Food Chemistry 145 (2014) 859–865

Contents lists available at ScienceDirect

Food Chemistry

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

Cereal foods are the major source of betaine in the Western diet – Analysis of betaine and free choline in cereal foods and updated assessments of betaine intake

Alastair B. Ross [a,b,*], Alicia Zangger [b], Seu Ping Guiraud [b]

[a] Food Science, Department of Chemical and Biological Engineering, Chalmers University of Technology, 412 96 Gothenburg, Sweden
[b] Nestlé Research Centre, Vers chez les Blanc, 26 Lausanne 1000, Switzerland

ARTICLE INFO

Article history:
Received 9 July 2013
Received in revised form 27 August 2013
Accepted 28 August 2013
Available online 7 September 2013

Keywords:
Betaine
Choline
Dietary intake
Whole grain
Wheat
Quinoa
Pseudocereals

ABSTRACT

Betaine and its precursor choline are important components of one-carbon metabolism, remethylating homocysteine into methionine and providing methyl groups for DNA methylation. Cereals are the main source of betaine in the diet, though there is little literature available on the content of betaine in cereal products, nor on betaine intake from cereals. Betaine and free-choline concentrations were measured by liquid-chromatography with tandem mass spectrometry in a wide range of commercially available cereal foods and cereal fractions. Whole grain wheat and related fractions were the best overall common source of betaine, while the pseudocereal quinoa had the highest amount of betaine measured (3900 μg/g). Based on estimates of dietary intake data cereal foods provide approximately 60–67% of betaine in Western diets, and 20–40% of betaine in South-East Asian diets. Average intake of betaine was 131 mg/d, well below those used in intervention studies using betaine to lower blood homocysteine.

1. Introduction

Betaine (glycine betaine, *N*,*N*,*N*-trimethylglycine) has critical functions as an osmolyte and methyl donor in the human body, but is not considered to be an essential nutrient as it can be irreversibly synthesised from free-choline by choline dehydrogenase (Craig, 2004). As an osmolyte, betaine allows waste products to pass against the concentration gradient into urine, and also protects cells against osmotic stress, reaching milimolar concentrations in some cell types (Burg, Ferraris, & Dmitrieva, 2007). As a methyl-donor betaine provides a methyl group for the remethylation of homocysteine to methionine which mainly occurs in the liver (Craig, 2004). Reflecting these two main functions, the highest concentrations of betaine in mammals are found in the kidneys and the liver (Slow, Lever, Chambers, & George, 2009). The role of betaine in reducing circulating homocysteine has received most attention over recent years in human nutrition, as elevated concentrations of plasma homocysteine are a biomarker for elevated vascular disease risk and increased risk of cognitive impairment (Bates, Mansoor, Pentieva, Hamer, & Mishra, 2010; Smith et al., 2010). Both oral betaine and choline supplementation can reduce circulating homocysteine (Olthof, Brink, Katan, & Verhoef, 2005a; Olthof, van Vliet, Verhoef, Zock, & Katan, 2005b), though it is unclear if there are any systemic benefits beyond reduction of homocysteine.

Betaine, and betaine supplements are with varying degrees of scientific substantiation proposed to have a wide range of health benefits (Craig, 2004; Lever & Slow, 2010), though comparatively little work has been done on dietary sources of betaine and its precursor choline and their potential effect on health (Atkinson, Elmslie, Lever, Chambers, & George, 2008; Price et al., 2010; Ross et al., 2011). High dose dietary betaine (500–845 mg/d) appears to have the same effect on homocysteine metabolism as supplementation of with purified betaine (Atkinson et al., 2008; Atkinson et al., 2009). Betaine also functions as an osmolyte in plants (Ahmad, Lim, & Kwon, 2013), and plant-based foods are the best dietary sources of betaine, though the number of commonly consumed plant foods that are high in betaine (>150 μg/g) is limited (de Zwart et al., 2003). Of commonly consumed foods, refined and whole grain wheat are the best sources of betaine and whole

Abbreviations: USDA 1, United States Department of Agriculture Database for the Choline Content of Common Foods release 1; USDA 2, United States Department of Agriculture Database for the Choline Content of Common Foods release 2.

* Corresponding author. Address: Department of Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden. Tel.: +46 31 772 3822.
E-mail address: Alastair.Ross@chalmers.se (A.B. Ross).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.08.122

grain wheat contains 4–5 times the amount of betaine found in refined wheat; 239 vs. 1086 μg/g (Bruce, Guy, Rezzi, & Ross, 2010).

Estimates of betaine intake and the main dietary sources of betaine vary, in part due to errors in the betaine concentration reported in the United States Department of Agriculture Database on Common Choline Containing Foods release 1 (USDA 1) released in (Howe, Williams, Holden, Zeisel, & Mar, 2004). These errors were corrected in the second release of the database in 2008 (USDA 2) (Patterson, Bhagwat, Williams, Howe, & Holden, 2008), but not before a number of papers reporting betaine intake based on USDA 1 data were published.

Choline has had increasing prominence as a conditionally important nutrient, especially for normal lipid metabolism and membrane function (Zeisel & Caudill, 2010). Some recent studies have linked plasma choline concentrations to cardiovascular disease risk (Konstantinova et al., 2008; Wang et al., 2011), probably via a high intake of phosphatidylcholine containing foods and an interaction with gut microbiota (Wang et al., 2011) leading to the production of trimethylamine-*N*-oxide (TMAO). Given these divergent outcomes for plasma choline status, the beneficial effect of sufficient supply of dietary choline being altered into a risk factor for cardiovascular disease is probably more complex than currently understood. Cereal foods generally contain moderate amounts of free choline, and unlike betaine, whole grains are not substantially higher in free-choline compared to refined grains (Bruce et al., 2010).

In this work, we present the results from the analysis of betaine and free-choline in cereal grains and commercially available products in order to help address the paucity of data on the range of betaine and choline in cereal grains. In order to put the amount of betaine measured in cereal products in context, we review the literature around betaine intake and have recalculated estimates of betaine intake based on USDA1. In addition, we follow up on a previous observation that gluten-free cereals appeared to be were largely devoid of betaine (Bruce et al., 2010) with a more comprehensive analysis of betaine and free-choline in gluten-free foods and cereals.

2. Materials and methods

2.1. Cereal samples

Cereal samples were either donated from intervention studies that included cereal foods (see acknowledgements) or were purchased from local supermarkets (Lausanne, Switzerland and Jönköping, Sweden) in order to get coverage of commonly consumed cereal foods, and gluten-free cereal products.

2.2. Sample preparation

Cereal samples were analysed based on the previously developed extraction protocol (Bruce et al., 2010). All food samples were milled to a uniform powder using a laboratory mill (M20, IKA, Germany). Samples with a high water content (e.g. bread) were freeze dried prior to milling. Approximately 50 mg of flour or milled food sample was weighed into 2 mL microcentrifuge tubes. Then 800 μL of internal standard solution in methanol was added to the sample (d_{11}-betaine and d_9-choline, both 100 μM), followed by 800 μl of water. Samples were manually shaken to homogenise them, and then vortexed for 5 min in a multitube vortex mixer and centrifuged (11 min, 4 °C, 5350*g*). A 50 μL aliquot of supernatant was then transferred into an LC–MS vial and diluted with 450 μL of methanol prior to analysis.

2.3. Liquid-chromatography–tandem mass spectrometry (LC–MS/MS)

The LC–MS/MS analyses have been previously described (Bruce et al., 2010). In brief, the analysis were performed using a Transcend TLX1 high pressure LC system (ThermoFischer Scientific) coupled to a TSQ Quantum Ultra AM triple quadrupole MS/MS system (ThermoFischer Scientific). The LC system was coupled with an Atlantis HILIC column (2.1 × 50 mm, 3 μm, Waters) kept at 30 °C. The mobile phase consisted of 10 mM ammonium acetate in water containing 0.005% acetic acid (A) and 100% acetonitrile (B). The flow rate was 600 μL/min, and the injection volume was 3 μL. Analytes were eluted using the following linear gradient conditions: 5% (A) from 0 to 1.0 min, ramped to 98% (A) at 7.0 min, maintained at 98% (A) to 11.0 min, stepped back to the initial conditions (5% (A)) at 11.5 min until 15.50 min. Data was acquired using positive electrospray ionization in multiple reaction monitoring mode by monitoring two transition reactions per analyte (betaine: 118 -> 58, 25 eV and 118 -> 59, 19 eV; choline: 104 -> 60, 17 eV and 104 -> 58, 31 eV). The first transition was used for quantification, and the second as the qualifier ion. The scan width was 0.6 m/z.

The spray voltage was 4000 V and the vaporizer and capillary temperatures were both set to 325 °C. Nitrogen was used for the sheath and auxiliary gases and set at 60 and 45, respectively. The tube lens voltage was optimized for each analyte (betaine: 58 V, choline: 60 V). Aria (version 1.6) and Xcalibur (version 2.1.0) software were used for the data acquisition. Xcalibur was also used for all peak integration and quantification. All the samples were analysed in triplicate and reanalysed if the coefficient of variation for triplicate samples was above 10%, or if the average of an inter-batch reference sample (brown wheat flour) deviated more than 15% from an average determined from 10 different batches.

2.4. Dietary intake data

Literature estimates of the intake of betaine from diet were searched for using Pubmed (www.pubmed.org) and Scopus (www.scopus.com) using the search term 'betaine AND intake'. Papers focusing on population studies and estimating either total betaine intake in a general or healthy population were selected and further reviewed for estimates of betaine intake. Papers where the method of determining betaine intake was unclear, or the database used not stated, were not included in the analysis. The initial USDA 1 database on betaine and choline in foods released in (Howe et al., 2004) overestimated betaine concentration in some foods leading to potential overestimation of betaine intake. This was corrected in the second release of the USDA database in (Patterson et al., 2008). Several papers have reported estimates of betaine intake based on the first release of the database or citing the original paper on which the database was based (Zeisel, Mar, Howe, & Holden, 2003). As these data probably substantially overestimate betaine intake, we estimated a 'corrected' intake from these data if they stated which foods were the most important sources of betaine in the diet, giving a broader overview of betaine intake. Correction was based on the re-estimating the betaine intake for each food group reported based on the difference reported between USDA 1 and USDA 2. Total betaine intake was also recalculated based on these data. The correction is only partial as most papers only reported the top five food sources of betaine, so that between 40% and 90% of betaine intake was corrected, depending on the cohort. Overall betaine intake values were estimated from the average of reported and corrected values, weighted by the number of subjects in each study. Only control subjects were included in the case of case-control studies.