Original Communication

Sorbitol enhances the physicochemical stability of B_{12} vitamers

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Abstract: The stability of B_{12} vitamers is affected by interaction with other water-soluble vitamins, UV light, heat, and pH. This study compared the degradation losses in cyanocobalamin, hydroxocobalamin and methylcobalamin due to the physicochemical exposure before and after the addition of sorbitol. The degradation losses of cyanocobalamin in the presence of increasing concentrations of thiamin and niacin ranged between 6%-13% and added sorbitol significantly prevented the loss of cyanocobalamin ($p < 0.05$). Hydroxocobalamin and methylcobalamin exhibited degradation losses ranging from 24%–26% and 48%–76%, respectively; added sorbitol significantly minimised the loss to 10% and 20%, respectively ($p < 0.05$). Methylcobalamin was the most susceptible to degradation when co-existing with ascorbic acid, followed by hydroxocobalamin and cyanocobalamin. The presence of ascorbic acid caused the greatest degradation loss in methylcobalamin (70%-76%), which was minimised to 16% with added sorbitol ($p < 0.05$). Heat exposure (100 °C, 60 minutes) caused a greater loss of cyanocobalamin (38%) than UV exposure (4%). However, degradation losses in hydroxocobalamin and methylcobalamin due to UV and heat exposures were comparable (>30%). At pH 3, methylcobalamin was the most unstable showing 79% degradation loss, which was down to 12% after sorbitol was added ($p < 0.05$). The losses of cyanocobalamin at pH 3 and pH 9 (~15%) were prevented by adding sorbitol. Addition of sorbitol to hydroxocobalamin at pH 3 and pH 9 reduced the loss by only 6%. The results showed that cyanocobalamin was the most stable, followed by hydroxocobalamin and methylcobalamin. Added sorbitol was sufficient to significantly enhance the stability of cobalamins against degradative agents and conditions.

Keywords: Stability, vitamin B_{12}, thiamin, niacin, ascorbic acid, sorbitol

Introduction

Vitamin B_{12} and cobalamin are generic terms used interchangeably to represent an array of naturally occurring vitamers in foods, such as methylcobalamin, adenosylcobalamin, hydroxocobalamin, cyanocobalamin and aquocobalamin. The chemical structure of cobalamin is characterised by a cobalt atom linked to a corrinoid ring, α-glucoside and nucleotide moieties (Figure 1). As a co-enzyme, methylcobalamin plays a significant role in the single carbon transfer of methionine synthesis, and 5'-deoxyadenosylcobalamin has a function in the metabolism of amino acids and propionate.

It is a widely held view that cobalamin is sourced from animal tissues, such as meat and milk, because the vitamin is synthesised by the ruminal or intestinal microflora [1]. Recent studies show that cobalamins are present in minimal amounts in some plant based-foods, such as in sea buckthorn [2], fermented soy beans and vegetables, and edible algae [3].

Pre-requisite prior to quantitation of cobalamins is sample preparation, where cobalamins are initially released from various matrices such as foods, dietary supplements and biological samples and converted to cyanocobalamin in excess potassium cyanide. Cyanocobalamin concentrations are then determined using liquid chromatography [2, 4, 5]. With the advancement in analytical instruments offering a higher sensitivity of cobalamin detection, researchers attempted to determine cobalamin vitamers in milk [6] and in dried edible algae, bovine livers and clams [3]. Although during sample preparation and analysis degradation of cobalamins occurs and could affect the accuracy of measurement, evidence on the stability of B_{12} vitamers other than cyanocobalamin is still lacking.

When exposed to light, vitamin B_{12} undergoes degradation and irreversible conversion to different vitamers. Under UV exposure, methylcobalamin and adenosylcobalamin are converted to more light-stable hydroxocobalamin, and they also undergo oxidation reactions by free radicals that have been generated during the exposure [7]. Acidic

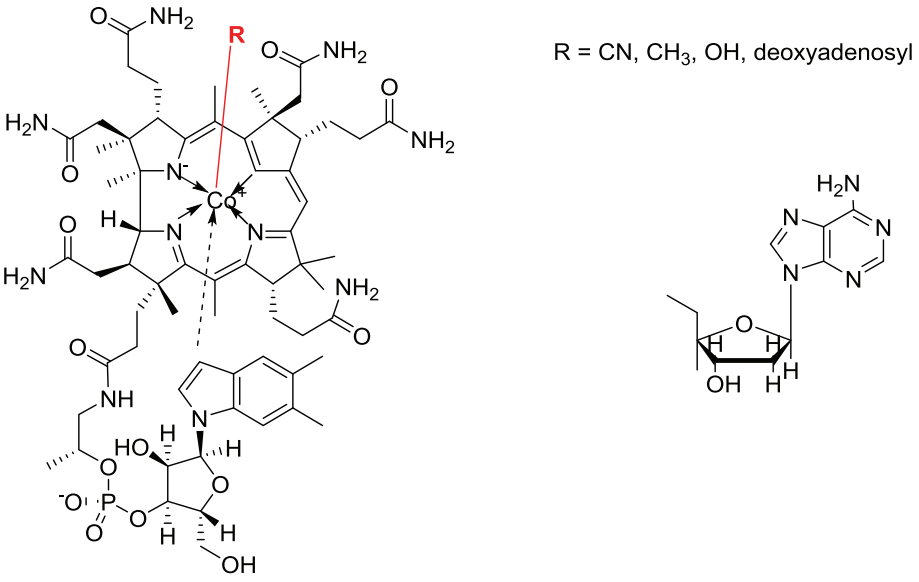

Figure 1. The chemical structure of cobalamin consists of a cobalt-centred corrin ring. The β-axial ligand depicted by R (in red colour) that could be attached to cobalt includes cyanocobalamin (CN), methylcobalamin (CH_3), hydroxocobalamin (OH) and deoxyadenosylcobalamin. The cobalt atom is also joined to 5,6-dimethylbenzimidazole.

pH conditions cause cobalamins to hydrolyse [8], and the presence of sucrose also reduces vitamin B_{12} content [9]. Barr et al (1957) found that degradation of vitamin B_{12} at 100 °C can be mitigated by the addition of sorbitol, which reduces the amount of available water that facilitates hydrolysis of cobalamins [9]. However, the cobalamin vitamer tested in this study is unknown and there is a paucity of evidence in the field. Sorbitol is known as a preferred alternative to sucrose and high fructose corn syrup in the processing of dried fruit products [10] which also enhances preservation effects [11].

The objective of this study was to investigate the effect of sorbitol if used for its preservative role on the stability of cyanocobalamin, hydroxocobalamin and methylcobalamin. Particularly, the stability of cobalamins co-existing with selected water-soluble vitamins, such as thiamin, niacin and ascorbic acid, was determined using high performance liquid chromatography (HPLC). Additionally, the work compared the degradation losses of cobalamins when exposed to UV light, heat, and pH conditions. Quintessentially, this paper offers important insights into the role of added sorbitol on the stability of cobalamins, which needs to be taken into account for the quantitation of vitamin B_{12}, as well as processing and storage of foods.

Materials and methods

Chemicals

Cyanocobalamin, methylcobalamin, hydroxocobalamin, thiamin hydrochloride, nicotinic acid, ascorbic acid, sorbitol, HPLC grade methanol, ammonium acetate, and acetic acid were purchased from Sigma-Aldrich (Sydney, NSW, Australia).

Standard solutions

Stock standard solutions of cyanocobalamin, hydroxocobalamin and methylcobalamin (500 μg/mL) were prepared in Milli-Q water in a UV-free laboratory. Aliquots of stock standard solution (500 μL) were flushed with nitrogen gas and stored in amber vials in a −20 °C freezer. Working standard solutions of cobalamins (5 μg/mL) were freshly prepared on the day of the experiment, pipetted into the glass vials and subjected to various treatments described in the following sections. Similarly, stock standard solutions of thiamin, niacin and ascorbic acid were prepared in Milli-Q water (2 mg/mL) and treated as previously described.

Interaction of cobalamins with thiamin, niacin and ascorbic acid

Initially, increasing concentrations of thiamin and niacin (5, 10 and 50 μg/mL), and ascorbic acid (5, 10 and 50 μg/mL) were pipetted into the glass vials containing either cyanocobalamin or hydroxocobalamin or methylcobalamin (5 μg/mL; Figure 2) to determine the degradation loss due to any interaction with other water-soluble vitamin(s). To assess the effect of sorbitol on the stability of cobalamins, increasing sorbitol concentrations (250, 500 and 1000 μg/mL) were added to individual cobalamin (5 μg/mL) pre-mixed with either both thiamin and niacin (50 μg/mL) or ascorbic acid (50 μg/mL), as illustrated in Figure 2. All samples were prepared in triplicate, and along

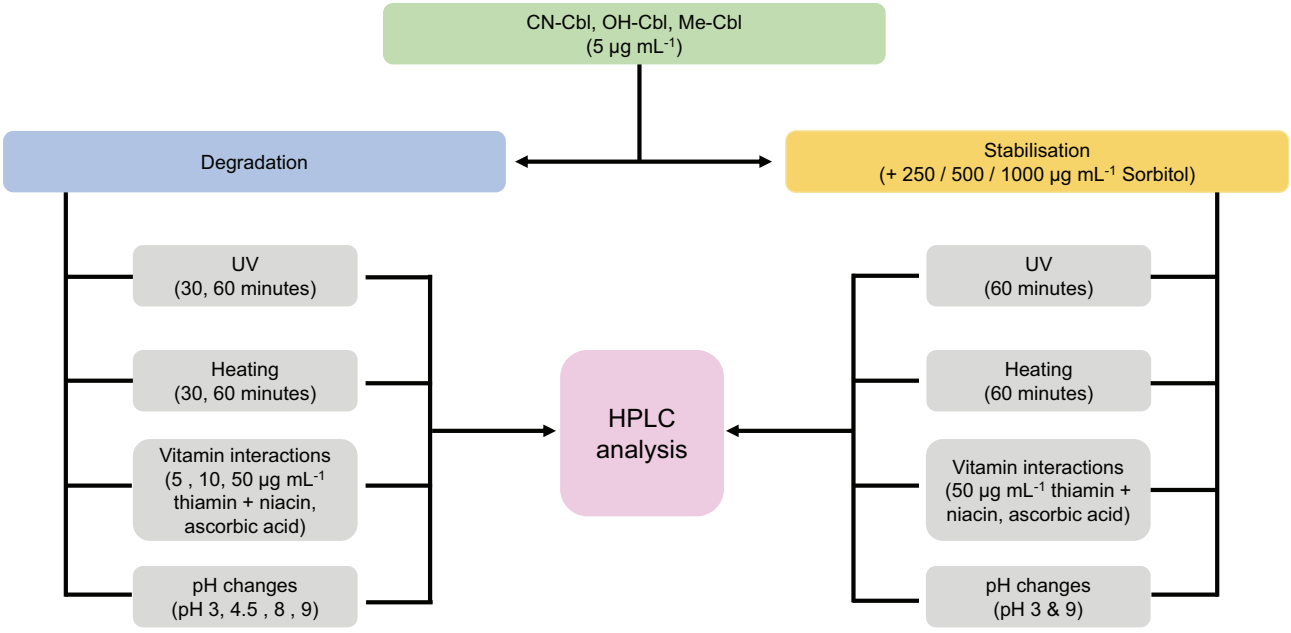

Figure 2. The stability of the B_{12} vitamers were assessed following UV exposure, heating, interactions with other B vitamins and pH changes, in the presence and absence of sorbitol. CNCbl, OHCbl and MeCbl correspond to cyanocobalamin, hydroxocobalamin and methylcobalamin, respectively.

with controls, the samples were immediately analysed using HPLC and each sample was injected twice.

UV-light and heat treatment

The glass vials containing cobalamins were placed in a UV-light cabinet, fitted with an "F8T5BLB" bulb (custom-manufactured by the UNSW Chemical Engineering Workshop). Samples (in triplicate) were exposed to UV light either for 30 minutes or 60 minutes. To assess the impact of heat on the stability of cobalamin vitamers, samples were placed in a 100 °C vacuum drying oven (Townson and Mercer, Croydon, UK) for up to 60 minutes. Samples with increasing sorbitol concentrations (250, 500 and 1000 µg/mL) were exposed to UV light or placed in a 100 °C oven for 60 minutes (Figure 2).

pH changes

The stability of cobalamins in acidic and alkaline pH conditions was determined by preparing the working standard solutions at certain pHs. For this purpose, 0.1 M HCl was used to obtain pH 3.0 and pH 4.5 and 0.1 M NaOH for pH 8.0 and pH 9.0. Addition of sorbitol concentrations to cobalamin vitamers was also carried out at pH 3 and pH 9 (Figure 2) and compared with control samples. The signal intensities representing the peak areas were used to determine the percentage of loss after each treatment. Mean peak areas of treated samples (a) were subtracted from those of untreated samples (b), and the stability was computed as (b − a)/b*100%.

HPLC analysis

A HPLC system (Shimadzu Prominence UFLC, Kyoto, Japan) equipped with a photodiode array detector was used for the study. Chromatography was performed on a reversed-phase Kinetex Biphenyl column (4.6 mm × 150 mm, 5 µm, Phenomenex, Sydney, Australia). The mobile phase system consisted of 50 mM ammonium acetate buffer (pH 5.0, mobile phase A) and methanol (50% v/v in Milli Q water) as solvent B [12]. The initial condition was set at 90% solvent A and 10% solvent B. The composition of solvent B was then raised to 10% in 4 minutes, then was held until 10 minutes before returning to the initial condition (10% B) at 15 minutes. The system was re-equilibrated until 25 minutes before the next injection. The flow rate and the injection volume were set at 0.8 mL/min and 100 µL, respectively. The characteristic peak for cyanocobalamin was detected based on its absorption maxima at 361 nm, and for hydroxocobalamin and methylcobalamin at 351 nm.

Statistical analysis

To determine whether data were normally distributed, Grubb's test was used to identify and verify any outliers,

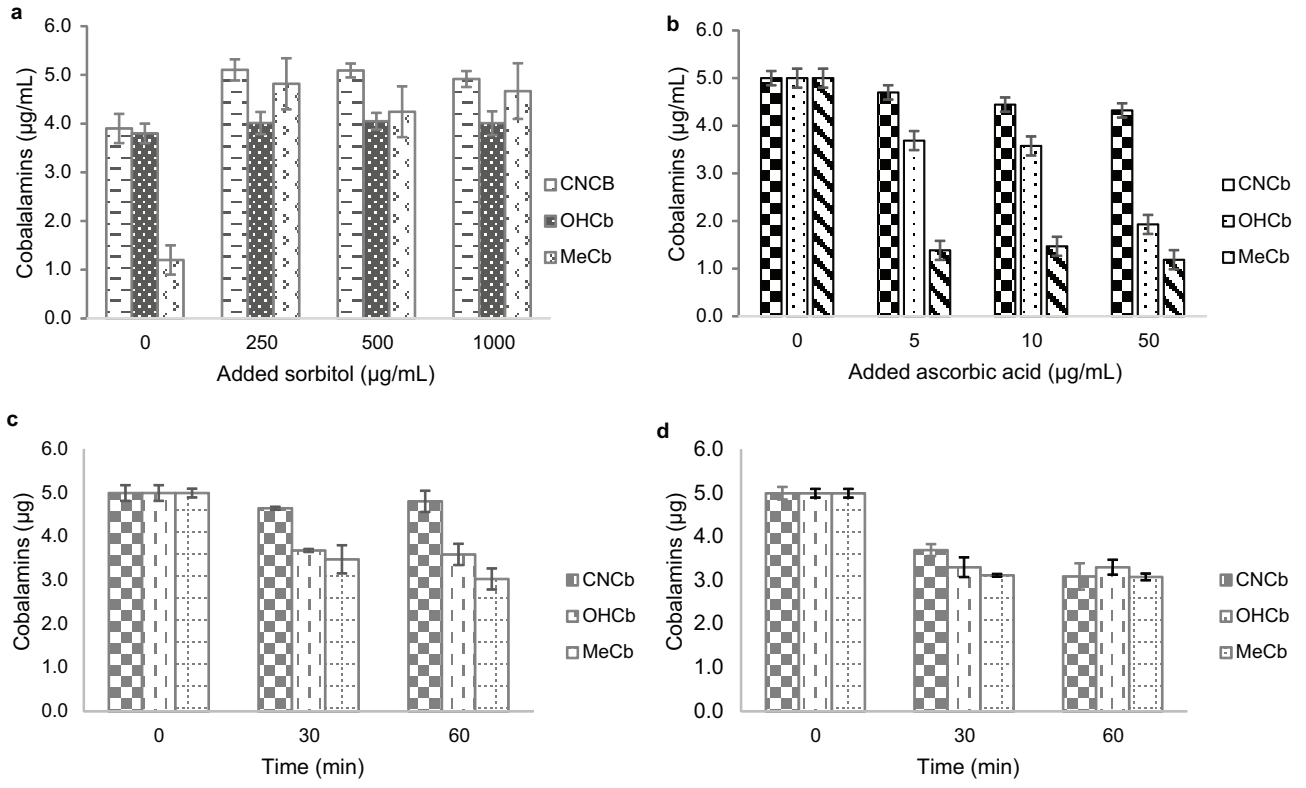

Figure 3. Added sorbitol improved the stability of cobalamins when co-existed with thiamin and niacin (a). The presence of increasing ascorbic acid concentrations accelerated the loss of cobalamins (b). The effect of UV exposure to cobalamin vitamers (c) was less pronounced than the effect of 100 °C heating (d). Error bars represent standard deviations (n = 3, each replicate was injected twice). CNCbl, OHCbl and MeCbl correspond to cyanocobalamin, hydroxocobalamin and methylcobalamin, respectively.

afterwards the data set were analysed using SAS Enterprise Guide 6.1 (SAS Institute Inc. Cary, NC, USA). Bartlett's test was employed to assess the homogeneity of variances, one-way analysis of variance (ANOVA) and Tukey's Studentized Range (HSD) test were used to compare the mean values. The p-value of 0.05 was chosen to report the level of significance.

Results

Interaction of cobalamins with thiamin and niacin

The presence of both thiamin and niacin generally resulted in lower analysed cyanocobalamin, hydroxocobalamin and methylcobalamin. Increasing the concentrations of thiamin and niacin significantly increased the loss of cyanocobalamin from 6% to 21% ($p < 0.05$), and hydroxocobalamin exhibited greater degradation losses between 24% to 26% than cyanocobalamin. Analysed hydroxocobalamins were 3.8 µg/mL and 4 µg/mL before and after the addition of sorbitol, respectively. Before sorbitol was added, the measured methylcobalamin with added thiamin and niacin was 1.2 µg/mL, corresponding to 76% loss. After addition of sorbitol (250 µg/mL), mean methylcobalamin increased to 4 µg/mL and the loss was minimised to approximately 8% (Figure 3).

Interaction of cobalamins with ascorbic acid

Ascorbic acid exerted higher degradation losses of hydroxocobalamin in comparison to cyanocobalamin ranging between 7% and 34%, while methylcobalamin had the highest loss of 72%-76% (Figure 3). Addition of sorbitol was found to be protective against degradation of cyanocobalamin caused by the presence of ascorbic acid (Table 1). Measured cyanocobalamin without sorbitol was 4.3 µg/mL and with sorbitol 5 µg/mL, thus preventing 13% degradation loss when sorbitol (250 µg/mL) was added. In the presence of ascorbic acid, degradation losses

Table 1. The degradation losses (%) in cyanocobalamin (CNCbl) with and without added sorbitol after exposure to UV, heat, other B vitamins **(50 µg/mL)** and pHs are compared with hydroxocobalamin (OHCbl) and methylcobalamin (MeCbl).

	Sorbitol (µg/mL)	60 min UV	60 min Heating	Ascorbic acid	Thiamin + niacin	pH 3	pH 9
CNCbl % loss	0	3.8^{a}	38^{g}	13^{m}	21^{s}	16^{1}	15^{4}
	250	$1.6^{a,b}$	1.7^{g}	$0.7^{m,n}$	$0^{s,t}$	8^{1}	0^{4}
	500	$0^{a,b}$	0.5^{g}	$0^{m,n}$	$0^{s,u}$	5^{1}	1^{4}
	1000	$0.3^{a,b}$	2.4^{g}	$3.2^{m,n}$	$2^{s,t,u}$	3^{1}	0^{4}
OHCbl % loss	0	28^{c}	34^{h}	34^{o}	24^{v}	20^{2}	24^{5}
	250	13^{c}	$10^{h,i}$	$20^{o,p}$	20^{v}	21	18^{5}
	500	10^{c}	$10^{h,j}$	$20^{o,q}$	18^{v}	15^{2}	18^{5}
	1000	14^{c}	$17^{h,i,j}$	$12^{o,p,q}$	18^{v}	17^{2}	18^{5}
MeCbl % loss	0	39^{d}	38^{k}	76^{r}	76^{w}	79^{3}	63^{6}
	250	$0.9^{d,e}$	$20^{k,l}$	32^{r}	5^{w}	13^{3}	$0^{6,7}$
	500	1.8^{d}	$32^{k,l}$	42^{r}	15^{w}	16^{3}	$13^{6,7,8}$
	1000	$2.5^{d,e}$	$29^{k,l}$	16^{r}	8^{w}	12^{3}	$0^{6,8}$

The same alphabetical and numerical superscripts within each column correspond to significant differences in degradation losses.

of hydroxocobalamin solutions were between 7%-34%. Unlike cyanocobalamin, when hydroxocobalamin co-existing with ascorbic acid increasing the sorbitol concentrations did not improve the stability ($p > 0.05$). The presence of ascorbic acid caused 72%-76% loss of methylcobalamin, and the loss was minimised to 32%-43% after sorbitol was added ($p < 0.05$). Notably, the stability of methylcobalamin in the presence of ascorbic acid was significantly improved by adding the highest sorbitol concentration (1000 µg/mL) with the loss of 16%.

Exposure of heat and UV on the stability of cobalamins

Overall, the experimental data showed that degradation losses of cyanocobalamin and hydroxocobalamin due to 100 °C heat treatment were significantly faster compared to UV exposure (Figure 3). The losses of methylcobalamin due to UV exposure and heat were not significantly different ($p > 0.05$). Measured cyanocobalamin after heating was 3.1 µg/mL and after UV exposure 4.8 µg/mL, indicating that cyanocobalamin degraded faster due to heat exposure (Figure 3). In contrast, degradation losses of hydroxocobalamin (3.3 µg/mL) and methylcobalamin (3.0 µg/mL) were not significantly different after 60 minutes UV exposure and heat ($p > 0.05$). Measured cyanocobalamin after 30 minutes of heat treatment was 3.7 µg/mL, corresponding to 26% of the loss. Degradation losses due to 30 minutes of heat exposure in hydroxocobalamin (3.3 µg/mL) and methylcobalamin (3.1 µg/mL) were 34% and 37%, respectively (Figure 3). The result showed that generally cobalamins were not heat-stable, and cyanocobalamin was more heat-stable than hydroxocobalamin and methylcobalamin.

Effect of pH conditions on cobalamins

At pH 4.5, there was a 3.5% loss of cyanocobalamin in comparison to cyanocobalamin dissolved in Milli-Q water. Decreasing the pH to 3.0 and increasing the pH to 9.0 resulted in 15% loss of cyanocobalamin. The losses of hydroxocobalamin at low pHs (3.0 and 4.5) and high pHs (8.0 and 9.0) were significantly greater than cyanocobalamin, which were 20% and 25%, respectively. The losses due to pH changes were the highest for methylcobalamin, where at lower pHs the mean loss was 78% and at higher pHs, 64%. Our study also evaluated the stability of cobalamins at pH 3.0 by adding increased concentrations of sorbitol, in which sorbitol exerted a protective effect on cobalamins at pH 3.0. It was found that increasing the sorbitol concentrations (250, 500 and 1000 µg/mL) conferred cyanocobalamin a significant protective effect ($p < 0.05$). When 1000 µg/mL sorbitol was present at pH 3.0, the loss of cyanocobalamin was 3%, which implied that sorbitol was able to protect cyanocobalamin against acid hydrolysis (at pH 3.0). Addition of sorbitol to hydroxocobalamin at pH 3.0 did not significantly minimise the degradation losses, confirming that sorbitol did not provide a sufficient protective effect for hydroxocobalamin at pH 3.0. In contrast, sorbitol was significantly protective against the loss of methylcobalamin at pH 3.0, minimising the loss from 79% to 12%-16%, hence improving the stability significantly ($p < 0.05$).

Cyanocobalamin at pH 9.0 was stable when sorbitol was present in the solutions, and the stability was not dependent on the concentration of sorbitol. The loss of hydroxocobalamin at pH 9.0 was not significantly different to that at pH 3.0, confirming that addition of sorbitol did not improve the stability of hydroxocobalamin. Sorbitol protected

effectively against degradation of methylcobalamin at pH 9.0; the loss was only 13% when 500 μg/mL was added compared to 63% without sorbitol.

Discussion

Interaction with other B vitamin(s)

It was noted that 5 μg/mL of thiamin and niacin was sufficient to cause the degradation of hydroxocobalamin, and 50 μg/mL did not cause any further degradation, suggesting that hydroxocobalamin was more sensitive compared to cyanocobalamin. Addition of thiamin and niacin led to significant degradation losses of methylcobalamin and higher concentrations of thiamin and niacin caused losses ranging from 48% to 75% ($p < 0.05$). The results showed that methylcobalamin was the least stable vitamer compared to hydroxocobalamin and cyanocobalamin when co-existing with thiamin and niacin (Figure 3) and is consistent with a previous study [13].

The stability of cobalamins mixed with thiamin and niacin (50 μg/mL) significantly improved with added sorbitol (Figure 3). Without added sorbitol, measured cyanocobalamin was 3.9 μg/mL and with sorbitol was 5 μg/mL, thus confirming that addition of 250 μg/mL of sorbitol was sufficient to enhance the stability of cyanocobalamin in the presence of thiamin and niacin. Increasing sorbitol concentrations did not improve the stability of hydroxocobalamin (Figure 3), and the degradation loss remained consistent at 18%. A previous study suggested that the decomposition product of thiamin, specifically from its thiazole moiety, may be responsible for the degradation of cobalamins [14]. Also, interaction of thiamin and cyanocobalamin caused the conversion of cyanocobalamin into hydroxocobalamin [14], which could lead to higher hydroxocobalamin measured here.

Adding an equal concentration of ascorbic acid to cyanocobalamin caused 6% degradation loss and increasing the concentrations of ascorbic acid also increased the loss to 13% (Figure 3). This was consistent with a previous finding by Ichikawa et al (2005), despite the fact that in their research 100 mM acetate buffer pH 4.8 was used to prepare vitamins standards [15]. Therefore, it can be suggested that the presence of ascorbic acid had a greater impact on the stability of cyanocobalamin than the pH of solutions. The result suggested that ascorbic acid might disintegrate the corrin ring by reducing the cobalt atom into cob (II)alamin, which could be observed by a colour change from red to brown, and slowly to yellow when the cobalt has been "released" [16]. Overall, our results suggested that sorbitol had a protective effect on cobalamins by minimising their degradation due to the presence of ascorbic acid.

Degradation loss by UV light and heat

Exposure of cyanocobalamin to UV light for 30 minutes caused 7% degradation loss and for 60 minutes 4%, suggesting that cyanocobalamin was transformed to the less stable hydroxocobalamin as photoaquation occurred [17]. The cyanocobalamin loss due to 30 minutes UV exposure (4.7 μg/mL) was not significantly different compared to 60 minutes exposure (4.8 μg/mL), similar to a previous finding [18]. Photodegradation losses of hydroxocobalamin and methylcobalamin after 60 minutes UV exposure were 28% and 39%, respectively, and greater than when cyanocobalamin was exposed (Figure 3). This exposure significantly reduced the hydroxocobalamin concentration to 3.6 μg/mL and methylcobalamin to 3.0 μg/mL ($p < 0.05$). This result may be explained by the fact that during photolysis, the bond between the cobalt-carbon (Co-C) atoms in cobalamin molecules disrupted easily due to the low bond enthalpy [19]. Our data showed that cyanocobalamin was more photo-stable than hydroxocobalamin. However, it has been reported that hydroxocobalamin is apparently more stable than cyanocobalamin [7] and both cobalamins (non-alkyl cobalamins) are equally photo-stable [18]. A possible reason for the inconsistent finding would suggest that hydroxocobalamin could be more stable in Dulbecco's phosphate buffered saline [7] and a buffer solution at pH 10.3 [18]. Our data on photodegradation of methylcobalamin (39% loss) was consistent with an earlier study that proposed that methylcobalamin and adenosylcobalamin (alkyl cobalamins) converted to cob(II)alamin [18]. Another possible reason was that methylcobalamin undergoes a "homolytic fission" reaction, in which methylcobalamin converts to aquocobalamin and 5'-deoxyadenosylcobalamin producing alkyl radicals [17].

Degradation of cobalamins caused by high temperature depends on bond dissociation energies. The bond dissociation energy for methylcobalamin was 36 kcal/moL [20], while the bond dissociation energy of cyanocobalamin is still unknown. The stability of cyanocobalamin after 30 minutes of heat exposure supported the idea that cyanocobalamin had a greater bond dissociation energy than both hydroxocobalamin and methylcobalamin [21]. The loss of cyanocobalamin following 60 minutes heating was 38% and was not significantly different from hydroxocobalamin (34%) and methylcobalamin (38%). The result showed that the stability of cobalamins was affected by higher temperatures and the length of heat exposure. In this study, we compared for the first time the presence of sorbitol with cobalamins and showed its significant protective effect against UV exposure or heating. Overall, addition of sorbitol significantly improved the stability of cobalamin vitamers (Table 1). The results suggested that adding sorbitol to cobalamin vitamers might have reduced the amount

of available water that would decrease the rate of hydrolysis resulting in decomposition of vitamin B_{12} [9].

pH changes

The results showed that stability of cobalamins was significantly affected by pH changes, and cyanocobalamin was the most stable to withstand pH changes compared to hydroxocobalamin and methylcobalamin. This was particularly consistent with the impact of pH changes on methylcobalamin, as acidic and alkaline environments facilitated the hydrolysis of the methyl side chain from the molecules [22]. Data presented in this study indicate that sorbitol was useful to enhance the stability of cobalamins and should be added during sample preparation prior to quantitation of cobalamins in fortified and unfortified samples, so that the degradation loss could be minimised. Sorbitol is a common ingredient used in multivitamin syrups and injections to stabilise vitamins during prolonged storage [23]. Also, sorbitol, being a hexavalent sugar alcohol, is involved in the formation of molecules between sugar alcohol and vitamins [24] by inhibiting the catalytic oxidative reactions [25].

The sensitivity of methylcobalamin to pH changes would imply that the pH of solutions would affect the stability of cobalamins during their release from food matrices. Currently, there is no agreement on the pH of the extraction buffer, on treatment with enzyme(s) to release endogenous cobalamins and/or added cyanocobalamin from various food matrices. Cobalamin vitamers were extracted from dairy produce (UHT skim milk and full cream milk, fermented milk) into sodium acetate buffer, pH 4.5 and converted to cyanocobalamin in the presence of excess KCN [26]. Cyanide is added to the extraction buffer to convert naturally occurring cobalamin vitamers to cyanocobalamin. Even though cyanocobalamin is stable, the pH of the extraction buffer is suggested to be between 4.0 and 7.0. In another study, cyanocobalamin was extracted from fortified foods and beverages using enzymatic digestion (α-amylase and pepsin) and 50 mM sodium acetate buffer, pH 4 [27]. The activity of α-amylase at pH 4 to hydrolyse starch and to assist in the release of cyanocobalamin from fortified starchy foods is open to discussion, because this enzyme (Sigma-Aldrich A2771) exerts its effect at pH 6.9. Furthermore, several studies have used sodium acetate as the extraction buffer with varying molarity, such as, 200 mM and pH 4 [4], 100 mM and pH 4 [28], 50 mM and pH 4 [2, 29] and 50 mM pH 4.6 [6]. Although the molarity of sodium acetate buffer differs between studies, pH 4 is commonly used for cobalamin extraction. When enzymatic digestion is carried out to release cobalamins from food matrices, enzymes should work at their optimal pH condition, for example: protease, pepsin and papain [4], and taka-diastase [28]. Heat treatment at 100 °C for 30 minutes has also been reported to successfully release cyanocobalamin from various food matrices [4]. Our experimental data showed that the loss of cyanocobalamin after 15 minutes of heating (11%) significantly increased to 26% (30 minutes) and 38% (60 minutes). The results suggested that extraction efficiency studies should be carried out to determine the recovery of the vitamin and to accurately measure cyanocobalamin in various matrices. As varying extraction protocols are documented in literature, the susceptibility of cobalamin vitamers to degradation reported here is crucial and needs to be considered for quantitation of cobalamins. HPLC was employed in our study because the sensitivity of the HPLC method was fit for the purpose. However, HPLC methods may not be adequate to detect and quantify B_{12} vitamers in foods that occur naturally in low concentrations, and mass spectrometry would be ideal for detection and accurate quantification of the vitamers. Also, the differing degrees of stability can present a challenge during the processing of foods fortified with vitamin B_{12}. Other factors that need to be taken into account include the exposure to UV light and heat which occurs commonly throughout the food supply chain, and also the presence of other endogenous vitamins and acidic or alkaline ingredients being used in food processing.

This is the first study to assess and compare the effect of sorbitol on physicochemical stability of cobalamin vitamers against exposure to UV light, heat, pH and other water-soluble B vitamins. The strength of this study is that the results are valuable and applicable for liquid samples involving multivitamin syrups and vitamin fortified beverages, because the protective effect of sorbitol was tested on standard solutions of cobalamins, thiamin, niacin and ascorbic acid. However, there are several imitations. Firstly, sorbitol concentrations lower than 250 μg/mL were not evaluated, so that its protective effect on the stability of cobalamins was not able to be compared with the current data. Secondly, no real samples (e.g. vitamins fortified beverages) were prepared either with or without added sorbitol for the quantification of cyanocobalamin. Therefore, further research should be undertaken to investigate the stability of cobalamins in various food matrices, which requires optimised protocols in the cobalamin extraction and sample-clean up. Future work also needs to examine more closely the effect of storage on the stability of cobalamins after the addition of ascorbic acid.

Conclusions

We have highlighted the importance of the stability of cobalamin vitamers and to minimise the degradation loss,

which is potentially a very useful contribution to the current literature. This study offers some insight into the protective effect of sorbitol as a stabilising agent for B_{12} vitamers due to interactions with thiamin, niacin and ascorbic acid, and degradative physical conditions. The data presented here would be beneficial for cobalamin analyses in multivitamin liquids and for manufacturers of vitamin fortified beverages, in order to improve the stability or to retain the nutrients, thereby reducing degradation losses that commonly occur during processing.

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Conflict of interest
The authors declare that they have no conflict of interest.

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