Note

The Stability of Thiamine and Thiamine Tetrahydrofurfuryl Disulfide Added to Table Wines

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Summary Both thiamine hydrochloride and thiamine tetrahydrofurfuryl disulfide were added separately to table wines at concentrations equivalent to 0.3 and 1.5 μg of free thiamine per kJ of caloric energy. The resultant mean increments in thiamine activity, measured by Lactobacillus fermenti microbiological assay after 21 months of storage, were in the range 55 to 103% of the added vitamin, indicative of high bioavailability of thiamine from this source.

Key Words thiamine hydrochloride, thiamine tetrahydrofurfuryl disulphide, stability, table wines, microbiological assay

Wernicke's encephalopathy (WE), a condition seen in Australia almost exclusively in alcoholics after they have been acutely thiamine-deficient, has been commonplace (1) and remains so (2). In many cases, WE is succeeded by Korsakoff's psychosis (KP), characterized by a variably severe but often permanent impairment of short-term memory. Before developing WE, drinkers characteristically eat practically nothing, taking in little except alcoholic beverages, which are totally inadequate sources of thiamine.

In Australia there has been some support for Centerwall and Criqui's assertion (3), based on U.S. data, that it would be cost beneficial to supplement alcoholic beverages with thiamine in order to prevent the Wernicke-Korsakoff (WK) syndrome. However, support for this measure is not universal (4).

Any thiamine supplementation of alcohol beverages would need to be targeted to those beverages preferred by drinkers in the critical period of dietary neglect four to six weeks prior to the onset of WE. At present in Australia, those beverages are beer and wine, particularly white table wine sold in casks (5). To be useful,

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thiamine supplements would need to be stable in the beverage. Satisfactory stability has already been reported for thiamine added to beer (1). Here we report the stability of thiamine hydrochloride and thiamine tetrahydrofurfuryl disulfide (TTFD) added to table wines, measured by microbiological assay. TTFD is included as a representative of the lipid-soluble allithiamines, potentially better absorbed than free thiamine, given the previous reports (6-8) of malabsorption of water-soluble thiamine in malnourished alcoholics.

The selected four table wines were popular, inexpensive “cask” wines, sold in a 4 L collapsible, opaque, plastic bag equipped with a faucet and packed into a cardboard box. Wine A was a Cabernet Sauvignon (red), wine B a Grenache (rosé), wine C a Spatlese Rhine Riesling (Moselle-style sweet white) and wine D was a Rhine Riesling (dry white). Using aseptic technique in a laminar flow cabinet, 10 ml samples of wine were measured into sterile high-density polyethylene containers to which were added sterile-filtered thiamine hydrochloride in water or TTFD in methanol to achieve molar concentrations equivalent to final free thiamine concentrations of approximately 0.3 or 1.5 μg/kJ of caloric energy (9) in the wine. The exact added concentrations of thiamine hydrochloride or TTFD are indicated in Table 1. Samples were prepared in triplicate and stored under the indicated conditions of temperature and illumination for 21 months before assay for thiamine activity, which was by an automated adaptation (10) of the microbiological assay (11) using Lactobacillus fermenti. Within the one assay batch, the L. fermenti growth responses to equimolar concentrations of thiamine hydrochloride and of TTFD were identical, yielding superimposable standard curves in the range 1.6 to 160 nmol/liter. The assay of thiamine by measurement of the fluorescence of the thiochrome derivative (12) was also investigated. Taste preference, as to the presence or absence of a thiamine supplement, was tested in an experiment double-blinded by methods reported previously (13). Volunteer students and laboratory staff members (n = 30) each nominated a preference, in respect to bouquet and flavor, for one of two samples of wine D, one of which contained thiamine hydrochloride added at 3.0 μg/kJ of energy.

The pH values of the wines A, B, C, and D were respectively 3.2, 2.9, 2.9, and 2.9. Independent of any addition, some small amounts of thiamine activity were found by bioassay in each of the wines after the 21 months storage period (Table 1). The amounts did not differ between the tested storage conditions, but did differ between the wines, being greater in wine A (0.4 μmol/liter) than in the other wines (0.1 μmol/liter). The addition of either thiamine hydrochloride or TTFD resulted in increased thiamine activity on bioassay of the wines after 21 months storage (Table 1).

The thiamine hydrochloride was most stable in all the wines when they were stored at 4°C in darkness (residual thiamine activity increments 93 to 103% of the addition), but even storage at ambient temperature in the light resulted in residual thiamine increments equivalent to 59 to 77% of the thiamine hydrochloride added (2.8 to 3.9 μmol/liter) at the rate of 0.3 μg/kJ, and 78 to 100% of the thiamine.

Table 1. Concentrations of bioassayed thiamine activity in four wines after 21 months storage at indicated temperature and illumination.

<table>
<thead>
<tr>
<th>Addition storage temperature and illumination*</th>
<th>Concentration of thiamine activity in wines</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Wine A (red) (1 SD)</td>
</tr>
<tr>
<td></td>
<td>(µmol/liter)</td>
</tr>
<tr>
<td>Nil addition</td>
<td>0</td>
</tr>
<tr>
<td>4°C, darkness</td>
<td>0.41 (0.01)</td>
</tr>
<tr>
<td>Ambient, darkness</td>
<td>0.36 (0.06)</td>
</tr>
<tr>
<td>Ambient, daylight</td>
<td>0.37 (0.07)</td>
</tr>
<tr>
<td>Thiamine HCl addition</td>
<td>2.8</td>
</tr>
<tr>
<td>4°C, darkness</td>
<td>2.99 (0.24)</td>
</tr>
<tr>
<td>Ambient, darkness</td>
<td>2.31 (0.12)</td>
</tr>
<tr>
<td>Ambient, daylight</td>
<td>2.04 (0.08)</td>
</tr>
<tr>
<td>Thiamine HCl addition</td>
<td>14</td>
</tr>
<tr>
<td>Ambient, daylight</td>
<td>11.32 (1.42)</td>
</tr>
<tr>
<td>TTFD addition</td>
<td>3.0</td>
</tr>
<tr>
<td>4°C, darkness</td>
<td>2.52 (0.14)</td>
</tr>
<tr>
<td>Ambient, darkness</td>
<td>2.11 (0.10)</td>
</tr>
<tr>
<td>Ambient, daylight</td>
<td>1.94 (0.32)</td>
</tr>
<tr>
<td>TTFD addition</td>
<td>15.1</td>
</tr>
<tr>
<td>Ambient, daylight</td>
<td>8.68 (0.19)</td>
</tr>
</tbody>
</table>

*“Ambient” temperature in the Brisbane laboratory varied from 18°C (winter nights) to 40°C (summer days). “Daylight” includes exposure to fluorescent laboratory lights.

hydrochloride added (14 to 19.5 µmol/liter) at 1.5 µg/kJ. Thus the losses of activity were less when the thiamine hydrochloride was added at the higher of these two concentrations. Storage at ambient temperature in darkness resulted in residual thiamine activity increments in the range 68 to 91%, indicating stability intermediate between that at 4°C in darkness and that at ambient temperature in daylight.

The residual bioassayed thiamine activity increments due to added TTFD (3.0 to 4.2 µmol/liter) stored for 21 months in the wines was almost as impressive as that of added thiamine hydrochloride, being 64 to 78% at 4°C in darkness, 57 to 71% at ambient temperature in darkness and 51 to 59% at ambient temperature in daylight. Comparable results (55 to 73%) were obtained after addition of TTFD at 15.1 to 21.1 µmol/liter to wine stored at ambient temperature in darkness. Again, stability was a little better at 4°C than at ambient temperature, and a little better in darkness than in daylight.

The thiochrome fluorescent assay can measure thiamine but not TTFD. Even so, the wines contained substances which fluoresced under the conditions of the
assay, but whose fluorescence excitation spectrum exhibited a maximum at 330 nm, different from the 365 nm maximum of thiochrome. For these reasons, thiochrome fluorescence proved to be unsuitable to the measurement of thiamine or TTFD in wine.

Since this microbiological assay by *L. fermenti* does not differentiate between thiamine and TTFD, it is also unlikely to differentiate between thiamine and other disulfide adducts of thiamine, including adducts with natural substances of the wines. Therefore, it is quite possible that the measured thiamine activity after addition of thiamine hydrochloride to the wines is due not to free thiamine but to its disulfide adducts. Nevertheless, the growth response of *L. fermenti* to the wines demonstrates the bioavailability of the thiamine activity, whatever its true chemical nature, to this organism. Due to their lipophilicity, orally administered disulfide adducts of thiamine, such as TTFD and thiamine disulfide, actually exhibit greater bioavailability to man than does free thiamine (14–16). We conclude, therefore, that the measured thiamine activity in the wines is bioavailable to man, whether or not it is derivatized.

The low pH values of these wines are ideal for storage of thiamine and its derivatives. Indeed, the residual thiamine activities in these wines are impressive, even after 21 months storage. While losses of thiamine activity could be minimized by storage at 4°C, they are still small at ambient temperature in darkness. Under these conditions, usual for wine storage, a two-fold excess of supplement over the desired concentration would guarantee sufficient residual thiamine activity in these wines for up to 21 months.

The group of 30 subjects asked to discriminate between the bouquet and flavor of wine without added thiamine and that of wine with added thiamine expressed a “no preference” result. Since this result was obtained on the addition of thiamine to the dry white wine D at 3 μg/kJ, it is unlikely that its addition at 0.3 or 0.6 μg/kJ would significantly affect these qualities in any of these wines.

In the absence of unambiguous chemical identification of the residual thiamine-active substances, we can draw no conclusion as to the superiority of thiamine hydrochloride or TTFD as thiamine supplements in wines. While the lipid-soluble allithiamines are better absorbed from the gastrointestinal tract than are the water-soluble thiamines (14–16), it is possible that antioxidants added during winemaking (17) could convert some allithiamine to free thiamine or other thiamine derivatives, thus negating the potential benefit of the allithiamines. Methods of chemical analysis which measure the unaltered allithiamines (18) would be required to test for derivatization of these compounds in wines.

We conclude that the addition of either thiamine hydrochloride or TTFD to table wines could be an effective way to ensure that drinkers do not become thiamine-deficient. Further, since the cheap cask wines studied here, along with beer, are popular (5) with Australian drinkers in the period leading to presentation with WE, it is in precisely these wines, and in beer, that thiamine supplementation could be most effective in the prevention of the Wernicke-Korsakoff syndrome in
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drinkers.

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REFERENCES


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