Food Research International 53 (2013) 174–179

Contents lists available at SciVerse ScienceDirect

Food Research International

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

Effect of edible plants combination on mineral bioaccessibility and bioavailability, using in vitro digestion and liposome-affinity extraction

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ARTICLE INFO

Article history:
Received 4 November 2012
Accepted 19 April 2013

Keywords:
Edible plants
In vitro techniques
Mineral bioavailability
Mineral bioaccessibility

ABSTRACT

Edible plants were usually consumed by people to satisfy their nutritional requirements. However, how such combination affected mineral bioaccessibility and bioavailability was unknown. *Persicae Semen* (A) was combined with *Carthami Flos* (B) in different ratios and then used for the preparation of the soups. Following in vitro digestion, trace minerals in the soups were transformed into their final mineral coordinated complexes and mineral concentration in the chyme was used for mineral bioaccessibility assessment. As similar as the biomembrane between gastrointestinal tract and blood vessels, liposome was used as a biomembrane model. The mineral bioavailability was assessed by the content of liposome-affinity mineral in the chyme, which was controlled by the combination ratio and gastrointestinal digestive enzymes. The optimal combination ratio was 1A:1B (w/w) with a safe dosage of 421.0 g/day and 500.0 g/day for males and females, respectively, and a maximum dosage of 2368.4 g/day for both adult.

1. Introduction

The past decade has witnessed a tremendous resurgence in the interest of edible plant products, which has been used as a food additive. The minerals in plants are partially responsible for their medicinal and nutritional properties as well as the toxic ones (Maiga, Diallo, Bye, & Paulsen, 2005; Turchia, Alagonab, & Lubrano, 2009). Minerals are associated with human metabolism and biosynthesis. The essential minerals in edible plants can satisfy our nutritional requirements (Maiga et al., 2005; Ouzouni, Veltsistas, Paleologos, & Riganakos, 2007; Ródenas, Sánchez-Muniz, Gómez-Juaristi, Larrea, & Marín, 2009). *Persicae Semen* is the seed of *Prunus persica Batsch*. *Carthami Flos* is the flower of *Carthamus tinctorius*. *Persicae Semen* and *Carthami Flos*, as edible plants, are commonly used in pair for improving blood circulation and decreasing blood stasis (Jiang, 2005; Li, Deng, & Zheng, 2004; Liu et al., 2012).

Iron, manganese, and zinc are essential to human health and development (Gerber, Leonard, & Hantson, 2002; Thorp, 1998). Low intake of essential minerals can result in nutritional deficiencies (Tapiero et al., 2001; Goldhaber, 2003), but excessive intake causes potential toxicity (Barroso et al., 2009). For example, excessive iron leads to tissue damage, coronary heart disease, and cancer (Li, Deng, & Zheng, 2003; Morris, Earl, Trenam, & Blake, 1995). Relatively high intake of manganese can cause mammalian cell gene mutation, DNA damage, and chromosomal aberration (Gwiazda, Lee, Sheridan, & Smith, 2002; Li, Zheng, Liu, & Cai, 2005). Zinc overload can induce neuronal death (Chen, Wang, Wu, Yu, & Zhu, 2009). Thus, in terms of potential toxicity, mineral bioaccessibility and bioavailability in edible plants should be evaluated. The combination ratio of edible plants greatly affected mineral ligands in edible plants so that mineral bioaccessibility and bioavailability may be significantly different (Zheng, Li, & Lin, 2007).

Mineral research in edible plants have been focused on determining the total content (Dawczynski, Schäfer, Leiterer, & Jahreis, 2007; Hu, Huang, Chen, & Wang, 2010; Kim et al., 2008; Ródenas et al., 2009) or the concentration of inorganic and organic (Oomen et al., 2003), dissolved and particulate, and exchangeable and nonexchangable minerals (Versantvoort, Oomen, Vande, Rompelberg, & Sips, 2005). However, only the faction of mineral complexes which could be released from edible plants into the chyme was bioaccessible. In vitro mineral bioavailability refers to the fraction of mineral complexes that affinitied with liposome.

Mineral bioavailability has been assessed by animal experiment (Lonnerdal, Mendoza, Brown, Rutger, & Raboy, 2011) and in vitro digestion/Caco-2 cell model (Aluru, Rodermel, & Reddy, 2011; Pixley, Palacios-Rojas, & Glahn, 2011). However, animal experiments are costly and time-consuming. Caco-2 cell model is rather complicated to perform (Eisenbrand et al., 2002; Hemalatha, Gautam, Platel, & Srinivasan, 2009; Shen, Luten, Robberecht, Bindels, & Deelstra, 1994).

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http://dx.doi.org/10.1016/j.foodres.2013.04.004

So an in vitro digestion and simulated absorption system is important as a convenient and low-cost analytical platform for rapidly identifying mineral bioavailability in edible plants. As similar as the biomembrane between gastrointestinal tract and blood vessels, liposome was a promising simulated biomembrane. Mineral bioavailability was assessed by the content of liposome-affinity mineral in the chyme. The influence of gastrointestinal digestive enzymes and edible plant combination on mineral bioaccessibility and bioavailability was discussed for the first time.

2. Materials and methods

2.1. Apparatus

An Agilent 7500cx series inductively coupled plasma mass spectrometer (Agilent Technologies Co., USA) was used for mineral determination. The pH values were measured using a Mettler Toledo 320-S pH meter (Mettler Toledo Co., Shanghai, China) with a combined electrode. Milli-Q purified water was obtained from a Milli-Q-purified water apparatus (Millipore Co., USA). A RE-52 rotator evaporator (Yarong Co.,Shanghai, China), a SHA-B temperature consistent oscillating water-bath (Guohua Co., Changzhou, China), a MK-III microwave digestion system (Sineo Microwave Chemistry Technology Co., Shanghai, China), an 86C ULT ultra low temperature freezer (Thermo Electron Co., USA), a DM LB2 microscope (Leica Microsystems Wetzlar GmbH, Germany), and an agate mortar were used.

2.2. Chemicals

The edible plants (*Persicae Semen* and *Carthami Flos*), in dry form, were obtained from the Lao-rui-lin drugstore in Zhangzhou, Fujian, China. Their origins were authenticated by Professor Chen Yu-lin. Vegetal standard reference materials, NIST 1573 (tomato leaves) and NIST 1515 (orchard leaves), were supplied from the National Institute of Standards Technology. Concentrated nitric acid (69–70%, Merck KGaA, Germany) and hydrogen peroxide (30%, Merck KGaA, Germany) were used for the digestion of edible plants. Agilent ICP-MS multi-element standards (10 mg/L, Nos. 2A, USA) and internal standards (100 mg/L of 45Sc, 72Ge, 103Rh, 115In, and 209Bi) were used for making the calibration curve and mineral determination by ICP-MS. Biological chemicals, including uric acid, mucin, albumin bovine, pepsin, pancreatin, lipase, and bile, were purchased from Sigma (St. Louis, MO, USA) and used for preparing digestive juice. All other chemical reagents were purchased from Shanghai Experiment Reagent Co., China. Milli-Q purified water (18.2 M Ω) was used for all sample preparations. To avoid mineral contamination, all of the polyethylene flasks and plastic containers were washed and kept soaked for 48 h in 10% (v/v) nitric acid, then rinsed 3 times with ultra-pure water before use.

2.3. Sample preparation

Persicae Semen and *Carthami Flos* samples were rinsed with Milli-Q purified water for 3 times, dried at 80 °C to constant weight, and then carefully ground in an agate mortar. Following the Chinese traditional method of preparing soups, each edible plant soup was prepared as follows: (a) 60.0 g of *Persicae Semen*, (b) 60.0 g of *Carthami Flos*, and (c) a pair of *Persicae Semen* (Plant A, 40.0 g) and *Carthami Flos* (Plant B, 20.0, 25.0, 30.0, and 40.0 g, respectively) in different ratios as 2A:1B, 8A:5B, 4A:3B, and 1A:1B, respectively. According to 401 prescriptions of traditional Chinese medicine, these combination ratios were chosen. The edible plants were decocted respectively as follow: the samples have been decocted for 1 h with addition of 300 mL ultrapure water respectively. First part soup was filtered and kept, then the residues were decocted two more times with addition of 300 mL ultrapure water. The three parts of soups were mixed and concentrated to 0.1 g/L (m/v).

The amounts of gastrointestinal inorganics, organics, and digestive enzymes, the process of digestion, and the digestion time were designed on human physiology (Versantvoort et al., 2005; Oomen et al., 2003). The details of the components of gastric juice, duodenal juice, and bile fluid were described previously by us (Li, Lin, & Zheng, 2011), inorganics and organics were included for semi-bionic digestion (SBD), and supplementary digestive enzymes were included for whole-bionic digestion (WBD). To study the effect of digestive enzymes on mineral bioaccessibility and bioavailability, two kinds of in vitro digestion, i.e., SBD and WBD, were applied for the digestion of edible plants.

Edible plant soups were digested by in vitro digestion (SBD or WBD) at 37 °C on a gentle oscillation at 100 rpm as follows (Li et al., 2011). Simulated gastric digestion process was initiated with the addition of 100 mL of gastric juice to 250 mL soup and incubated for 2 h. Intestinal digestion is continued on base of gastric digestion. The pH value of the chyme was adjusted to 7.8 ± 0.2 with 1 mol/L NaOH. The chyme was mixed with 100 mL of duodenal juice and 50 mL of bile, then incubated on a gentle rocking shaker for 7 h. Blank comparison was also obtained by using the same digestion procedure without the addition of the soup in each set of experiment. All chymes were filtered with 0.45 μm membrane.

2.4. Mineral species distribution in chyme from edible plant soups

Egg-derived lecithin (0.10 g) was dissolved in chloroform and then transferred into a rotatory evaporator to evaporate chloroform. Twenty-five milliliters of chyme was mixed with liposome, shook at 100 rpm to form a homogeneous liposome suspension, frozen at −71 °C in a super low freezer for 30 min, and then thawed at 37 °C to form liposome. The frozen-thawed process was repeated three times to promote mineral species distribution in the liposome-water system. Liposome-Affinity Minerals (LAMs), as bioavailable mineral species, could be extracted by liposome and then entered into the liposome phase. Water-Soluble Minerals (WSMs) were remained in water phase. According to the measurement by Leica microscope, the average diameter of liposomes was ranged from 0.3 to 0.35 μm. So, LAMs can be separated from WSMs by 0.22 μm membranes.

2.5. Mineral analysis in edible plants, edible plant soups, chyme, LAMs, and WSMs

The decomposition method of edible plant, edible plant soups, the filtrate of the chyme from in vitro digestion of the soups, the LAMs, and the WSMs were: (a) the sample amount was about 0.25 g for dried edible plant material, 5 mL for the soups, 5 mL for the filtrate of the chyme, all LAMs and WSMs; (b) in a Teflon digestion vessel, the sample was added to 2.0 mL of concentrated HNO_3 and 1.0 mL of H_2O_2 (30%) and was decomposed under microwaves for 10 min under 10 atm pressure; and (c) after being cooled naturally to room temperature, the decomposed solution was diluted to 25 mL for the determination of mineral concentration by ICP-MS. The calibration curve and analytical conditions for mineral measurement were showed in Fig. 1 and Table 1, respectively.

Analysis of variance was calculated by using SASPROC MIXED (Littell, Milliken, Stroup, & Wolfinger, 1996). For all analyses, significance was assigned at the $P < 0.05$ level.

3. Results and discussions

3.1. Accuracy of the method

The validity of the proposed method was checked by analyzing standard reference materials NIST 1571 and NIST 1573. The results