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Betaine, choline and folate content in different cereal genotypes

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ABSTRACT

The importance of dietary methyl donors, e.g. betaine, choline and folate, is increasingly being recognised. This study examined variations in methyl donor concentrations in different cereals grown in Sweden. Fourteen cereal samples, representing different genera and cultivars, were analysed using HPLC-UV/FLD. The content of methyl donors in the cereals varied significantly due to cereal genotype. Betaine content varied most, with 28 mg/100 g DM in oats and 176 mg/100 g DM in rye. Total choline varied less, with 67 mg/100 g DM in rye and 149 mg/100 g DM in naked barley. In wheat, the lowest concentration of folate with 36 μg/100 g DM was found, and the highest of 91 μg/100 g DM in barley. Esterified choline was the major contributor to total choline content (80–95%) in the cereals. Free choline was less abundant, ranging from 3 to 27 mg/100 g DM. 5-CHO-H_4folate was the dominant folate form in all cereals, amounting to approx. 35–50% of the sum of folates, as determined after pre-column conversion. Due to the limited number of available cultivars, no interpretation regarding effects from cultivar can be made. In conclusion, the studied cereal genotypes are good sources of methyl donors, but concentrations show considerable variation between different cereals.

1. Introduction

Methyl donors are required for normal cell function, DNA methylation, phosphatidylcholine synthesis and protein synthesis as reviewed by Obeid (2013). Insufficient dietary intake of methyl donors is associated with a number of health risks. For example, folate deficiency is linked to development of neural tube defects, macrocytic anaemia and cardiovascular disease (Obeid, 2013). Choline deficiency is assumed to have an effect on liver disease, atherosclerosis and neurological disorders (Zeisel and Da Costa, 2009). The US Institute of Medicine (IOM, 1998) established dietary reference intakes for choline of 550 mg/day 425 mg/day for men and women, respectively. There is no recommendation for betaine. Both methyl donors, folate and betaine, are required for the remethylation of homocysteine to methionine. It was shown that supplemental folic acid, besides lowering homocysteine concentrations, even increased plasma betaine concentrations in healthy adults (Melse-Boonstra et al., 2005). Choline can be metabolised to betaine through a two-step oxidation process in the mitochondria. Dietary choline could therefore, in principle, supply all the requirements for both choline and betaine, but the converse is not true. However, dietary betaine is important for its choline-saving effect (Dilger et al., 2007).

Choline, betaine and folate are mainly obtained from the diet and therefore the relative importance of food sources varies with dietary pattern. Cereal foods are the major source of dietary betaine and folate in the Western diet, because of both relatively high content and high cereal consumption (Ross et al., 2014). Since betaine is also a major plant osmolyte, its content in plant foods depends on growing conditions (Corol et al., 2012). In addition, the content of folate in cereals has been found to be significantly affected by genotype, maturity stage and growing conditions (Giordano et al., 2016; Piironen et al., 2008). The majority of choline in the diet appears in esterified form as phospholipid, e.g. glycerophosphocholine, phosphocholine, phosphatidylcholine and sphingomyelin, and only a small part is present as free choline (Patterson et al., 2008), which makes quantification difficult (Phillips, 2012). While LC-MS/MS is considered the golden standard for measuring individual choline forms, not all laboratories have access to this instrumentation but rather rely on UV/FLD-based methods. To our knowledge, most published choline data, except for data in the USDA database (Patterson et al., 2008), are for free choline only. Therefore, the USDA database is commonly used to

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calculate dietary intake of methyl donors, although this can lead to inaccuracies due to lack of data on traditional foods from other regions. Another major challenge in analysis of choline content in foods is extraction and/or hydrolysis. A number of methods have been developed for extraction of choline from foods, including the use of acid, alkali and/or enzymes (Phillips, 2012). Simplification and improvement of a previous extraction method (Hefni et al., 2015) can allow routine quantification of total choline content in a wide range of foods using a simple HPLC-FLD based method. In addition, difficulties in quantification of 5-CHO-H_4folate (the dominant folate form in cereal) by HPLC-UV/FLD have been reported, due to co-elution and weak fluorescence detection (FLD) and ultraviolet (UV) absorbance of the molecule (Gujska and Kuncewicz, 2005; Hefni and Witthöft, 2012; Kariluoto et al., 2006).

The aims of this study were to (1) quantify the content of the methyl donors choline, betaine and folate in different cereal genera and cultivars and (2) improve the methodology for quantification of both 5-CHO-H_4folate and total choline in cereal foods.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and enzymes were purchased from Sigma-Aldrich (St. Louis, USA) except for acetonitrile, which was purchased from VWR International (Stockholm, Sweden). All reagents were of p.a. grade except acetonitrile and methanol, which were of HPLC grade. Water was purified using a Milli-Q system (Merck Millipore, USA). The folate standards folic acid (PteGlu), (6S)-5,6,7,8-tetrahydrofolate sodium salt (H_4folate), (6S)-5-formyl-5,6,7,8-tetrahydrofolate sodium salt (5-HCO-H_4folate), 10-formylfolic acid sodium salt (10-HCO-PteGlu) and (6S)-5-methyl-5,6,7,8-tetrahydrofolate sodium salt (5-CH_3-H_4folate) were purchased from Merck Eprova AG (Schaffhausen, Switzerland). All were stored at −80 °C until use.

The actual concentration of folates and folic acid was determined using the molar extinction coefficients (Hefni and Witthöft, 2012). Standard stock solutions of folates (≈200 μg/mL) were prepared in 0.1 M sodium acetate (pH 4.6) containing 10% sodium chloride, 1% ascorbic acid and 0.1% 2,3-dimercapto-1-propanol under subdued light and stored under nitrogen atmosphere at −80 °C for a maximum of three months. Choline and betaine stock solutions (1 mg/mL for each) were prepared in Milli-Q water, stored at −20 °C and used within 1 month.

Thermostable α-amylase suspension (Sigma-Aldrich, St. Louis, USA) was used for folate extraction without additional preparation. Rat serum was obtained from the animal research house at Linnaeus University (Kalmar, Sweden) and used for folate deconjugation as a source of γ-glutamyl hydrolase. The serum was prepared by dialysis using 0.05 M phosphate buffer (pH = 6.1) containing 0.1% 2,3-dimercapto-1-propanol at 4 °C and stored portioned at −80 °C. A phospholipase D solution (400 U/mL) was prepared using 50 mM Tris-HCl buffer (pH = 8), stored at −80 °C and used to check the efficiency of acid hydrolysis during choline analysis.

2.2. Cereal samples

Grain from two cultivars each of rye (genus *Secale*), barley (genus *Hordeum*) and oat (genus *Avena*) and eight cultivars of wheat (genus *Triticum*) (Table 1), harvested during 2015 and 2016, was obtained from different mills and producers: Västgötarna in Västergötaland county, Warbro mill and Saltå mill in Söderman county, Nibble farm in Söderman county and Isgärde farm on Öland, Sweden. Grain samples (250–500 g) were vacuum-packed in polyethylene bags and stored at −20 °C until analysis. Before analysis, the grain was milled using an ultracentrifuge mill (Cyclotec 1093 sample mill, Foss Tecator, Höganäs, Sweden).

2.3. Analysis

2.3.1. Betaine extraction, derivatisation and quantification

Betaine extraction was carried out in duplicate based on the method of Hefni et al. (2016). Briefly, milled cereals (0.15 g) were homogenized in 5 mL Milli-Q water, shaken for 5 min and centrifuged (5 min, 2000g). The supernatant (containing the betaine) was transferred into a plastic tube. The extraction was repeated once more, and the supernatants combined. After extraction, 4 mL dichloromethane were added to the combined extracts and the mixture was shaken for 5 min in order to remove any hydrophobic compounds. After centrifugation (5 min, 2000 g), the aqueous (top) layer was transferred to another tube and stored (−20 °C) for derivatisation and analysis by HPLC-UV within one week. Betaine in

Table 1
Betaine and free and total choline content (mg/100 g DM) in cereal samples.

Cereal genus	Species	Cultivar (origin)	Harvest year	Dry matter (g/100 g)	Betaine content (mg/100 g)	Choline content (mg/100 g)	
						Free	Total
Rye	*S. cereale*	Schmidt rye (Västgötarna)	2015	87	176 ^{A}	14	70 ^{A}
Secale	*S. cereale*	Rye (Saltå mill)	2016	92	153 ^{B}	11	67 ^{A}
Wheat *Triticum*	*T. spelta*	Spelt (Warbro mill)	2016	89	143 ^{A}	4	108 ^{A}
	T. spelta	Spelt (Saltå mill)	2016	92	137 ^{A}	4	103 ^{A}
	T. aestivum	Öland spring wheat (Isgärde farm)	2016	88	115 ^{BC}	18	101 ^{A}
	T. aestivum	Wheat (Saltå mill)	2016	92	132 ^{AB}	3	97 ^{A}
	T. aestivum	Jacoby Borst lantvete (Nibble farm)	2015	88	123 ^{AB}	7	109 ^{A}
	T. aestivum	Wheat Dala lantvete (Västgötarna)	2015	89	98 ^{CD}	9	106 ^{A}
	T. dicoccum	Emmer (Västgötarna)	2015	88	94 ^{CD}	9	108 ^{A}
	T. dicoccum	Emmer (Warbro mill)	2016	88	83 ^{D}	8	117 ^{A}
Barley	*H. vulgare nudum*	Naked barley (Warbro mill)	2016	86	98 ^{A}	27	149 ^{A}
Hordeum	*H. vulgare*	Barley (Saltå mill)	2016	90	46 ^{B}	5	119 ^{A}
Oat	*A. sativa nuda*	Naked oat (Warbro mill)	2016	91	44 ^{A}	13 ± 2	135 ^{A}
Avena	*A. sativa*	Oat (Saltå mill)	2016	93	28 ^{B}	4	101 ^{B}

Different superscripts within cereal genus represent significant differences ($P < 0.05$). N = 2-4.