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Original research paper

THE BETAINE CONTENT IN COMMON CEREAL-BASED AND GLUTEN-FREE FOOD FROM LOCAL ORIGIN

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ABSTRACT: In this study, betaine content in cereal grains, cereal-based products, gluten-free grains and products of mainly local origin was surveyed. Estimates of betaine are currently a topic of considerable interest. The principal physiologic role of betaine is as an osmolyte and methyl donor. Inadequate dietary intake of methyl groups causes hypomethylation in many metabolic pathways which leads to alterations in liver metabolism and consequently, may contribute to numerous diseases such as coronary, cerebral, hepatic and vascular. Cereals are the main sources of betaine in human diet. Results showed that betaine content in grains is variable. Spelt grain was found to be a richer source of betaine (1848 μg/g DM) than that of common wheat (532 μg/g DM). Gluten-free ingredients and products were mainly low in betaine (<150 μg/g DM). Amaranth grain is a remarkable gluten-free source of betaine (5215 μg/g DM). Beet molasses is an ingredient which may increase betaine content in both cereal-based and gluten-free products.

Key words: *betaine, cereals, gluten-free products, dietary intake, amaranth, beet molasses*

INTRODUCTION

Betaine is a non-essential nutrient which has been hypothesized to decrease the risk of vascular and cognitive diseases because of its role as methyl donor in the one-carbon metabolism. Betaine is proposed to have many other beneficial effects such as increased body endurance, antiinflammatory effect, reduced risk for neural tube defects, depression, and liver and kidney diseases. Betaine has been recommended as an ingredient in dietary supplements because of its ability to increase nutrient absorption from the digestive tract into the bloodstream, hence increasing the effectiveness of nutrient uptake (Cappello, 2013).

Betaine (glycine betaine) is a small zwitterionic compound (quaternary amine), first isolated from sugar beet. It has dual function in human organism. Firstly, it is an osmolyte which preserves the osmotic equilibrium and tertiary structure of macromolecules in the kidney tissue and allows the passing of waste products against concentration gradient into urine. Secondly, it is a donor of methyl groups required for the remethylation of homocysteine to methionine which mainly occurs in the liver (Craig, 2004). Glycine betaine intake was found effective in lowering plasma homocysteine levels in patients suffering from homocystinuria (Wilken et al., 1983) and chronic renal failure combined with hyperhomocysteinemia (McGregor et al., 2002) as well as in healthy subjects (Brouwer et al., 2000). Mildly elevated plasma homocysteine levels are considered to be independent risk factor for cardiovascular, cerebral and peripheral vascular diseases (Welch and Loscalzo, 1998). Elevated plasma homocysteine concentrations have also been implicated in the development of birth defects and various forms of dementia (Seshadri et al., 2002).

Betaine can be formed endogenously by a two-step oxidation of choline, however, it may not be synthesized in adequate amounts and generally needs to be included in the diet. Humans obtain betaine from foods that contain either betaine or choline-containing compounds (Craig, 2004). Betaine is present in foods in variable amounts depending on the source and processing. Among commonly consumed foods, high betaine foods (>150 μg/g) are rather limited and mainly include wheat bran and germ, spinach, beet, bread and other baked products, shrimps (Craig, 2004). Ross et al. (2014) and de Zwart et al. (2003) implicated cereal foods as the major sources of betaine in the Western diet. Plant-based food may contain various glycine betaine analogues such as proline betaine, trigonelline, dimethylsulfoniopropionate (de Zwart et al., 2003). The effects of analogues in the metabolism are not entirely known and glycine betaine was the compound primarily found at higher levels in foods whereas analogues were discovered only in small quantities (Slow et al., 2005).

Betaine can be added to the diet as a supplement. In the US, betaine is self-affirmed as GRAS ingredient whereas in Europe, it received an approval for use in foods by the European Commission (Commision Regulation EU 432, 2012) which allows a health claim to be made on food containing at least 500 mg betaine per portion. Health claim is related to contribution of betaine to normal homocysteine metabolism. Betaine is commercially available in three different forms: natural betaine anhydrous, synthetic betaine anhydrous and betaine hydrochloride. On an industrial scale, natural grade betaine is obtained from sugar beet molasses. Betaine is the primary nitrogenous compound in sugar beet and is extracted from molasses by water-based chromatographic separation and crystallization.

In this work, results from the analysis of betaine in a variety of cereal-based foods with an emphasis on wheat and wheat products (*aestivum*, spelt, durum wheat) are presented. The analysed food items also included gluten-free (GF) ingredients and products. The origin of most analysed items was local or from the wider region (Slovenia).

MATERIAL AND METHODS

Sample collection

Majority of the food samples analysed in this report was randomly obtained from the local market and food stores in Novi Sad (Serbia). Wheat grain (*Triticum aestivum*), all durum wheat (*T. durum*), triticale (*xTriticosecale*), barley (*Hordeum vulgare*), and rye (*Secale cereale*) samples were from the collection of sample reserves of the Laboratory of the Institute of Food Technology. Amaranth grain and related samples were provided from the local producer. Plain GF biscuits and specialty bread enriched with molasses were experimentally obtained and the details of their preparation are described elsewhere (Filipčev et al. 2015, 2010).

Chemicals and reagents

Anhydrous betaine was used as an external standard (98% purity, AlfaAesar GmbH&KG, Germany). All other solvents and reagents were of analytical grade (E. Merck, Darmstadt, Germany).

Extraction and analysis

Betaine is completely soluble in water. The solubility trend is water>methanol>ethanol>n-propanol>n-butanol>2-propanol (Wang et al. 2012). Despite good solubility in water, water was not found appropriate for betaine extraction from the samples because of simultaneous dissolution of impurities which aggravate further chromatographic separation of the analyte. Methanol was found to be more suitable extraction solvent due to better removal of undesirable impurities from the matrix.

Two grams of ground and homogenized sample was weighted and suspended in 25 mL methanol and then it was vortexed for 10 min. After a 30-min ultrasonic extraction in an ultrasonic bath (ATU Ultrasonidos, Spain), the sample was vigorously shaken and centrifuged for 10 min at 5000 r/min (Eppendorf Centrifuge 5804R). Three mL of upper methanol layer was removed and evaporated to dryness. Then the residue was reconstituted in 2 mL of water and filtered through a membrane filter (regenerated cellulose, pore size 0.22 µm, diameter 25 mm, Agilent Technologies, USA).

Betaine is a polar zwitterionic compound. Due to its physicochemical characteristics, it is not suitable to be analysed by conventional reverse-phase HPLC with UV detector. Lee et al. (2012) developed a HPLC method for betaine quantification using ELSD detector. Du Shin et al. (2012) proposed HILIC column in combination with ELSD for betaine analysis.

Betaine analysis was performed using a HPLC system Agilent (Agilent Technologies Inc., USA) equipped with a Kinetex®HILIC (Phenomenex, Germany) column (2.6µm, 100 x 2.1 mm) and ELSD detector. Separation was performed at a flow-rate 0.5 ml/min with a mobile phase of acetonitrile and 10 mM acetate buffer at pH 3.7 following a gradient regime: 90% acetonitrile for 3 min, decrease to 60% acetonitrile during 15 min maintaining this proportion for 6 min and then final increase to 90% acetonitrile. Total runtime was 30 min. Injection volume was 5 µL with autosampler injection mode. Injector was at room temperature. Detector parameters were as follows: evaporator temperature 40 °C; nebulizer temperature 55 °C; gas flow rate 1.60 SLM, PMT gain 3.0.

The method of external calibration was applied. The calibration of the chromatograph was carried out by the addition of anhydrous betaine standard within the range expected in the samples. The betaine area and concentrations were considered as the variables to obtain the linear regression equation. Since large betaine variation in samples was expected, low and high concentration calibration curves were prepared. For the betaine range between 0.05-0.2 mg/mL, equation Y=15573 * X-358.74 (r^2=0.9859, n=4) was used whereas for the range 0.5-1.5 mg/mL, the equation was Y=19606 * X+3509.1 (r^2=0.9821, n=4). All analyses were performed in duplicate.

All values were reported on a dry matter (DM) basis. DM was determined by drying samples in the oven at 105 °C overnight until the constant mass, cooling in a dessicator, and then weighing. All DM analysis was carried out in duplicate.

Statistical analysis

All calculations were performed using Microsoft Excel.

RESULTS AND DISCUSSION

The analysed products were characterized by a variable content of betaine. The majority of samples analysed fell into the range expected from previous reports (Tab. 1, 2) but some discrepancies were observed as well. The highest content of betaine was determined in amaranth grain (7420 μg/g DM), wheat bran (5215 μg/g DM) and biscuits enriched with beet molasses (4421 μg/g DM) (Tab. 3, 4).

Betaine in cereal products

In the refined wheat products, betaine amounts ranged from 218 to 529 μg/g DM whereas in the wholegrain products somewhat higher amounts, but lower than expected, were registered (532-675 μg/g DM). Ross et al. (2014) reported 2-4 times higher betaine in wholegrain wheat products but also emphasized that discrepancies among samples may be due to variation of betaine in the field. Slow et al. (2005) noted that betaine level in grain is highly dependent on the stress level of the crop. For example, growth under drought may cause higher grain betaine levels in comparison to well-irrigated crops. Varietal differences may also be the cause of different betaine levels (Corol et al., 2012). There was a wide variation in the betaine contents of durum flour and semolina; durum flour was much lower in betaine (278 μg/g DM) than the semolina sample (1227 μg/g DM) (Table 3). Literature data mainly reports higher betaine contents (700-1400 μg/g DM) in pasta products which are mainly prepared from durum wheat (Tab. 1).

Table 1.
Betaine content in various cereal-based food reported by other studies

Food		Betaine content (μg/g)	Reference
Flour (origin and type not specified)		270-1110	de Zwart et al., 2003
Pasta (origin and type not specified)		480-1350	
Oats		200-1000	
Pasta			
	Spirals, uncooked	1449	
	cooked	228	
	Organic, uncooked	1472	
	cooked	352	
	Fresh, uncooked	1139	
	cooked	271	
Scones			
	Before baking	296	
	Baked	245	
Bread			Ross et al., 2014
	Refined	200	
	wholegrain	550-1000	
Pasta			
	Refined	650	
	wholegrain	700-1200	
Sweet snacks			
	Refined	100-200	
	wholegrain	300-500	
Breakfast cereals			
	Refined	100-200	
	wholegrain	600-1100	
Oatmeal		62-139	
Pasta			
	Refined	700	
	wholegrain	700-1100	
Bread			Slow et al., 2005
	White	360-520	
	Wholemeal	670-790	
	wholegrain	560-620	
Wheat bran		2300-7200	
Muesli		270-440	
Biscuits		160-430	
Plain cake		170-270	
Plain cracker		1000-1300	
Cheese scones		440-500	
Pasta			
	Fresh fettuccini	1300-1400	
	Instant noodles	990-1400	

Spelt wheat grain and wholegrain flour seems to be a more abundant source of betaine in comparison to common wheat counterparts although this was not confirmed for the refined flour. Spelt bread, in average, contained more betaine than the wheat breads. Other cereals such as triticale, barley and rye showed higher betaine levels in grain in comparison to common wheat. Oat grain performed similarly to common wheat grain. De Zwart et al. (2003) and Ross et al. (2014) reported much lower betaine levels for rolled oats (oatmeal) (60-130 μg/g) but this may be due to processing effects. Mixed grain products such as breakfast cereals widely varied in betaine content, from below detection limit to 471 μg/g DM which is due to variant ingredient composition of this product type which is based on various cereal flakes, dry fruits and bran fractions. If based mainly on oats, the betaine levels are expected to be low (<150 μg/g) as inferred by Ross et al. (2014). In cooked pasta, lower betaine levels were found because betaine is water soluble and easily leach into the cooking water. De Zwart et al. (2003) reported 60-80% betaine losses during boiling (Tab. 3).

Table 2.
Betaine content in various gluten-free ingredients and products reported by other studies

Food		**Betaine content (μg/g)**	**Reference**
Based on corn			
	Grain	<5	de Zwart et al., 2003
	Corn flakes	6-7	Slow et al., 2005
	Extruded corn snacks	21-73	
Commercial gluten-free food (not specified)		trace	Ross et al., 2014
Quinoa		4000	
Rice			
	White, raw	2	
	Brown, raw	3	

Table 3.
Betaine in refined- and wholegrain cereal products

Food	**n**	**Betaine content (μg/g DM)**[*]	**Food**	**n**	**Betaine content (μg/g DM)**
Wheat (*T.aestivum*) and wheat products			Triticale (*x Triticosecale*)		
grain	2	532±60 (490-574)	grain	1	1030
bran	2	5215±237 (5047-5383)	Barley (*Hordeum vulgare*) and products		
flour					
refined	4	529±70 (457-593)	pearled	1	424
wholegrain	1	540			
bread					
white	6	218±187 (108-595)			
specialty (molasses enriched)	2	314±33 (291-338)	bran	1	372
biscuits			flour		
classic	2	354±19 (335-373)	wholegrain	2	899±174 (776-1023)
wholegrain	2	606±98 (537-675)	from hulless var.	1	574
pasta					
dry[**]	3	238±93 (132-306)	Rye (*Secale cereale*) and products		
cooked[***]	2	152±49 (117-186)			
Wheat (*T.durum*) and products			grain	1	2213
flour	2	278±36 (253-303)	wholegrain flour	1	1182
semolina	1	1227	Oats (*Avena sativa*)		
Spelt wheat (*T.aestivum* spp. spelta) and products			grain	3	542±142 (404-688)
grain	4	1848±977 (973-2723)			
flour			Various breakfast cereals	2	236±333 (n.d.-471)
wholegrain	2	1369±103 (1296-1442)			
refined	3	552±37 (522-593)			
bread, refined	2	444±24 (427-461)			

n-number of samples
n.d. – not detected
[*]Mean±SD (range in parenthesis)
[**]plain pasta, pasta enriched with spinach, wholegrain pasta
[***]pasta enriched with buckwheat

Table 4.
Betaine in various gluten-free (pseudo)cereals and products

Food	n	Betaine content (µg/g DM)*	Food	n	Betaine content (µg/g DM)
Maize (*Zea mays*) and products			Proso millet (*Panicum miliaceum*)		
grain	2	205±139 (107-304)	grain, dehulled	1	281
bran	1	104	Buckwheat (*Fagopyrum esculentum*)		
flakes	3	110±9 (103-120)	wholegrain flour	1	108
starch	2	n.d.	pasta	1	390
expanded grain	1	n.d.			
Rice (*Oryza sativa*) and products			Soy bran	1	62
starch	1	n.d.	GF bread mix	2	110±7 (105-115)
pasta	1	n.d.	Sweet biscuits	2	110±5 (107-115)
expanded grain (rice crackers)	3	n.d.	Savoury biscuits	2	52±73 (n.d.-104)
Amaranth (*Amarantus cruentus*) and products			Plain biscuit	2	110±3 (108-113)
grain	1	7420	Enriched plain biscuit**	2	4730±664 (4228-5232)
expanded grain	1	669	Extruded products***	16	364±62 (284-504)
flour	2	1060±233 (895-1225)			

n - number of samples
n.d. – not detected
*Mean±SD (range in parenthesis)
**enriched with beet molasses
***based on corn, enriched with molasses

Betaine content in gluten-free cereals and related products

Ross et al. (2014) and Bruce et al. (2010) found that gluten-free products are generally low in betaine. They reported that most commercially available gluten-free commodities contained less than 150 µg/g betaine. Our study showed that starch, expanded grain, pasta, flakes based on maize and rice contained betaine below limit of detection (Tab. 4). Commercially available GF products (bread mix, biscuits) contained less than 150 µg/g DM betaine which confirms the findings of Ross et al. (2014). Among gluten-free grains, remarkably high level of betaine was found in the amaranth grain (7420 µg/g DM), much higher than that reported by Ross et al. (2014) for amaranth (646 µg/g) and quinoa (3930 µg/g). Expanded amaranth seed was lower in betaine by an order of magnitude but still higher than the range reported for the common wheat. Proso millet and buckwheat grain and products contained moderately high betaine levels whereas soy bran was low in betaine.

Beet molasses addition to formulation of plain GF biscuits markedly increased the betaine content (from 110 to 4730 µg/g DM). Beet molasses is rich in betaine (5-6%) (Higginbotham and McCarthy, 1998) and is used as a raw material for betaine extraction on industrial scale. These results suggest that it would be advisable to include amaranth, quinoa, beet molasses, proso millet and buckwheat into GF product formulations in order to improve the diet of those who follow gluten-free or vegan diet.

Dietary betaine intake

Data on the intake of betaine are limited. Estimations differ across the studies due to differences in methodology used to collect food consumption data and unsystematic overestimation of betaine in some foods in earlier versions of databases (which were corrected in later issues). Surveys encompassed different populations in the USA (Long Island, Massachusetts, Hawaii, Texas), New Zealand, Australia, Canada, Taiwan, China, the Gambia, and Seychelles. Nevertheless, data provided useful information on betaine dietary intake which may serve as early indicators in rating whether the intakes are sufficient or not. From these studies, average betaine intake of 131 mg/d was estimated with large variation among the studied populations (40 mg/d in Texas, 84 mg/d in China and Taiwan, 134 mg/d in Massachusetts) (Ross et al., 2014). Betaine intake was lower for the female population.

According to Olthof et al. (2003), betaine intake in the range of dietary intake is able to provide health effects related to lowering plasma homocysteine and substantiated that diet rich in betaine (≈ 2 g/d) is more effective than a diet poor in betaine (0.5 g/d). Atkinson et al. (2009) reported that a betaine-rich meal providing 800 mg/d betaine exhibited acute health effects similar to a supplement providing 1 g/d betaine. Oral betaine supplementation at high doses (>6 g/d) lowers plasma homocystein in patients with homocystinuria (Perry et al., 1968) but has been associated with some gastrointestinal discomfort (Knopman and Patterson, 2001). It seems that normal betaine dietary intake is more likely to be on the low side indicating possible inadequate betaine status in a general population.

The most consumed food commodity in the diet of an average Serbian citizen is white bread. Average daily per capita consumption of bread is around 300 g (Škrbić and Filipčev, 2007). Considering data from Table 3 for the betaine content in white, specialty and spelt bread and respective moisture contents (not presented here), it can be calculated that a 300-g portion of bread would provide approximately 43.2, 63.2, and 87.1 g/d betaine, respectively. This corresponds to 33, 48 and 66% of the average estimated betaine intake. On the other hand, a 100 g portion of the molasses-enriched biscuit is able to provide around 413 mg betaine, which is half of the amount required to shift diet into the category of betaine-rich. To accomplish a higher betaine intake (at least 800 mg/d), dietary adjustments are recommended that include wheat bran, spelt wheat, buckwheat, amaranth and molasses-enriched biscuits. Food fortification with betaine may also be a feasible way to improve the betaine status in general population.

CONCLUSIONS

Knowing more about betaine content in food is important for the reason to approximate the human intake of betaine and enable planning of meals to provide higher betaine intake. Betaine is a nutrient which seems to be efficient in lowering plasma homocysteine. Elevated plasma homocystein levels are considered to be independent risk factor for cardiovascular diseases. Endeavour towards keeping stable betaine levels in blood by an adequately composed diet might be beneficial for general population and may complement the nutritional strategies focused on lowering cardiovascular risks.

The results from this study confirmed the previous findings that wheat and wheat-based products are good sources of betaine. Spelt wheat is a richer source of betaine than common wheat. However, those who avoid gluten may be in increased risk to have a very low betaine intake. An excellent non-glutenous alternative dietary source of betaine is amaranth. Beet molasses can be viewed as an ingredient which may remarkably increase the betaine content in baked food. The results also showed that betaine content in grains is variable, depending on source which may be associated to varietal differences and environmental factors.

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САДРЖАЈ БЕТАИНА У ЖИТИМА, ПРОИЗВОДИМА ОД ЖИТА И БЕЗГЛУТЕНСКИМ ПРОИЗВОДИМА ПОРЕКЛОМ ИЗ НАШЕГ ПОДНЕБЉА

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Сажетак: У овом раду је приказан садржај бетаина у сировинама и производима на бази жита, као и безглутенским житарицама и производима који су пореклом са нашег поднебља или ширег региона или су комерцијално доступни на домаћем тржишту. Процењивање садржаја бетаина је у последње време у фокусу интересовања истраживача. Бетаин је неесенцијална хранљива материја са двоструком физиолошком функцијом у организму: бетаин је важан осмолит и донор метил група. Недовољан прехрамбени унос метил група изазива хипометилацију у многим метаболичким циклусима, што доводи до промена у метаболизму јетре и самим тим до бројних болести као што су коронарне, церебралне, хепатичне и васкуларне болести. Жита су главни извори бетаина у људској исхрани. Резултати показују да је садржај бетаина у узорцима био променљив. Зрно спелта пшенице садржи више бетаина (1848 μг/г с.м.) од обичне пшенице (532 μг/г с.м.). Безглутенски производи се одликују веома ниским садржајем бетаина (<150 μг/г с.м.). Семе штира је изузетан безглутенски извор бетаина са чак 5215 μг/г с.м. бетаина. Меласа шећерне репе је састојак који може ефикасно да се користи за обогаћивање глутенских и безглутенских производа бетаином.

Кључне речи: *бетаин, жита, безглутенски производ, прехрамбени унос, штир, меласа шећерне репе*

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