Analytica Chimica Acta 1026 (2018) 51–61

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Flow-through dynamic microextraction system for automatic *in vitro* assessment of chyme bioaccessibility in food commodities

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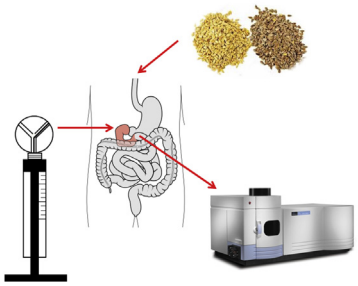
HIGHLIGHTS

- First work reporting on-line/dynamic *in-vitro* chyme bioaccessibility of metal species.
- Use of physiologically relevant duodenal and bile fluids.
- Full automation of the Versantvoort's method, specifically designed for foodstuff.
- High temporal resolution of chyme/gastrointestinal extraction by hyphenation to ICP OES.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 February 2018
Received in revised form
15 April 2018
Accepted 27 April 2018
Available online 2 May 2018

Keywords:
Bioaccessibility
In-vitro assay
Dynamic extraction
Chyme analysis
Automation
ICP OES

ABSTRACT

An automatic flow-through dynamic extraction method is proposed for the first time for *in vitro* exploration, with high temporal resolution, of the transit of the chyme from the gastric to the duodenal compartment using the Versantvoort's fed-state physiologically relevant extraction test. The flow manifold was coupled on-line to an inductively coupled plasma optical emission spectrometer (ICP OES) for real-time elucidation of the bioaccessible elemental fraction of micronutrients (viz., Cu, Fe and Mn) in food commodities across the gastrointestinal tract. The simulated intestinal and bile biofluid (added to the gastric phase) was successively pumped at 1.0 mL min^{-1} through a large-bore column (maintained at 37.0 ± 2.0 °C) initially loaded with a weighed amount of linseed (250 mg) using a PVDF filter membrane (5.0 μm pore size) for retaining of the solid sample and in-line filtration of the extracts. The lack of bias (trueness) of the on-line gastrointestinal extraction method coupled to ICP OES was confirmed using mass balance validation following microwave assisted digestion of the residual (non-bioaccessible) elemental fraction. Mass balance validation yielded absolute recoveries spanning from 79 to 121% for the overall analytes and samples. On-line dynamic extraction was critically appraised against batch counterparts for both gastric and gastrointestinal compartments. Due to the lack of consensus in the literature regarding the agitation method for batch oral bioaccessibility testing, several extraction approaches (viz., magnetic stirring, end-over-end rotation and orbital shaking) were evaluated. Improved gastric extractability of Fe along with bioaccessible data comparable to the dynamic counterpart based on the continuous displacement of the extraction equilibrium was obtained with batchwise magnetic stirring, which is deemed most appropriate for ascertaining worst-case/maximum bioaccessibility scenarios.

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https://doi.org/10.1016/j.aca.2018.04.067
0003-2670/

1. Introduction

Linseed (*Linum usitatissimum L.*) is currently regarded as a functional food that serves as a source of omega-3 fatty acids, lignans, dietary fiber, proteins, carbohydrates, lipids and essential elements (e.g., Ca, Cu, Fe, K, Mn, P, Na and Zn). These components are important to maintain human body homeostasis, in addition to ensure beneficial effects on hormonal regulation in the prevention of diseases, such as cancer and diabetes [1,2]. Nevertheless, the mere occurrence of nutrients in foodstuff does not guarantee by its own its availability by the human body after ingestion. To assess the actual pools of nutrients that are released into the gastrointestinal (GI) tract and are available for intestinal absorption, *in-vivo* methods have been developed over the past decades. However, *in-vivo* tests that use animal models are ethically controversial, time-consuming, cost expensive and require specialized and trained personnel [3,4]. Therefore, EU regulations (e.g., REACH) [5] suggested the replacement of *in vivo* assays by *in vitro* counterparts as a proxy for oral bioaccessibility with no need of animal models. The Bioaccessibility Research Group of Europe launched a standardized and validated operational procedure (so-called unified bioaccessibility method (UBM)) [6,7] that harmonizes the various bioaccessibility tests for health risk assessment of metals from contaminated soils. *In vitro* digestion methods dedicated to bioaccessibility studies in food samples are also available in the literature [8–10]. As is the case with the UBM method, the so-called Versantvoort's test [10] embraces two consecutive extraction steps to mimic two digestion compartments: (i) the gastric compartment in which the gastric fluid and saliva are incorporated to the solid sample, and (ii) the GI compartment involving the addition of the bile and duodenal body fluids to the mock chyme. The extraction method is performed with biorelevant digestive fluid surrogates the composition of which resembles human physiology. As compared with the UBM test, which is performed mimicking fasted state, the Versantvoort's method capitalizes upon fed state conditions whereby operational modifications are undertaken based on the physiological modifications occurring within the GI tract with food components, such as change in pH and GI residence times, and the increase in the secretion of gastric acid, bile and pancreatic fluids [11]. It should be however noted that overly simplistic *in vitro* bioaccessibility methods [12–15], e.g., those based in compendial body fluids endorsed by the United States Pharmacopeia (USP) [16], have been sometimes proposed in the literature for food commodities. USP-based digestive fluids do not properly simulate the real composition of the human GI tract, and predominantly are applied individually, that is, the food is first exposed and extracted with the gastric fluid and then the solid residue is exposed to the duodenal phase, whereby information about the chyme transit is lost. Two common limitations of conventional oral bioaccessibility tests based on batchwise extraction are (i) the lack of immediate removal of desorbed compounds from the surface of the matrix, which is inherent to the bioaccessibility concept [17], and (ii) the absence of insight into the leaching kinetics of target species at real-time [18].

To tackle the above shortcomings, several teams performed *in vitro* dynamic bioaccessibility assays with the aid of flow setups that are able to bring fresh portions of body fluids (usually gastric fluid) through the solid sample as contained in a dedicated flow-through chamber. For example, Beauchemin's group used on-line dynamic extraction for the speciation of bioaccessible arsenic [19–21] and the identification of bioaccessible pools of Cr, As, Cd and Pb in bread [22]. Notwithstanding the simplification of the body fluids using USP recommendations, the authors were unable to analyse the duodenal phase because of clogging of the flow conduits [23]. Rosende et al. [24] investigated the risk exposure of metal species in soil materials under worst-case extraction scenarios with a flow-through system using UBM bio-relevant gastric phase, yet mucin that generates turbid and viscous milieu was omitted from the biofluid after statistical data processing. However, this enzyme might behave as a vital component of body fluid surrogates in the GI digestion of foodstuff. Further, previous flow systems using small-scale column setups [18–23,25] were unable to handle sample amounts > 200 mg, which might not assure the sample representativity in bioaccessibility assays of food commodities.

In this work, an automatic flow-through dynamic microextraction system is proposed for the first time for exploring the transit of the chyme from the gastric to the duodenal compartment based on the Versantvoort's fed-state method for on-line elucidation of the bioaccessible elemental fraction of food commodities with high temporal resolution. The proof of concept applicability of the novel flow approach hyphenated to an inductively coupled plasma optical emission spectrometer (ICP OES) was demonstrated by on-line analysis of bioaccessible concentrations of micronutrients (viz., Mn, Fe and Cu) in commercially available golden and brown linseed.

2. Experimental

2.1. Reagents, solutions and samples

All solutions were prepared from analytical reagents using Milli-Q water (18.2 MΩ cm, Millipore Synthesis A10, Billerica, USA). A multi-element standard solution 5 (TraceCERT, Fluka, Sant Louis) was used for ICP OES calibration. Gastrointestinal/chyme extracts were analyzed by a matrix-matched protocol using dilute working solutions of the multi-elemental standard.

The polyethylene containers (Corning®) and glassware were soaked in 10% (v/v) nitric acid for ca. 24 h and rinsed three times with Milli-Q water pending use.

Physiologically-based digestive fluids were composed of organic reagents, enzymes and salts according to UBM [6], yet with the increase in enzyme concentrations by a factor of 4–10 (conservative conditions) throughout the various body fluids in the mimicry of the fed-state system, as previously suggested by Versantvoort's [10] and FOREhsT [11] methods. In short, the *in vitro* saliva (pH 6.8 ± 0.2) is composed as follows: 298 mg L^{-1} NaCl, 200 mg L^{-1} KSCN, 896 mg L^{-1} KCl, 1694 mg L^{-1} $NaHCO_3$, 570 mg L^{-1} Na_2SO_4, 888 mg L^{-1} NaH_2PO_4, 200 mg L^{-1} urea, 30 mg L^{-1} uric acid, 580 mg L^{-1} alpha amylase from *Bacillus* sp (1594 units mg^{-1} protein, Sigma, A-6814), and 50 mg L^{-1} mucin from porcine stomach (type II, Sigma, M2378). The simulated gastric phase (pH 1.30 ± 0.02) consists of 2752 mg L^{-1} NaCl, 824 mg L^{-1} KCl, 266 mg L^{-1} NaH_2PO_4, 306 mg L^{-1} NH_4Cl, 400 mg L^{-1} $CaCl_2 \cdot 2H_2O$, 85 mg L^{-1} urea, 20 mg L^{-1} glucuronic acid, 650 mg L^{-1} glucose, 330 mg L^{-1} glucosamine hydrochloride, 6.5 mL L^{-1} HCl (37%), 2000 mg L^{-1} albumin from bovine serum (BSA, Merck, 112018, Darmstadt, Germany), 5000 mg L^{-1} pepsin from porcine gastric mucosa (0.7 FIP-U mg^{-1}, Merck, 107185) and 6000 mg L^{-1} mucin. The *in vitro* duodenal fluid (pH 8.1 ± 0.2) consists of 7012 mg L^{-1} NaCl, 564 mg L^{-1} KCl, 200 mg L^{-1} $CaCl_2.2H_2O$, 50 mg L^{-1} $MgCl_2$, 3388 mg L^{-1} $NaHCO_3$, 80 mg L^{-1} KH_2PO_4, 100 mg L^{-1} urea, 180 μL L^{-1} HCl (37%), 2000 mg L^{-1} BSA, 18,000 mg L^{-1} pancreatin from porcine pancreas (24,000 FIP-U g^{-1} lipase, 1400 FIP-U g^{-1} protease, 30,000 FIP-U g^{-1} amylase, Merck, 107133), and 3000 mg L^{-1} lipase from porcine pancreas (114 units mg^{-1} protein, Sigma, L-3126). The surrogate bile fluid (pH 8.2 ± 0.2) contains 5260 mg L^{-1} NaCl, 376 mg L^{-1} KCl, 222 mg L^{-1} $CaCl_2.2H_2O$, 5785 mg L^{-1} $NaHCO_3$, 250 mg L^{-1} urea, 150 μL L^{-1} HCl (37%), 3600 mg L^{-1} BSA, and 60,000 mg L^{-1} bile from porcine pancreas (Sigma, B-3883). All of the