



ORIGINAL ARTICLE

Chemical and Biological Liberation of Trimethylamine from Foods

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Knowledge of the chemical composition of foods is essential before attempting dietary manipulation to manage an inborn error of metabolism. These data are usually obtained by chemical means within a laboratory. Avoidance of the ingested precursors of trimethylamine would greatly aid the control of a condition known as the “fish-odour syndrome” (trimethylaminuria), but the appropriate dietary data are limited. In this context, 30 common foods were investigated to assess their “trimethylamine-producing capacity” following both chemical (alkaline hydrolysis) and biological (ingestion and subsequent urine analysis) liberation techniques. All resultant samples were examined for trimethylamine by head-space gas chromatography. With the exception of fish and seafood (cod, mackerel, prawn), no association was observed between chemical and biological liberation values. Some foods (beef, biscuit, chicken, lamb, peanut, pork, soya) gave greater values following chemical hydrolysis that were not reflected in their biological results. Thus, data concerning trimethylamine levels in foods obtained from chemical hydrolysis studies cannot be used to predict successfully which particular foods will actually provide trimethylamine in the biological situation following ingestion.

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INTRODUCTION

Dietary manipulation is a major tool in the management of many inborn errors of metabolism and clinical decisions concerning the choice of which particular foods to exclude from the diet are usually based on sometimes-scant knowledge of their chemical components. The “fish-odour syndrome” (trimethylaminuria) is such an inherited disorder in which the flavin-monooxygenase enzyme system (FMO3 isozyme) is unable to catalyse the formation of the non-odorous N-oxide of trimethylamine leading to the excessive excretion of the malodorous amine in the urine, sweat and breath (Ayesh *et al.*, 1993; Dolphin *et al.*, 1997; Mitchell, 1996;

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Mitchell and Smith, 2001). The exclusion of trimethylamine from the diet, the effective removal of the enzyme substrate, would ease this problem considerably.

Very little free trimethylamine is ingested owing to its strong fish-like odour and the majority of the body's intake is derived within the gut by microbially assisted liberation from various precursors present in the diet. Trimethylamine N-oxide, choline and carnitine, when fed in a chemically pure form, are known to provide trimethylamine (De La Huerga and Popper, 1951; Rebouche and Chenard, 1991; Strack *et al.*, 1963; Zeisel *et al.*, 1983, 1989a, b) and other chemicals such as phosphorylcholine, glycerophosphorylcholine and lecithin (phosphatidylcholine) are suspected of this role. However, there are other potential precursors containing a trimethylamino function, such as ergothioneine, sinapine and the sphingomyelins, together with presumably as yet unidentified chemicals, which may contribute to the total trimethylamine-producing capacity of foods.

Owing to the relatively widespread and uncertain nature of these trimethylamine precursors, an apparently suitable diet for "fish-odour syndrome" patients compiled from available food analysis tables would lead to the omission of many foods, and may be nutritionally unsound, unnecessarily restrictive, and hence unpopular and probably ineffective owing to non-compliance.

To obtain more information, a relatively destructive procedure of alkaline hydrolysis has been attempted to determine the overall "trimethylamine-producing capacity" from the combination of all the various precursors within the foods. This should permit the foods to be tentatively placed into a rank order. However, this line of deduction may be flawed as it assumes that the actual biologically mediated liberation (mainly via enzymes) of trimethylamine from precursors within the ingested food will be identical with, or at least be similar to, that achieved by chemical degradation in the laboratory. To explore this issue, the foods were additionally examined for urinary trimethylamine production following their ingestion by volunteers. This permitted the chemical and biological treatments to be compared.

MATERIALS AND METHODS

Chemicals and Foods

Isopropylamine (2-aminoethanol), trimethylamine hydrochloride (trimethylammonium chloride) and trimethylamine N-oxide dihydrate (trimethyloxamine) were obtained from Sigma Chemical Co. Ltd (Dorset, U.K.). Aqueous titanium trichloride (titanous chloride; 30% (w/v) in 24% (w/v) aq. HCl) was obtained from British Drug House (Merck) Ltd (Dorset, U.K.). All other chemicals and reagents were of analytical grade and freely available in the laboratory. Foods were purchased from local stores and were cooked, where appropriate, in the normal manner.

Chemical Liberation of Trimethylamine

A known weight (up to 1 g) of food, homogenized if necessary in dilute hydrochloric acid (0.1 M), was taken and added to a screw-capped glass vial containing acidified isopropylamine as internal standard (0.5 mL; 0.0386 g/L of 0.1 M HCl). Pelleted potassium hydroxide (3 g) was added before sealing the vial with an airtight PTFE-lined septum cap and the vial was left on ice to cool. The vial was then vortex-mixed and heated at 120°C in an aluminium heating block for 24 h after which an aliquot (0.5 mL) of the head-space gas was injected directly onto the analytical column of the gas chromatograph (Zhang *et al.*, 1992).

Biological Liberation of Trimethylamine

Six healthy male volunteers were recruited (age 32 ± 5 years, weight 85.4 ± 8.2 kg, mean \pm S.D.) for the studies. None were smokers and four took moderate amounts of alcohol (approx 15 units/week; approx 210 g ethanol/week), but not immediately before or during the test periods. All subjects were free from medication during the test periods and fortuitously no one was required to take antibiotics during the entire study period. The protocols were fully explained to the volunteers before the commencement of the investigations and ethical approval was obtained from the local ethics committee.

The volunteers fasted overnight and discarded all urine voided in the morning. A light breakfast was consumed (no seafood) plus the particular food (227 g, 8 oz) under investigation and the following 0–8 h urine collected, during which time only water was allowed. This was repeated for all the foods examined. At least 1 week separated each individual study. In previous investigations with chemical precursors, a 0–8 h urine collection period was sufficient to demonstrate an increase in trimethylamine output. Urine was collected into plastic bottles containing hydrochloric acid (10 mL, 6 M) that acted as a preservative and also kept volatile amines in solution as their water-soluble hydrochloride salts. Multiple aliquots (20 mL) of the measured total urine volumes were stored frozen (-20°C) in the dark until analysis. Thawed urine samples (2 mL) were chemically reduced with aqueous titanous chloride (0.2 mL; 30% (w/v) in 24% (w/v) aq. HCl) in a vial at 30°C for 30 min to convert trimethylamine N-oxide to trimethylamine. The treated samples were then diluted with water (up to 10 mL, depending upon final concentration) as appropriate. Aliquots (5 mL), spiked with isopropylamine (30 μL , 20.8 μg) as internal standard, were then placed into a screw-capped glass vial (15 mL) and pelleted potassium hydroxide (2 g) added before sealing the vial with an airtight PTFE-lined septum cap and the vial was left on ice to cool. The vial was then vortex-mixed and heated at 90°C for 20 min in an aluminium heating block, after which an aliquot (2 mL) of head-space gas was injected directly onto the analytical column of the gas chromatograph (Zhang *et al.*, 1992).

Chromatographic Quantification of Trimethylamine

Gas chromatography was performed on a Pye Unicam 4500 series gas chromatograph (Pye Unicam, Cambridge, U.K.) with a flame ionization detector. The silanized glass column (170 cm \times 4 mm i.d.) was packed with 4% (w/w) Carbowax 20 M and 0.8% (w/w) potassium hydroxide on Carbopack B (60–80 mesh) graphitized support (Supelco Inc., PA, U.S.A.). The operating temperatures of the column, injection port and detector unit were 70°C isothermal, 150 and 200°C respectively, with a nitrogen carrier gas flow-rate of 60 mL/min. The use of authentic trimethylamine, and trimethylamine N-oxide dihydrate, added to distilled water, urine and sample food homogenates, permitted the construction of calibration curves (0.1–150 $\mu\text{g}/\text{mL}$; 0.0001–0.15 g/L), which enabled quantification of the amine. All analyses were undertaken in duplicate, samples with high amine concentrations were diluted before analysis as appropriate (Zhang *et al.*, 1992).

RESULTS AND DISCUSSION

The results obtained from the chemical hydrolysis studies enabled the various foods to be placed into a rank order with respect to their “trimethylamine-producing

TABLE 1

Trimethylamine-producing capacity of various foods (μg trimethylamine/g food) following chemical hydrolysis and biological liberation

Food substance	Chemical hydrolysis ¹	Biological liberation ²
Sugar	0.0	n.d.
Cucumber	2.2	n.d.
Apple	2.6	10.1
Onion	4.2	n.d.
Banana	4.3	5.7
Tomato	4.5	22.4
Carrot	5.4	33.5
Lettuce	9.3	n.d.
Orange	12.7	7.3
Milk	13.3	18.5
Potato	15.3	36.4
Peas	22.6	49.9
Butter	25.2	n.d.
Bread	26.4	34.6
Cauliflower	29.5	45.0
Cheese	30.6	30.4
Rice	39.1	12.5
Biscuit	52.8	8.1
Pasta	70.5	n.d.
Chicken	74.0	18.7
Mushroom	81.7	45.0
Pork	90.0	24.4
Egg	147.3	36.4
Beef	163.0	20.0
Prawn	169.0	948.2*
Peanuts	180.9	29.4
Soya	194.0	22.9
Lamb	353.0	16.4
Mackerel	821.0	679.4*
Cod	2800.0	1334.7*

¹The values are the mean of three separate determinations, coefficient of variation = 5%.

²These values were derived from the amounts of trimethylamine measured in the urine (0–8 h) voided following the ingestion of a particular food substance (227 g). Values are the mean of six subjects.

*These values were significantly greater (Student's *t*-test, $P < 0.01$) than control values obtained following the ingestion of the light breakfast alone (equivalent to $39.2 \pm 13.5 \mu\text{g}$ trimethylamine/g).

Note: n.d.=not determined.

capacity" (Table 1). As a rough guide, odour problems in "fish-odour syndrome" patients begin to appear in concordance with a urinary concentration of trimethylamine of $10 \mu\text{g/mL}$ ($18\text{--}20 \mu\text{mol/mmol}$ creatinine). This threshold is a minimum value and many patients do not experience problems until reflective urine concentrations rise above this level (Mitchell and Smith, 2001). Assuming a zero flavin-monoxygenase N-oxidation capacity (most patients have between 10 and 50% capacity), that all of the liberated trimethylamine is uniformly voided over an 8 h period (480 mL urine) and that 227 g (8 oz) of the food was consumed, then any food producing more than $21.1 \mu\text{g/g}$ of trimethylamine should create a potential problem. Clinical observations suggest this is not the case (Ayesh *et al.*, 1993; Mitchell, 1996).

When the trimethylamine values obtained from the food substances via chemical hydrolysis and biological liberation were compared, a significant correlation was found (product moment correlation coefficient, $r=0.83$, $P < 0.001$). This correlation

was diminished on ranking (Spearman's rank correlation coefficient, $R=0.42$, $0.1 > P \geq 0.05$) suggesting a distorting effect of the high numerical values obtained for fish and seafood. When these latter three values were removed, a random association was observed ($r=0.04$, $P > 0.5$; $R=0.18$, $P > 0.5$).

In the majority of instances, the trimethylamine levels obtained following the biological liberation experiments were not significantly different (Student's *t*-test) from the measured background levels of $39.2 \pm 13.5 \mu\text{g}$ (range 5.9–79.5 μg). This control value was obtained from 180 urine samples (six volunteers, 30 samples each), with no statistically significant difference (Student's *t*-test) being observed between the individual volunteers. It appeared that in these cases there was no significant response to the ingestion of these particular food items and that the biological data may merely reflect a variable basal trimethylamine level arising from the light breakfast alone or residual material from a previous meal. In some instances (e.g., carrot, tomato), the biological values did appear to substantially exceed those obtained via chemical hydrolysis but these cases did not attain statistical significance when compared to background levels.

It was observed that some values obtained from chemical hydrolysis were greater than those found following biological liberation. Particularly noticeable were the meat products (lamb, 22-fold greater; beef, eight-fold; chicken, four-fold; pork, four-fold) and a few others (soya, eight-fold; biscuit seven-fold; peanut six-fold). This is suggestive of potential trimethylamine-releasing precursors that are susceptible to chemical hydrolysis but not enzymatic liberation. These chemicals may be in a combined form within the foods. Indeed, chemically pure lecithin (phosphatidyl choline) is known not to elevate urinary trimethylamine levels when administered to humans, whereas choline has a significant elevatory effect (De La Huerca and Popper, 1951). It has been suggested that most of the choline that we are presumed to eat is actually in the form of lecithin (Wurtman, 1979). Carnitine has also been shown to increase urinary trimethylamine levels (Rebouche and Chenard, 1991; Strack *et al.*, 1963), but when present within skeletal muscle, it may also be in some chemically associated form and not readily accessible to biological liberation. The only compound that appeared to behave similarly in both situations was trimethylamine N-oxide, the major precursor within fish and seafood. The general role of gut microflora and their differences between individuals must also be borne in mind.

CONCLUSION

The results obtained from this present study suggest problems with the total alkaline hydrolyses of foods. Data obtained from such studies cannot be used to predict successfully which particular foods will provide trimethylamine in the biological situation. Dietary modification based on chemical hydrolysis results for the treatment of "fish-odour syndrome" patients, with the exception of avoidance of fish and seafood, will be of little clinical use. This raises a general question concerning the overall relevance of composition data obtained via chemical means within the laboratory if it is to be applied to the more complex biological situation. A direct "chemical-biological equivalence" with regard to the liberation of chemical components from foods must not be automatically presumed.

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