: state of the art

Over the past decade, many studies on food nutrients and contaminants have taken into account bioaccessibility using the latest physiological knowledge and the most recent technological breakthroughs (Briones-Labarca, Venegas-Cubillos, Ortiz-Portilla, Chacana-Ojeda, & Maureira, 2011; Cabañero, Madrid, & Cámara, 2004; Colle *et al.*, 2013; Courraud, Berger, Cristol, & Avallone, 2013; Gawlik-Dziki *et al.*, 2012; Kulp, Forston, Knize, & Felton, 2003; Mandalari *et al.*, 2013; Plaimast, Sirichakwal, Puwastien, Judprasong, & Wasantwisut, 2009; Pugliese *et al.*, 2013; Sun, Van de Wiele, Alava, Tack, & Du Laing,