Food Chemistry 197 (2016) 1275–1279

Contents lists available at ScienceDirect

Food Chemistry

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

Iron binding efficiency of polyphenols: Comparison of effect of ascorbic acid and ethylenediaminetetraacetic acid on catechol and galloyl groups

Poonkodi Tamilmani *, Mohan Chandra Pandey

Freeze Drying and Animal Product Technology Division, Defence Food Research Laboratory, Siddartha Nagar, Mysore 570011, Karnataka, India

ARTICLE INFO

Article history:
Received 11 August 2015
Received in revised form 29 October 2015
Accepted 10 November 2015
Available online 11 November 2015

Keywords:
Ascorbic acid
EDTA
Iron binding ability
MANOVA
Polyphenols

ABSTRACT

Dietary polyphenols are markedly studied for their antioxidant activity. They also have a negative impact on nutrition whereby they interfere with iron absorption. Common dietary polyphenols include: catechins, flavonols, flavanols, flavones, anthocyanins, proanthocyanidins and phenolic acids. Ascorbic acid (AA) and Ethylenediaminetetraacetic acid (EDTA) are commonly used to counter act this reaction and increase iron bioavailability. This study was aimed at determining the effect of AA and EDTA on the catechol or galloyl iron binding ability of pure phenolics, coffee and tea. Phenolic concentrations of 40, 80, 610, 240, 320, 400, 520 and 900 μg/ml were tested against six levels of AA and EDTA. These effects were studied in detail using Multivariate Analysis of Variance (MANOVA) with the hypothesis that there would be one or more mean differences between the ratio of enhancer and the different concentrations of samples tested. AA was found to be more efficient than EDTA in a way that lesser quantity is required for completely overcoming negative iron binding effects of polyphenols and similar samples.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Iron exists in the human diet, predominantly as non-heme iron. Iron bioavailability has been exhaustively studied and effect of inhibitors and enhancers on iron absorption characterized. The wide prevalence of iron deficiency can be attributed to it being more reactive and thus affected by many diet components. Polyphenols, minerals, phytic acid, fiber are common deterrents of iron uptake (Vitali, Vedrina Dragojevic, Sebecic, & Vujic, 2007; Samman et al., 2001; Hallberg, 1998; Sandstrom, 2001; Rossander-Hulthen, Brune, Sandstrom, Lonnerdal, & Hallberg, 1991; Sharp, 2004). In order to counter act these inhibitors and to make iron more bioavailable, compounds such as Ascorbic acid (AA), Ethylenediaminetetraacetic acid (EDTA), meat etc (Cook, Watson, Simpson, Lipschitz, & Skikne, 1984; Hurrell, Reddy, Juillerat, & Cook, 2006; Reinhold, Garcia, & Garzon, 1981; Siegenberg et al., 1991), are used in food and pharmaceutical formulations.

Dietary polyphenols are widely studied for their antioxidant activity, wherein they stop progression of Reactive Oxygen Species (ROS) production. As result of this activity, polyphenols render iron un-absorbable by forming insoluble complex. AA and EDTA are the two enhancers being studied here exert their beneficial effect by reversibly or irreversibly forming a more soluble complex with iron as opposed to the complexes formed by polyphenols. Iron binding ability of polyphenols is measured using a two wavelength spectroscopic method based on the principle that polyphenols bind iron through the catechol (Smith & Martell, 1989) and galloyl (Brune, Rossander, & Hallberg, 1989) groups.

There is huge progress in the application of statistical analysis in food science and technology owing to the achievable efficiency in experimentation and design and also the ease of interpretation when dealing with huge data. In this case Multivariate Analysis of Variance (MANOVA) was employed to study, differentiate and compare iron binding ability as affected by multiple levels of polyphenol concentrations in conjugation with different ratios of AA and EDTA.

Past studies on effect of ascorbic acid and EDTA, two potential enhancers of iron absorption, have been carried out with specific foods and meals (Cook, Reddy, Burri, Juillerat, & Hurrell, 1997; Davidsson, Walczyk, Zavaleta, & Hurrell, 2001; Hallberg & Rossander, 1984; MacPhail, Patel, Bothwell, & Lamparelli, 1994; Siegenberg et al., 1991) which are poorly characterized in terms of composition, processing conditions and other variables. Well characterized food samples are required to fully resolve effect of enhancers. For more generalized reporting, it is essential to study factors affecting metabolism and absorption of iron in presence of enhancers/inhibitors of iron uptake. Work directed in this

* Corresponding author.
E-mail addresses: poonkodi.09@gmail.com (P. Tamilmani), pandeymc@yahoo.com (M.C. Pandey).

http://dx.doi.org/10.1016/j.foodchem.2015.11.045
0308-8146/© 2015 Elsevier Ltd. All rights reserved.

direction will comprise prime *in vitro* studies in order for maximally predicting *in vivo* events. This study aims at elucidating the effect of polyphenols (tannic acid, gallic acid, chlorogenic acid and catechin), coffee and tea extract as inhibitors of iron absorption at different concentrations and the counter effect of ascorbic acid and EDTA as enhancers of iron absorption at different ratios. Coffee and tea comprises a mixture of phenolics and form a common part of human diet and hence was chosen for the study. Because these beverages have a relatively simple matrix compared to other phenol rich dietary source, they can be considered analogous to the present study. Definite statistical analysis of the data was done to differentiate between the enhancing effect of the AA and EDTA against all samples studied. These results will help in (a) understanding chelator and phenol interactions behavior *in vivo* (b) help in deciding quantity of enhancers to be added to food products in accordance with their polyphenol content to help minimize iron chelation by polyphenol (c) help in preparation of iron supplements with incorporated AA or EDTA.

2. Materials and methods

2.1. Reagents and chemicals

All chemicals used were of analytical grade. Distilled deionized water was used throughout the study. Four standard polyphenols used were – Gallic acid, Tannic acid, catechin and chlorogenic acid. Two market coffee (C1 & C2) and tea (T1 & T2) samples each were procured. Enhancers tested for counteracting negative effects of polyphenols were Ethylenediaminetetraacetic acid (EDTA) and Ascorbic acid (AA). All standards and chemicals were procured from Sigma Aldrich.

2.2. Sample preparation

Polyphenol standards and coffee samples were used as such in preparing appropriate concentrations. Sample preparation for tea involved extracting 2 g of tea leaves in 100 ml boiling water for 5 min. The extract was filtered, pre concentrated in a rotary evaporator at 50 °C. The pre concentrated extract was then subjected to lyophilization to get powdered sample which was used for analysis.

2.3. Iron binding ability of polyphenols

The iron binding ability of polyphenols was determined using the method as designed by Khokhar & Apenten (2003) which measures iron binding ability as catechol and galloyl equivalents. Polyphenol concentrations tested were 40, 80, 160, 240, 320, 400, 520 and 900 μg/ml. Ratios of polyphenol is to enhancer tested were 1:0, 1:1, 1:2, 1:4, 1:6 and 1:8. All samples were prepared in 50% DMF-acetate solution. 2 ml sample at different concentrations was added to 8 ml FAS reagent (89 parts of 50% urea-acetate prepared in 0.1 M acetate buffer, 10 parts 1% gum Arabic solution and 1 part 5% ferric ammonium sulfate in 1 M HCl) and absorbance was measured at 578 and 680 nm after 15 min incubation at room temperature. Analysis was performed in duplicates.

2.4. Statistical analysis

Statistical analysis of data is essential owing to the huge sample size and multiple comparisons done within (effect of eight concentrations and ratios) and between AA and EDTA data sets, helping in clear interpretation. Analysis was done in two stages. The first stage involved analyzing effect of AA and EDTA on pure phenolics and coffee–tea, giving a total of 4 separate MANOVAs with sample size of 192 each. Four individual super variables that maximally differentiate variation in each group were extracted from these analyses which were then subjected to a separate MANOVA, thus being able to get clear differentiation. Multivariate analysis of variance was carried out using SPSS (22.0). The statistical power was tested using G* Power (3.0.10) software.

3. Results and discussion

3.1. Iron binding ability of pure phenolics

The iron binding ability of polyphenols at different phenol-enhancer combinations was studied to find out the trend of reduction in the iron binding ability of phenolics in the presence of different concentrations of AA and EDTA (Fig. 1). Fig. 1 represent five of the total eight concentrations studied for clear observation of trend. Calibration curve was obtained using tannic acid and catechin for galloyl ($y = 0.002027x - 0.054308$; $R^2 = 0.994706$) and catechol ($y = 0.000890x - 0.004269$; $R^2 = 0.996244$) groups respectively. On observing the iron binding efficiency of the four polyphenols without the addition of any enhancer, it can be seen that low concentrations of polyphenols lead to higher efficiency than high concentrations.

It can be seen from Fig. 1 that at higher polyphenol concentrations, lesser AA is required for fully counteracting the iron binding by polyphenols. But invariably, all ratios of AA completely mitigate the iron binding at a ratio of 1:6. The action of iron binding by AA is either by chelating iron or by reducing Fe^{3+} to Fe^{2+}, making it unavailable to bind polyphenols. At concentration above 320 μg/ml the same trend is observed in all polyphenols studied. Whereas, EDTA shows a different pattern of action. EDTA is also known to exert its action by chelating iron. It is seen that irrespective of the concentration of polyphenol, high polyphenol: EDTA ratios are required for completely balancing iron binding by polyphenols. 1:5 and 1:6 ratios seem to favor its action and a more gradual decrease is seen with increasing polyphenol concentrations as opposed to AA. The same experiments representing coffee and tea show the same trend is observed but the magnitude of iron binding is slightly higher than that of pure phenolics. This can be due to presence of mixture of other chelators.

Thus, it can be inferred that the amount of enhancers used in different foods dependent on its inherent polyphenol concentration. This effect if more pronounced in AA where, food containing lower phenolic concentrations require higher AA, whereas, those containing higher concentrations require lower AA.

3.2. Statistical analysis

3.2.1. Assumptions and compliance

Prior to analysis, the four sets of data (AA ↔ pure phenols [I], AA ↔ coffee–tea samples [II], EDTA ↔ pure phenols [III] and EDTA ↔ coffee tea samples [IV]) were checked individually for compliance with assumptions for conducting a MANOVA. The dependent variable is the iron binding ability at various concentrations and independent variable is the ratio of enhancers added. Dependent variables were checked for correlations with a series of Pearson correlation to test for moderate correlations between dependent variables. Significant correlations were observed among most of the dependent variables (concentration of polyphenols). The data were also checked for normal distribution using Shapiro–Wilk test ($p < 0.05$) and those that did not comply with this assumption were subjected to transformation to generate normally distributed sample sets. Additionally, Box's M value ($p < 0.001$) was referred to check equality of covariance matrices. Levene's test of equality of variances was performed at a significance level of

Download English Version:

https://daneshyari.com/en/article/1183359

Download Persian Version:

https://daneshyari.com/article/1183359

Daneshyari.com