Analytica Chimica Acta 939 (2016) 1–9

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

On-line coupling of physiologically relevant bioaccessibility testing to inductively coupled plasma spectrometry: Proof of concept for fast assessment of gastrointestinal bioaccessibility of micronutrients from soybeans

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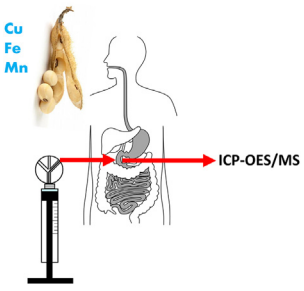
HIGHLIGHTS

- Automatic physiologically relevant extraction test mimicking gastrointestinal digestion.
- Real-time monitoring of bioaccessible fractions of micronutrients in soybean.
- Modelling of extraction kinetics of nutrients in the gastrointestinal tract.
- On-line sampling and handling of extracts as a front end to ICP OES.
- Elucidation of nutrient bioaccessibility in transgenic against non-transgenic soybean.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 17 May 2016
Received in revised form
7 July 2016
Accepted 21 July 2016
Available online 28 July 2016

Keywords:
Oral bioaccessibility
In-vitro assay
Automation
Flow system
Micro-nutrient
Soybean

ABSTRACT

In-vitro physiologically relevant gastrointestinal extraction based on the validated Unified BARGE Method (UBM) is in this work hyphenated to inductively coupled plasma optical emission spectrometry in a batch-flow configuration for real-time monitoring of oral bioaccessibility assays with high temporal resolution. A fully automated flow analyzer is designed to foster in-line filtration of gastrointestinal extracts at predefined times (≤15 min) followed by on-line multi-elemental analysis of bioaccessible micro-nutrients, *viz*., Cu, Fe and Mn, in well-defined volumes of extracts (300 μL) of transgenic and non-transgenic soybean seeds taken as model samples.

The hyphenated flow setup allows for recording of temporal extraction profiles to gain full knowledge of the kinetics of the gastrointestinal digestion processes, including element leaching and concomitant precipitation and complexation reactions hindering bioavailability. Simplification of the overall standard procedure is also feasible by identification of steady-state extraction conditions. Our findings indicate that reliable measurement of oral bioaccessible pools of Cu, Fe and Mn in soybean might be obtained in less than 180 min rather than 240 min as endorsed by UBM. Using a matrix-matched external calibration, limits of detection according to the 3s criteria were 0.5 μg/g for Mn, 0.6 μg/g for Cu and 2.3 μg/g for Fe. Trueness of the automatic bioaccessibility method was confirmed by mass balance validation with recoveries ranging from 87 to 116% regardless of the target element and sample. Cu was the micronutrient

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http://dx.doi.org/10.1016/j.aca.2016.07.030

with the highest oral bioaccessibility ranging from 73% to 83% (7.5–7.9 μg/g) for non-transgenic and transgenic soybeans, respectively, followed by Mn and Fe within the ranges of 29–31% (10.8–11.4 μg/g) and 11–15% (8–14 μg/g), respectively, regardless of transgenesis. The proposed kinetic method is proven suitable for fast and expedient estimation of the nutritional value of soybeans and elucidation of the potential effect of transgenesis onto bioaccessible fractions of elements.

1. Introduction

There is an increasing interest on nutritional aspects of food commodities, dietary supplements, functional foods and nutraceuticals and their impact on human health [1,2]. Release of food components (e.g., macro and micro-minerals, antioxidant and other bioactive components), availability for intestinal absorption and structural design of food-based delivery systems might be accurately explored by *in-vivo* testing [2,3]. *In-vivo* gastrointestinal methods are however restricted by animal ethical issues, associated expenses, and intensive resources [4,5]. Further, EU REACH guidelines promote the use of *in-vitro* models for the investigation of potential physiological effects of substances in order to reduce the number of tests on animals [6]. This has triggered the development of a plethora of *in-vitro* mock digestive gastrointestinal tests to simulate the physicochemical and physiological events in the human gastrointestinal tract (GIT) [5,7–12]. The aim behind is to get insight into the oral bioaccessibility of bioactive species or contaminants in solid matrices, which is defined as the fraction of the overall target species that can be released from its matrix in the GIT and thus become potentially available for intestinal absorption [13,14]. Responsive to user's needs, National and International regulations (e.g., ISO/TS 17924:2007) adopted recommendations and specifications for selection of appropriate oral bioaccessibility testing [5,15]. Intended to proxy the human digestion process as accurately as possible with the incorporation of the stomach and the small intestine compartments, and alternatively the mouth, esophagus and other compartments of the small intestine and colon as well, the four predominant biomimetic extraction tests for inorganic metal species under fasting or feeding conditions are [16–18]: (i) the physiologically-based extraction test (PBET), (ii) RIVM method by the Dutch National Institute for Public Health and the Environment, (iii) the simulation of human intestinal microbial ecosystem (SHIME) procedure and (iv) the TNO dynamic gastrointestinal model (TIM). Readers are referred to a comprehensive review by Wragg and Cave for detailed fundamentals and operational principles of in-vitro gastrointestinal testing using body fluid surrogates [19].

A survey of the literature has revealed that *in-vitro* human digestion models for food commodities are greatly dissimilar with varying types and concentrations of bile salts and enzymes (mucin, pepsin, pancreatin, trypsin, amylase, just to name a few), digestion times and the number of mock digestive compartments [7,17,20], even for similar food commodities from the same research group [10,21]. In the quest of standard operational procedures and harmonized biomimetic extraction tests, the BioAccessibility Research Group of Europe (BARGE) [22], an European network to bring together researchers and institutes using bioaccessibility tests in human health risk assessment and exposure, back to 2009, launched the standardized Unified BARGE method (UBM) [23]. Adapted from RIVM [24], UBM was aimed at ensuring conservative extraction conditions by means of an international inter-laboratory comparison exercise [25]. The standardized UBM was validated *in-vivo* against the gastrointestinal digestion of immature swine, because of resemblance with that of toddlers [26,27], for Pb, As and Cd in soils. Human digestion in UBM is simulated using surrogate digestive fluids, *namely*, saliva, gastric juice, duodenal juice and bile, for which their chemical composition is paralleled to human physiology. The UBM involves two sequential extraction steps under fasting conditions at human body temperature (37 °C): the gastric digestion for 1 h at a pH < 1.5, in which the saliva and gastric juice are added to the solid substrate (mouth + stomach compartments), followed by the gastrointestinal (GI) digestion for 4 h at a pH ca. 6.3, in which the duodenal fluid and bile salts are added to the gastric phase, thus simulating the mouth, stomach and small intestine compartments. Though UBM was originally conceived for risk assessment of hazards related to unintentional ingestion of soil or by children suffering pica [22,25], the harmonized bioaccessibility test may be extended to sediments, vegetables, dusts, ash, or any other solid matrix studied in an exposure assessment approach [23]. For example, a recent work demonstrated the UBM applicability for assessing bioaccessible fractions of macro and microelements in a variety of nutritional supplements [28]. The UBM and the majority of common *in-vitro* digestion models indicated above are batch tests aimed at producing a limited number of discrete GIT surrogate extracts per sample without time resolution of the human digestion process. Bearing this in mind, researchers have been over the past decade dedicated significant amount of effort to developing semi-automatic on-line continuous extraction methods (with manual replacement of digestive fluids) [29–31], or fully mechanized batchwise/flow-based platforms for simplification of bioaccessibility testing of trace elements in foodstuff and soil materials [32–34]. Unfortunately, flow-through oral bioaccessibility assays usually incorporate entirely independent body fluids as extractants (only saliva, or gastric or intestinal phases) [29–31] that do not mimic the transit of chyme from stomach to duodenum. For the sake of facile handling of enzyme-containing mock digestive fluids compendial dissolution media from US or EU pharmacopeias are frequently linked to on-line flow-through tests [29,30,35]. These are however overly simplistic buffer solutions that do not accurately reflect the contents of the human gut, whereby the biorelevance of experimental data for human risk assessment is debatable. Some enzymes, such as mucin, are usually neglected to prevent the manipulation of suspensions in flow setups that might lead to the build-up of backpressure [29,30,34]. In fact, some authors reported that leaching with intestinal fluid surrogates needs to be omitted from on-line continuous extraction because it might lead to clogging problems [36]. HPLC pumps rather than peristaltic pumps might alleviate the above shortcomings [35], yet the practical applicability of bulky HPLC pumps in routine assays is rather limited and makes the potential miniaturization of the flow-through assays cumbersome.

In this work, automatic batch-wise biomimetic testing capitalizing onto UBM but incorporating real-time monitoring of the human digestion process is proposed as a front end to inductively coupled plasma optical emission spectrometry (ICP OES) for rapid assessment of oral bioaccessible fractions of elements. To the best of our knowledge, this is the first report automating the UBM