Talanta 174 (2017) 605–609

Contents lists available at ScienceDirect

Talanta

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

Innovative, simple and green ultrasound assisted-enzyme based hydrolytic microextraction method for manganese at trace levels in food samples

Erkan Yilmaz, Mustafa Soylak*

Erciyes University, Faculty of Sciences, Department of Chemistry, 38039 Kayseri, Turkey

ARTICLE INFO

Keywords:
Ultrasound assisted-enzyme based hydrolytic microextraction
Pepsin
Manganese
Food samples
Micro-sampling flame atomic absorption spectrometer

ABSTRACT

We describe the usage of hydrolytic enzymes for the innovative, green and simple microextraction of manganese in food samples. The ultrasound assisted-enzyme based hydrolytic microextraction method (UA-EH-ME) was applied for the separation and determination of manganese in food samples prior to its micro-sampling flame atomic absorption spectrometric determination (MS-FAAS). Analytical parameters influenced on the enzyme based hydrolytic microextraction method such as pH, type of enzyme, amount of enzyme, temperature and time of ultrasonic waves were fully evaluated and optimized by using mg level of NCS ZC73033 scallion certified reference material. In the presented method, total manganese in 5.0 mg of food samples was extracted to 800 μL of aqueous phase at pH 1.0 by 0.75 mg of pepsin as microextraction agent (hydrolytic enzyme). The procedure can be completed in 15 min. These values are optimized conditions for the quantitative extraction of manganese from food samples. The most important advantage of the UA-EH-ME procedure is the using of mg level of pepsin for breaking down certain bonds of bio-molecules for extraction of manganese in very low levels of food samples. The limit of detection (LOD), the limit of quantification (LOQ), and relative standard deviation were found as 4.9 mg kg^{-1}, 16.3 mg kg^{-1} and 4.8%, respectively. The manganese contents of the analysed food samples were in the range of 26–345 mg kg^{-1}.

1. Introduction

Sample preparation techniques is a difficult issue that requests improved analytical techniques and highly proficient personnel. Therefore, the search for new methods in sample preparation techniques accomplishing simplicity, robustness, cheap, and rapidity is an important objective in analytical chemistry [1–3]. In order to determine the trace elements in food samples, comprehensive sample preparation methods often requires prior to instrumental analysis [4–9]. Up to now, many sample digestion method such as wet-ashing [10], dry-ashing [11], microwave-assisted acid digestion [12] or many separation preconcentration method such as solid phase extraction (SPE) [13–15], cloud point extraction (CPE) [16], liquid-liquid extraction (LLE) [17], liquid phase microextraction (LPME) [18], solid phase microextraction (SPME) [19] have been applied for that purpose.

In many of these methods, many solvents like mineral acids and oxidizing reagents, toxic organic solvents which causes environmental toxicity were used and also in these methods [20,21], expensive, complex and time-consuming laboratory equipments are needed. These adverse situations limit the application of these techniques. Therefore, the techniques are often not reproducible and/or comparable.

In recent years, concentrating on green chemistry changed expected requests in sample preparation techniques and an important effort has been shifted to the development of green, innovative, simple and cheap analytical methods. The development of novel sample preparation techniques provides an opportunity to extend the concepts and practices of green analytical chemistry [22,23].

Hydrolytic enzymes (pepsine, α-amylase, protease type XIV, lipase and pancreatine) act at specific bonds of the substrate, breaking biomolecules such as proteins, sugar and triglycerid through catalyzing the introduction of water. The breaks down for certain bonds of these biomolecules is to be achieved under suitable hydrolysis conditions such as pH, temperature, and ionic strength [24–26]. After enzymatic hydrolysis action, a variable fraction of analytes such as metal, organometallic and organic species is extracted from the food sample to water phase. The concentration of extracted analytes can be finally measured by either atomic spectrometric or chromatographic techniques. In enzymatic hydrolysis, polluting or toxic reagents, which cause secondary waste are not used and the extraction of analyte is achieved under mild temperature and water phase conditions. Hence, these methods are environmentally friendly and can be takes place in green

* Corresponding author.
E-mail address: soylak@erciyes.edu.tr (M. Soylak).

http://dx.doi.org/10.1016/j.talanta.2017.06.069
Received 7 April 2017; Received in revised form 23 June 2017; Accepted 24 June 2017
Available online 27 June 2017
0039-9140/

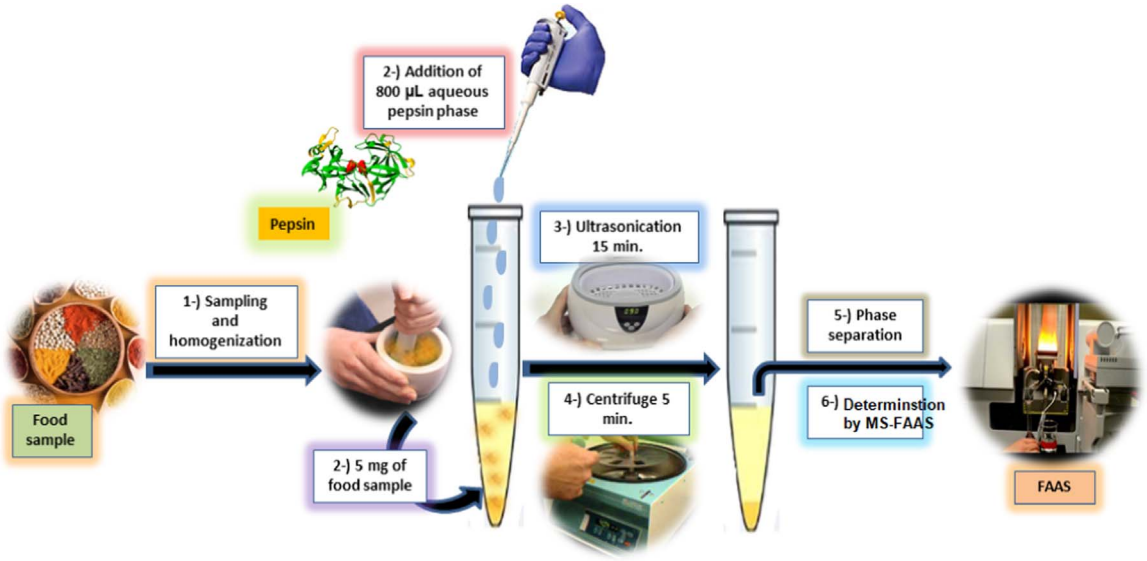

Fig. 1. Graphical representation of the ultrasound assisted-enzyme based hydrolytic microextraction method (UA-EH-ME).

chemistry.

Enzymatic hydrolysis extraction, based on the transfer of analyte from the solid food matrix to an aqueous phase, is employed for sample preparation [27,28]. Nevertheless, some shortcomings such as use of large amount of enzymes and samples and large aqueous phase which cause long extraction process. These drawbacks make enzymatic hydrolysis extraction studies to be intensive, expensive and time-consuming. It is of great importance in the development of microsized enzymatic extraction processes for real samples to eliminate these disadvantages.

The properties of ultrasonic radiation make it an effective appliance for analytical applications, as proved by the increasing number of analytical chemistry publication in recent years [29]. The ultrasonic radiation is used most effectively for the acceleration of the mass transfer between different phases and increase the extraction yield in extraction process. Hence, the use of ultrasonic energy in extraction methods provide a lot of advantage mentioned above [30].

In the presented work, an ultrasound assisted-enzyme based hydrolytic microextraction method (UA-EH-ME) has been developed for the separation and determination of manganese in food samples prior to its micro sampling flame atomic absorption spectrometric determinations.

2. Materials and methods

2.1. Instruments

A Perkin Elmer AAnalyst 800 flame atomic absorption spectrometer (Norwalk, CT, USA) equipped with hollow cathode lamp was used for measurement of manganese concentration. A home-made micro-sampling analysis system consist of Teflon funnel connected to the nebulizer of FAAS was used for measurements. In this system, 100 µL of the aqueous phase including manganese was injected into the FAAS by Eppendorf pipette and the peak height was measured.

All pH measurements were carried out using a Sartorius PT-10 digital pH meter (Germany) equipped with a combined glass–electrode. An ultrasonic water-bath (Bandelin, Germany) programmable for time and with ultrasound frequencies of maximum 35 kHz was used for assisting enzymatic extraction. The centrifugation for phase separation was performed using a Hettich Rotofix 32 centrifuge (Buckinghamshire, England). All solutions were prepared with reverse osmosis purified water (Millipore Milli-Q system18.2MΩ cm, resistivity).

2.2. Reagents and solutions

All chemicals were at analytical reagent grade unless otherwise stated. Ultra-pure water purified through reverse osmosis (18.2 MΩ cm, Millipore) was used as the working medium. To avoid metal contamination, all plastic and glass laboratory equipment used in procedure were washed and kept for 48 h in 10% (v/v) nitric acid and then rinsed several times with ultrapure water before use.

Pepsin from porcine gastric mucosa (Merck, Germany), pancreatin from porcine pancreas (Sigma, St. Lois MO) and α-amylase from *Bacillus* sp. (Sigma, St. Louis MO) were obtained. Analytical grade of HNO_3 65%, HCl 37%, NaOH, NaH_2PO_4, Na_2HPO_4 and NaCl were purchased from Merck (Germany). NCS ZC73033 Scallion and NCS ZC73032 Celery certified reference materials were supplied from China National Analysis Center for Iron and Steel, 1568a - Rice Flour, 1570a - Trace Elements in Spinach Leaves, 1515 - Apple Leaves and 1573a - Tomato Leaves certified reference materials were supplied from National Institute of Standards and Technology in U.S.

2.3. Ultrasound assisted-enzyme based hydrolytic microextraction procedure

Five mg of scallion certified reference material was weighed into a 15 mL centrifuge tube and then, 800 µL of a aqueous phase (extraction phase) containing 1.0% (w/v) NaCl and 0.75 mg of pepsin adjusted to pH 1.0 was added in the centrifuge tube. The mixture were processed by ultrasonic waves at maximum frequency of 35 kHz and an output power of 640 W max at 37 °C for 15 min in the ultrasonic bath. At this stage, the breaks down for certain bonds of this food matrix is to be achieved and manganese in food matrix was extracted to aqueous phase, and the mixture was centrifuged at 4000 rpm for 5 min to separate solid phase remain and aqueous phase. An aliquot 100 µL of the aqueous phase was taken with a micropipette and aspirated to the micro sampling unit connected to the nebulizer of the flame atomic absorption spectrometer and absorbance value was measured. The same way was followed for blank studies. A graphical representation of the ultrasound assisted-enzyme based hydrolytic microextraction method (UA-EH-ME) was shown in Fig. 1. The percentage recovery of manganese was calculated by using ratio of obtained value to certify value of manganese in NCS ZC73033 scallion certified reference material.