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# Glycine betaine and glycine betaine analogues in common foods

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**Abstract**

In this study we have surveyed the betaine content of a wide range of foods commonly found in the western diet. Glycine betaine, proline betaine (stachydrine), trigonelline and dimethylsulfoniopropionate (DMSP) were the only betaines to be found at ⩾150 μg/g. Glycine betaine was primarily found in shellfish, flour, and some vegetables, such as beetroot, spinach and silverbeet. Proline betaine was found in citrus fruit and alfalfa sprouts, while trigonelline was found in coffee, chick peas, lentils and rolled oats. Significant DMSP was only found in some shellfish. Different sources of individual foods showed variation in betaine content, and the way in which individual foods were cooked affected betaine content, with boiling causing the highest loss of betaine.


*Keywords:* Glycine betaine; Proline betaine; Stachydrine; Trigonelline; Dimethylsulfoniopropionate; DMSP; Dietary intake; Homocysteine; BHMT

## 1. Introduction

Glycine betaine (*N,N,N*-trimethylglycine) is a small zwitterionic compound which is produced by a wide variety of organisms (bacteria, plants, invertebrates, and mammals). It was first isolated from the sap of sugar beet, *Beta vulgaris*, hence the name betaine (Scheibler, 1869). In mammals, glycine betaine acts as an osmolyte in the inner medulla of the kidney, preserving osmotic equilibrium, while also maintaining the tertiary structure of macromolecules (Yancey & Somero, 1979; Yancey & Burg, 1990). In humans, glycine betaine can be readily absorbed through dietary intake or endogenously synthesised through the catabolism of choline in the liver (Flower, Pollitt, Sanford, & Smyth, 1972). The concentration of glycine betaine in human plasma is highly regulated (Chambers & Lever, 1996), although concentrations are lower in patients with renal disease (Lever, Sizeland, Bason, Hayman, & Chambers, 1994), and urinary excretion is elevated in patients with diabetes mellitus (Dellow, Chambers, Lever, Lunt, & Robson, 1999). Glycine betaine is also an important source of methyl groups required for the formation of methionine and *S*-adenosylmethionine (SAM) (Chambers & Lever, 1996; Barak, Beckenhauer, & Tuma, 1996).

Various glycine betaine analogues are found in plants. High levels (up to 100 μmol/g dry mass) of proline betaine (stachydrine) can be found in some citrus varieties (Nolte, Hanson, & Gage, 1997), while trigonelline can be found in coffee beans (Mazzafera, 1991), tomatoes (Rajasekaran, Aspinall, Jones, & Paleg, 2001), and alfalfa (Tramontano & Jouve, 1997). Levels of these betaines can be up to three-fold higher in plants that are under salt stress. Dimethylsulfoniopropionate (DMSP), a sulfonium analogue, and arsenobetaine, are found in marine bacteria, and phytoplankton, and are consequently accumulated by filter feeders and marine animals (Blunden & Gorden, 1986; Yoshida et al., 2001). Proline betaine, trigonelline and arsenobetaine have been detected in human plasma and urine samples. These are believed to be predominantly of dietary origin and are readily absorbed (Fowler, 1977; Sizeland, Chambers, Lever, Bason, & Robson, 1993). Unlike glycine betaine, these compounds are not accumulated in the kidney (Lever et al., 1994).

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Glycine betaine intake can lower plasma homocysteine levels in patients suffering from homocystinuria (Wilken, Wilken, Dudman, & Tyrrell, 1983), and in chronic renal failure patients with hyperhomocysteinemia (McGregor et al., 2002), as well as in healthy subjects (Brouwer, Verhoef, & Urgert, 2000). Homocysteine is derived through the metabolism of methionine, and has been recognised as an independent risk factor for the development of vascular disease (Wilken & Gupta, 1979; reviewed by Hankey & Eikelboom, 1999). In a reaction catalysed by betaine-homocysteine methyltransferase (BHMT; E.C. 2.1.1.5), a methyl group is transferred from glycine betaine to homocysteine, producing methionine and *N,N*-dimethylglycine (reviewed by Malinow, 1994).

As glycine betaine analogues also interact with BHMT, it is possible that they could interfere with, or enhance, homocysteine metabolism. Studies with purified enzyme indicate that proline betaine is a substrate for porcine BHMT in vitro (Mulligan, Laurie, & Garrow, 1998), while purified BHMT also utilises DMSP as a substrate at seven times the rate of glycine betaine (Goeger & Ganther, 1993; Garrow, 1996).

Prior to the present study, there was little information on the content of glycine betaine or glycine betaine analogues in the diet, other than for a small number of foods that have high levels. We present a survey of the betaine content of foods, identify the variation in a subset which contains significant levels ($\geqslant$150 μg/g) of any betaine, and determine the effect that different cooking methods have on the betaine content.

## 2. Materials and methods

### 2.1. Sampling and reagents

The foods included in the survey consisted of a combination of common foods and foods likely to contain betaine. Foods were purchased from local supermarkets, butchers, greengrocers and fishmongers. Where possible, at least two different sources of each food type were sampled [$2<n<10$, for the general food survey, $2<n<15$ for the variation study, and $2<n<6$, for the cooking study ($n$ = the number of samples analysed)].

All reagents were of analytical-reagent grade. Betaine standards were prepared using bidistilled water. Arsenobetaine was obtained from Fluka (Basel, Switzerland); betonicine, butyrobetaine, *N,N*-dimethylglycine, ergothionine, glycine betaine, and trigonelline from Sigma, (St Louis, USA). Dimethylsulfoniopropionate (DMSP), proline betaine (stachydrine), and propionobetaine (β-alanine betaine) were synthesized as described previously (Randall, Lever, Peddie, & Chambers, 1995; Samuelsson, Randall, Happer, Peddie, & Lever, 1998) (Fig. 1). Acetyl carnitine and carnitine were not quantified during this investigation, as nutritional data already exists (reviewed in Rebouche, 1992). All foods were extracted using dichloromethane, which removes hydrophobic compounds, increases column life and was shown not to remove betaines. All extracted samples were stored frozen until analysis.

### 2.2. Cooking methods

Cooking methods included boiling, steaming, microwaving, baking, and frying. Cooking times were as recommended on the packaging or taken from a cookbook (Edmonds, 1999).

### 2.3. Betaine extraction from foods

Edible portions of the uncooked and cooked solid foods were processed using a standard kitchen food processor (Moulinex Multimoulinette, Model T71) and approximately 1 g was further homogenised (Ultra Turrax homogenizer) to form a paste. If a homogeneous paste could not be achieved, water was added, and the volume recorded. The homogenates were shaken for a minimum of 5 min, then centrifuged for 5 min at 2000 *g*. The aqueous supernatant was removed and extracted using an equal volume of dichloromethane. After centrifugation at 2000 *g* for 5 min, the aqueous layer was stored for analysis.

Liquid foods were shaken with an equal volume of dichloromethane, centrifuged for 5 min at 2000 *g* and the resulting aqueous layer stored for analysis.

The method described above proved unsuitable for fatty foods (i.e. butter and oils). These foods were suspended in dichloromethane, and were extracted with an equal volume of bidistilled water, and shaken for 5 min. The two phases were separated by centrifugation for 5 min at 2000 *g* and the aqueous layer was stored for analysis.

### 2.4. Derivatization and HPLC

The derivatization of the extracted samples was based on the method of Lever, Bason, Leaver, Hayman, and Chambers (1992). A 5 μl aliquot of the extracted sample was added to 250 μl acetone, and vortexed prior to derivatization with 2-naphthacyl trifluoromethanesulfonate. Samples were transferred to capped HPLC vials for analysis.

Betaine analysis was performed by HPLC (SIL-10AD VP, Shimadzu, Japan) using Zorbax (Agilent, USA), Alumina (Merck, Germany), or Phenosphere (Phenomenex, Germany) columns (5 μm 4.6×250 mm). In order to ensure accurate identification of each betaine, at least two different HPLC systems were used for the analysis of each food. Separation was performed at a flow-rate of 1 ml/min, with a mobile phase

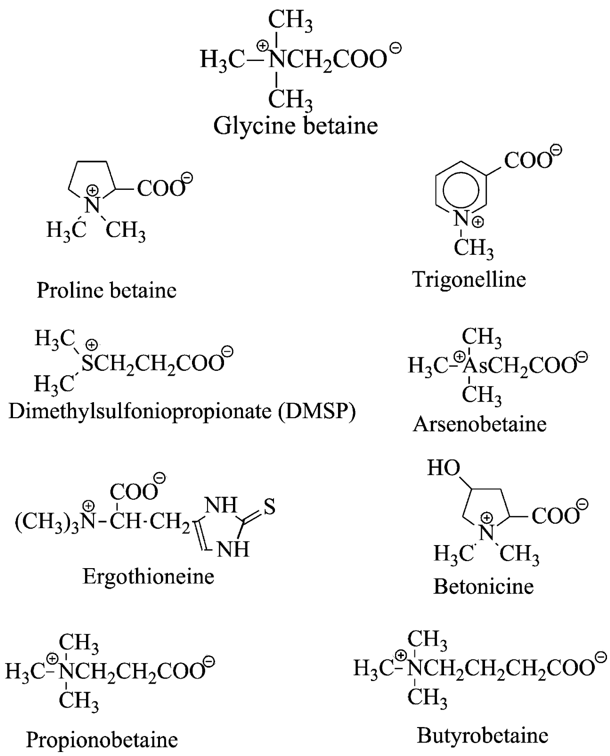


Fig. 1. Chemical structures of betaines analysed in this study. Trivial names are written under each structure.

of either tetramethylammonium hydroxide buffer (7 mM tetramethylammonium hydroxide, 14 mM glycolic acid), 10% water in acetonitrile (Zorbax and Phenosphere columns, 45 min run time), or 0.01 M succinic acid; 0.036 M triethylamine buffer, 4.5% water in acetonitrile (Alumina column, 60 min run time). The column temperature was maintained at 40 °C, and sample injection volume was 100 μl. The HPLC eluates were monitored at 249 nm, and the detection limit was 1 μg/g. The concentration (μg/g) of the various betaines was quantified by comparing to external standards.

## 3. Results

Quantitatively, the only betaines that were found at levels greater than 10 μg/g in the foods analysed were glycine betaine, proline betaine, trigonelline and DMSP. DMSP was only found in shellfish at values ranging from 20 to 200 μg/g and a high variability of DMSP content was observed in different shellfish varieties. It should be noted that foods in which DMSP was detected also had high levels (up to 10-fold higher) of glycine betaine. In addition, a small amount of arsenobetaine was detected in perch and gurnard, ranging from 2 to 10 μg/g. No other food had detectable levels of arsenobetaine. Betonicine, butyrobetaine, ergothionine and propionobetaine were not detected in any of the foods analysed.

Table 1 shows the glycine betaine, proline betaine and trigonelline contents of the foods that were analysed in the general food survey. Foods that are shown in bold had high levels ($\geqslant 150$ μg/g) of one or more, of either glycine betaine, proline betaine or trigonelline. High levels of glycine betaine were found in shellfish, flour and some vegetables (beetroot, silverbeet and spinach), while high levels of proline betaine were found in citrus fruits and legumes, and high levels of trigonelline were found in coffee, legumes (peas, alfalfa sprouts) and oats.

For some of the foods with high levels of betaine, more samples were obtained from different sources to determine if the betaine content varied, depending on the source of the food (Table 2). Food samples from the different sources showed variable levels of betaine. Furthermore, the betaine content was found to vary, depending on how the food had been processed, for example, canned beetroot (203–305 μg/g glycine betaine) compared to fresh (1680–2230 μg/g glycine betaine).

Cooking affected the betaine content of food (Table 3). No significant losses were observed during baking, microwaving, or frying, while only small to medium losses was observed during steaming. Large losses (60–80%) occurred during boiling; however the betaines could be recovered from the water. Glycine betaine was found to be heat-stable at 220 °C, and only small losses occurred (<14%) after 30 min at 250 °C.

## 4. Discussion

### 4.1. General

Betaines not only act as osmolytes, but may have a role as a possible therapy in lowering the plasma concentration of homocysteine in humans. Several studies have provided strong evidence that high plasma homocysteine levels are linked to an increased incidence of vascular disease (reviewed by Hankey & Eikelboom, 1999).

Nutrition has a major impact on the incidence of vascular disease and vascular health. Manipulating the dietary intake of beneficial compounds would provide a practical and inexpensive means of decreasing the incidence and cost of vascular disease (Gardner, 2001). To make this possible, quantification of the contents of beneficial compounds in typical foods is required. In this study, we have surveyed the betaine content of a wide variety of foods to ascertain which betaines are present and at what levels. This is the first step in assessing whether increasing dietary betaine intake is a practical means of reducing the risk of vascular disease development.

Table 1
Glycine betaine, proline betaine and trigonelline contents (μg/g) in different foods[a]

| Food | Glycine betaine | Proline betaine | Trigonelline |
|---|---|---|---|
| *Fruit & Vegetables* | | | |
| **Alfalfa Sprouts** | 35 | **240** | **181** |
| Avocado | <5 | – | <5 |
| Banana | <5 | – | 23 |
| Bean | 30 | – | 58 |
| **Beetroot** | **750** | <5 | <5 |
| Broccoli | <10 | – | – |
| Brussel Sprouts | <5 | 34 | 31 |
| Cabbage | – | – | – |
| Carrot | – | – | – |
| Cauliflower | <10 | – | – |
| Celery | – | – | – |
| **Chick Peas** | – | – | **350** |
| Corn | <5 | – | <5 |
| Courgette | – | – | 15 |
| Cranberry | – | – | – |
| Cucumber | – | – | <5 |
| Feijoa | – | – | – |
| Garlic | <10 | – | <10 |
| Grape | – | – | – |
| **Grapefruit** | – | **190** | – |
| Kiwifruit | <5 | – | – |
| Kumara | 110 | <5 | – |
| Leak | – | – | – |
| Lemon | – | 120 | – |
| **Lentils** | <10 | – | **250** |
| Lettuce | <10 | – | <5 |
| Mango | – | – | – |
| Mushroom | <10 | 15 | <5 |
| Nectarine | – | – | – |
| Onion | <5 | <5 | <10 |
| **Orange** | – | **510** | 18 |
| Parsnip | <5 | <5 | – |
| Passionfruit | – | – | – |
| **Pea** | <5 | – | **280** |
| Pear | <10 | <5 | – |
| Pepper | <10 | – | – |
| Plum | <5 | – | – |
| Potato | <10 | – | – |
| Pumpkin | – | – | 30 |
| **Silverbeet** | **910** | 50 | <5 |
| **Snow Pea Shoots** | <10 | – | **160** |
| **Spinach** | **740** | – | 100 |
| Swede | <5 | <5 | – |
| Tamarillo | – | – | – |
| Tomato | – | – | – |
| Yam | <5 | – | <10 |

| Food | Glycine betaine | Proline betaine | Trigonelline |
|---|---|---|---|
| *Meat* | | | |
| Beef | 58 | – | – |
| **Chicken** | **200** | – | <5 |
| Lamb | 72 | – | – |
| Pork | 41 | – | – |
| *Seafood* | | | |
| **Clams** | **2500** | 15 | <10 |
| Cod | 25 | <5 | – |
| Groper | 12 | – | – |
| Gurnard | <10 | – | – |
| **Monkfish** | **500** | 40 | 10 |
| **Mussel** | **1630** | 26 | 83 |
| Perch | 26 | – | – |
| Salmon | 19 | – | – |
| Terakihi | <5 | – | – |
| Tuna | <10 | – | – |
| *Other foods* | | | |
| Butter | – | – | – |
| Canola Oil | – | – | – |
| Cheese | – | – | <10 |
| Chocolate | <5 | – | <5 |
| **Coffee** | – | – | **2200** |
| Cottage Cheese | <5 | – | – |
| Cream | <5 | – | – |
| Cream Cheese | <10 | – | – |
| Egg | <10 | – | – |
| **Flour** | **730** | – | – |
| Honey | – | – | – |
| Margarine-made with olive oil | <5 | – | – |
| Marmite-a yeast extract | <10 | – | – |
| Milk | <10 | – | – |
| Milo | 34 | – | – |
| Olive Oil | – | – | – |
| **Pasta** | **820** | – | – |
| Peanuts | <5 | – | – |
| Red Wine | <5 | – | 14 |
| Rice | – | – | – |
| **Rolled Oats** | 130 | – | **230** |
| Sour Cream | <5 | – | – |
| Soy Sauce | – | <5 | – |
| Sugar | – | – | – |
| Tea | 24 | <10 | 16 |
| Vegemite-a yeast extract | <5 | – | – |
| Yoghurt | <5 | – | – |

[a] After repeated measurements, the values from the chromatographic system that gave the lowest concentration of betaine are reported. Foods highlighted in bold showed high levels (⩾150 μg/g) of one or more of the betaines.

### 4.2. General food survey

The results presented here show that, aside from acetyl carnitine and carnitine, (which were not quantified in this study; reviewed by Rebouche, 1992), the only other betaines that were found to be present in food at high levels were glycine betaine, proline betaine, trigonelline and DMSP. The levels of glycine betaine, proline betaine and trigonelline in the normal western diet can be attributed to a small number of foods and, generally, each food type tended to have only one betaine present at high levels (⩾150 μg/g,

Table 1). DMSP (20–200 μg/g) was only found in some shellfish.

### 4.3. Glycine betaine

High levels of glycine betaine were found in animal products, especially shellfish, and in some plants, specifically members of the beet family (e.g. beetroot and spinach), and grain. High levels of glycine betaine were also detected in chicken; however, it is not known whether the level observed is a result of endogenous synthesis, or a result of the chicken's diet, as they are largely grain-fed.

The main source of glycine betaine in the normal western diet would be from flour products (e.g. bread and pasta). There would only be a small contribution of glycine betaine from shellfish, as they are not consumed regularly, or in large quantities (Jones-Putnam & Allshouse, 1999), thus glycine betaine uptake could be substantially increased, in the western diet, by increasing shellfish consumption.

### 4.4. Proline betaine

Most proline betaine in the diet originates from citrus fruit, and to a lesser extent some legumes. Increased consumption of citrus fruit would increase proline betaine intake. In addition, because of its osmoprotectant and cryoprotectant properties, it has been suggested that the levels of proline betaine could be increased in various citrus varieties through genetic engineering (Nolte et al., 1997). It is therefore possible that, in the future, some citrus varieties may be engineered to have high proline betaine levels.

### 4.5. Trigonelline

Trigonelline was found primarily in coffee, and legumes (peas, chickpeas and lentils). Previous studies have found that the level of trigonelline in green coffee beans depends on the variety under investigation. The levels of the two most common varieties are reported to be in the range of 6000–13,000 μg/g (*Coffea arabica*), and 3000–11,000 μg/g (*Coffea robusta*) (Stennert & Maier, 1994). During roasting, trigonelline can be converted to nicotinic acid (Mazzafera, 1991). Stennert and Maier (1994) examined a range of roasted coffee beans, reporting an average of 6200 μg/g of dry matter, and other studies consistently report the level of trigonelline to be at approximately 1% (1000 μg/g dry matter) (Wu, Skog, & Jagerstad, 1997). The trigonelline content of instant coffees are reported to be in the range of 3000–26000 μg/g, with no significant differences between spray-dried, freeze-dried and decaffeinated extracts (Stennert & Maier, 1994).

The level of trigonelline found in this study was much lower (2000 μg/g) than previously reported values. The extraction method used for this study was infusion, using a standard household coffee plunger, whereas previous studies have used a more rigorous extraction method, in which coffee beans are boiled for 10 min. Although the method used in this study does not extract all the trigonelline present in the coffee, it more closely resembles what is likely to be consumed. The amount of trigonelline consumed per cup of coffee may therefore vary significantly depending on how the coffee is prepared.

Two recent intervention studies have shown that both filtered (Urgert, van Vliet, Zock, & Katan, 2000) and unfiltered (Grubben et al., 2000) coffee, consumed daily over a 2–4 week period, elicits a significant increase (approximately 10%) in plasma homocysteine concentration. Although there is little known about the acute effects or the mechanism(s) causing the observed homocysteine rise, it may be, at least in part, a result of the ingestion of trigonelline, which may interfere with the BHMT pathway.

In legumes, trigonelline functions as a cell cycle regulator during the growth of many legume root meristems (Tramontano & Jouve, 1997). It was therefore not surprising to see high levels of trigonelline in the legumes that were analysed in this study.

### 4.6. Dimethylsulfoniopropionate (DMSP)

In addition to the high levels of glycine betaine, shellfish also contained DMSP at approximately tenfold lower concentrations than glycine betaine. DMSP has been shown to be a more effective substrate for BHMT (Goeger & Ganther, 1993). Increasing shellfish consumption would not only increase glycine betaine uptake, but may also provide a source of DMSP, which may be more effective in lowering plasma homocysteine via BHMT.

### 4.7. Betaine variability

The betaine content of the same food type from different sources was highly variable (Table 2). If the biological function of betaines is taken into account this variability is not surprising. As a result of their osmoprotectant and cryoprotectant properties, the level of betaine would be expected to be dependent on the stress level of the organism. For example, grain crops grown during a drought would be expected to have higher levels of glycine betaine than crops that were grown on well-irrigated fields. Similarly, trigonelline has been found to increase two-fold in alfalfa during salt stress (Tramontano & Jouve, 1997).

The level of betaine was also found to vary widely, depending on the way in which food had been processed (e.g. canned beetroot compared with fresh), or cooked

Table 2
Variation in the levels of betaine (μg/g) in foods from different sources

| Food | Glycine betaine Min−Max±S.D.[a] | Proline betaine Min−Max±S.D.[a] | Trigonelline Min−Max±S.D.[a] |
|---|---|---|---|
| *Fruit & Vegetables* | | | |
| Alfalfa Sprouts | – | 299−363±24(6) | 120−235±31(6) |
| Beetroot-fresh | 1680−2230±261(5) | – | – |
| Beetroot-canned | 203−305±45(4) | – | – |
| Chickpeas | – | – | 366−556±87(4) |
| Grapefruit | – | 104−165±34(3) | – |
| Lemon | – | 113−175±33(3) | – |
| Lentils | – | – | 298−326±14(3) |
| Orange | – | 97−341±87(9) | – |
| Pea | – | – | 89−173±32(7) |
| Tangelo | – | 160−309±63(4) | – |
| Shellfish | | | |
| Mussel (unprocessed) | 9400−11600±120(4) | – | – |
| Mussel (smoked) | 1120−1680±590(8) | – | – |
| Mussel (cooked) | 1770−2120±120(7) | – | – |
| Oyster | 2780−2810±22(2) | – | – |
| Scallops | 640−1180±250(4) | – | – |
| *Other foods* | | | |
| Coffee | – | – | 3000−13000[b] |
| Flour | 270−1110±258(6) | - | – |
| Oats | 200−1000±360(4) | - | 150−260±55(4) |
| Pasta | 480−1350±278(10) | - | – |

[a] Minimum and maximum values (μg/g).
[b] Data taken from literature (reviewed by Stennert & Maier, 1994).
Numbers in parentheses show number of samples analysed.

Table 3
The betaine content of different foods after cooking using various methods

| Food | Cooking Method | Average betaine content (μg/g±S.D.) | | Betaines remaining (%) |
|---|---|---|---|---|
| | | Uncooked | Cooked | |
| *Vegetables* | | | | |
| Frozen Peas | Boiled | 203±171(2) | 116±22(3) | 57 |
| | Steamed | 203±171(2) | 152±17(3) | 68 |
| | Microwave | 203±171(2) | 188±12(3) | 90 |
| Spinach | Boiled | 268±53(5) | 82±3(3) | 30 |
| | Steamed | 268±53(5) | 169±30(6) | 63 |
| | Microwave | 268±53(5) | 289±41(6) | 108 |
| Silverbeet | Boiled | 1847±1247(2) | 490±52(3) | 27 |
| | Steamed | 1847±1247(2) | 1439±1169(3) | 78 |
| | Microwave | 1847±1247(2) | 1589±633(3) | 86 |
| *Other foods* | | | | |
| Pasta | | | | |
| -Spirals | Boiled | 1449±98(2) | 228±11(2) | 16 |
| -Organic | Boiled | 1472±3(2) | 352±19(2) | 24 |
| -Fresh | Boiled | 1139±1(2) | 271±25(2) | 24 |
| Falafel[a] | Fried | 202±9.5(3) | 220±10(3) | 109 |
| | Baked | 202±10(3) | 231±26(3) | 114 |
| Scones[b] | Baked | 296±46(3) | 245±93(3) | 83 |

[a] Food consisting primarily of chick peas.
[b] Dough made with flour.
Number in parentheses show number of samples analysed.

(e.g. boiling compared with microwaving, Table 3). As betaines are small, highly water-soluble molecules, large losses during certain types of food processing and cooking were not unexpected.

Some special diets are also likely to exclude major sources of betaines. For example, patients with coeliac disease are likely to have a lower than average glycine betaine intake because of the lack of wheat products in their diet, while vegetarians are likely to have higher than average intakes of trigonelline because they generally consume higher amounts of legumes (chickpeas, lentils).

From the results of this study, it is relatively simple to manipulate the diet such that it is possible to consume 500 mg/day of glycine betaine. A further 500 mg/day can be derived from choline catabolism (Zeisel & Blusztajn, 1994). The effects of other betaines, in particular trigonelline and proline betaine, are unknown, and need to be investigated. In addition, the levels of betaine in the diet can vary widely, depending on the source of the food, and the way in which the food is processed and/or cooked. The total level of glycine betaine, therefore, can be significantly increased through changes in dietary intake. Dietary manipulation of glycine betaine levels may provide a practical and inexpensive means of improving human health.

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