Comparison of measured and declared vitamin D concentrations in Australian fortified foods

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Abstract

Fortified foods are an important source of dietary vitamin D, since this nutrient occurs naturally in relatively low concentrations in a limited number of foods. Hence, we aimed to investigate the accuracy of the declared vitamin D content of Australian fortified foods. Vitamin D_3, 25-hydroxyvitamin D_3 (25(OH)D_3), vitamin D_2 and 25(OH)D_2 were measured in 30 fortified food samples (edible oil spreads, malted chocolate drink powders, soy milks and breakfast cereals) using liquid chromatography with triple quadrupole mass spectrometry. The measured vitamin D content ranged from -54% to +190% of declared values. One product had measured vitamin D content close to the declared value, while 10 of 14 products had vitamin D in excess of that declared. Label information proved an unreliable indicator of measured vitamin D content across all product categories which may be problematic for those relying on fortified foods as their main source of vitamin D.

Keywords: breakfast cereal; edible oil spreads; fortified foods; drink powder products; soy milk; vitamin D

Abbreviations

%CV	percentage coefficient of variation
25(OH)D	25-hydroxyvitamin D
EU	European Union

FSANZ	Food Standards Australia New Zealand
LC-QQQ	liquid chromatography with triple quadrupole mass spectrometry
LOD	limit of detection
NMI	National Measurement Institute of Australia
RPD	relative percent difference
UVB	ultraviolet-B

1. Introduction

Vitamin D plays a crucial role in bone health: severe vitamin D deficiency can result in rickets in infants and children, and osteomalacia in adults. It is, therefore, concerning that vitamin D deficiency (serum 25-hydroxyvitamin D (25(OH)D) <50 nmol/L) affects 20% of Australian adults aged ≥25 years (Malacova et al., 2019), 32% of young adults (18-24 years) (Horton-French et al., 2021) and 39% of remote-dwelling Aboriginal and Torres Strait Islander adults (Black et al., 2019). Exposure to ultraviolet-B (UVB) radiation from the sun offers the greatest potential source of vitamin D; however, diet is an alternative source of vitamin D when sun exposure is inadequate. As naturally occurring vitamin D exists in few foods and usually in low concentrations, vitamin D fortification of certain foods is mandated or permitted in many countries in order to improve vitamin D intakes and status at the population level (Calvo et al., 2004; Lips et al., 2019). In Australia, it is mandatory to fortify edible oil spreads, such as margarine and other water-in-oil emulsion spreads, with vitamin D (Food Standards Australia New Zealand [FSANZ], 2019). Other food products that are approved for voluntary vitamin D fortification include some dairy products and their plant-based alternatives, selected breakfast cereals and formulated beverages (FSANZ, 2019).

Food manufacturers sometimes add an excess of nutrients to supplements and fortified foods to compensate for potential losses during manufacture and potential instability of the food matrix (World Health Organization [WHO] and Food and Agricultural Organization of the United Nations [FAO], 2006). However, accurate information about the actual vitamin D content of fortified foods is important for estimating vitamin D intakes. Previous studies have analysed vitamin D in fortified foods (infant formulas, milk products, orange juice and edible oil spreads) in America, Canada, New Zealand and the Netherlands. Both under-fortification (underages) and over-fortification (overages) were observed in vitamin D-fortified foods, with the measured vitamin D content in some products varying considerably from that indicated on the label (Byrdwell et al., 2011; Nimalaratne et al., 2014; Patterson et al., 2010; Pehrsson et al., 2014; Thomson, 2006; Verkaik-Kloosterman et al., 2017).

To our knowledge, no previous study has investigated the accuracy of the declared vitamin D content in fortified foods in Australia. Hence, the primary purpose of this study was to measure the vitamin D content of fortified foods on the Australian market and compare this to the declared value. Secondary aims were to assess the measured content against the requirements of the Australia New Zealand Food Standards Code and international nutrient tolerance limits, and to investigate the variability in measured vitamin D content between batches and products. We further investigated whether remaining shelf life was a predictor of percentage overage or underage.

2. Methods

2.1 Product selection and transport

An inventory of vitamin D-fortified foods on the market in Perth, Australia, was conducted in May 2019. We identified a list of 61 vitamin D-fortified food products within the following categories: edible oil spreads, drink powders, milk and milk alternatives, and ready-to-eat breakfast cereals. Popular brands and products were identified by shelf-stocking density/product facings (Russell and Urban, 2010; Urban, 1969). Of these, 14 different food products with the greatest product facings were selected for analysis: four edible oil spreads, two malted chocolate drink powders, four soy milks and four breakfast cereals. Food products were chosen to cover a range of popular product categories and, where possible, to capture a range of declared vitamin D content within food types.

Samples were purchased from five supermarkets in Perth, Western Australia across three days in June 2019. To examine any potential variation in vitamin D content between different production batches of the same product, one product from each food category was selected and five samples from various batches were purchased for that food product. The five replicate samples had best before dates ranging between the following dates: edible oil spreads, October 2019 to December 2019; malted chocolate drink powders, March 2020 to November 2020; soy milks, August 2019 to December 2019; breakfast cereals, December 2019 to March 2020. The remaining food products were purchased as single items. All samples were purchased before the best before date. All products were made in Australia for the Australian market. Chilled edible oil spreads were packed in insulated containers with ice bricks and couriered overnight, while shelf-stable samples were boxed and sent by regular courier, to the National Measurement Institute (NMI) in Melbourne, Victoria, for analysis.

2.2 Chemical analysis

All samples were prepared, frozen and analysed within 9 weeks after purchase and prior to the best before date (Table 1). Samples were analysed for fat content using acid hydrolysis and Mojonnier tube extraction (AOAC International, 2005b) and moisture by NMI's in-house method that was based on an AOAC method (AOAC International, 2005a). Vitamin D_3, 25(OH)D_3, vitamin D_2 and 25(OH)D_2 were measured using liquid chromatography with triple quadrupole mass spectrometry (LC-QQQ), with saponification, extraction and derivatisation processes conducted under yellow/non-ultraviolet light to minimise risk of analyte deterioration (Gill and Indyk, 2018).The full LC-QQQ method and its validation have been described in detail previously (Dunlop et al., 2021). Briefly, a saponification mixture of sample, a known quantity of chemically labelled internal standard solution, 1 g sodium ascorbate, 10 mL deionised water, 30 mL ethanol and 2 g potassium hydroxide was made up

to 50 mL with additional deionised water in a 50 mL Falcon® tube. The quantity of sample (1-2.5 g solid sample; 5-15 g liquid sample) used was determined to produce a quantity of saponified fat ≤1 g in order that the volume of the saponification mixture did not exceed that of the Falcon® tube. The chemically labelled standard solution consisted of isotopically-labelled metabolites (vitamin D_3 [$^{13}C_5$] carbon-13 labelled standard, purity ≥97%; 25(OH) D_3 [$^{13}C_5$] carbon-13 labelled standard, purity ≥95%; vitamin D_2 [$^{2}H_3$] deuterated standard, purity ≥98%; and 25(OH)D_2 [$^{2}H_3$] deuterated standard, purity ≥98%), sourced from IsoSciences (Ambler, USA). The Falcon® tubes were placed in a shaker bath overnight (~16 h, 25°C). The D vitamer analytes were hydrolysed in the saponification mixture and absorbed onto diatomaceous earth (Chem Elut™ 10 mL unbuffered SPE cartridges, Agilent Technologies). They were then extracted into petroleum ether and evaporated to dryness using nitrogen gas. Resolvation of the resulting residue into a 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) in anhydrous acetonitrile solution induced formation of vitamin D-PTAD derivatives. Water was added to halt this derivatisation after 10 min. Extracts were centrifuged (10,000 rpm, 1 min) where necessary to separate out any precipitate, then placed in microvials.
The D vitamer analytes were separated by reverse phase chromatography (Supelco Ascentis® Express C18 column, 15 cm x 3 mm, 2.7 µm) with eluent A as 1 L Milli-Q® water, 1 mL 0.1% formic acid, 0.5 mL 6.4 nM methylamine and eluent B as 1 L methanol, 1 mL 0.1% formic acid, 0.5 mL 6.4 nM methylamine. The gradient profile as time (min:sec), % eluent B was as follows: 0.00 min, 80%; 1.00 min, 80%; 13.00 min, 97%; 13.01 min, 100%; 17.00 100%; 17.01, 80%, 20.00 80%, with a constant flow rate of 0.6 mL/min. The LC-QQQ (1290 Infinity Series LC System and 6460 Triple Quad LC-MS, Agilent Technologies, Santa Clara, USA) was operated in electrospray ionisation mode with positive polarity. The product ions used to quantify and qualify the D vitamers are detailed elsewhere (Dunlop et al., 2021). This process was repeated for calibration samples comprising the same isotopically labelled

metabolites to produce calibration curves (Supplementary Figure 1) against which the D vitamer analytes were quantitated. Previous validation of the method using National Institute of Standards and Technology (NIST) Standard Reference Material 1546a (meat homogenate) has demonstrated that the LC-QQQ method provides a mean value for vitamin D_3 within the NIST reference range and a mean value 0.01 μg/100 g outside the NIST reference range for 25(OH)D_3 (Dunlop et al., 2021).

For each sample, the process of saponification, extraction, derivatisation and quantitation was carried out in duplicate. The resulting replicate values were averaged to give a single mean value for each sample. For each vitamer, the relative percent differences (RPD) between duplicate analyses (acceptable range ≤25%) and percent recoveries (acceptable range 80-120%) were recorded. Limits of detection (LOD), which are matrix dependent and defined as the lowest concentration detected during the analytical run, were recorded.

2.3 Label data

The vitamin D content (μg/100 g or 100 mL), as declared by the manufacturer on the nutrition information panels, was recorded for each food. The declared vitamin D content was assumed to be the sum of added vitamin D and any naturally occurring vitamin D. For two of the eight edible oil spreads, vitamin D content was not displayed on the nutrition information panel and was only listed in the ingredient list. In these cases, the manufacturers were contacted to provide the amount of vitamin D that was added. This amount was used in place of a declared value with the assumption that it represented the total vitamin D content of the product. The form of vitamin D added was not specified on the product packaging of any samples.

2.4 Statistical analysis

All data were analysed using IBM SPSS Statistics version 25 (IBM Corp.). Fourteen different food products from four different food categories were analysed in this study. Five replicate purchases were made for ‘product 1’ of each food category to allow between-batch analysis, providing a total of 30 analytical samples.

Total vitamin D content for each analytical sample was calculated as the sum of all forms of vitamin D measured. For edible oil spreads, this measured content was compared to the mandated content of no less than 55 mg/kg (5.5 mg/100 g) of vitamin D (FSANZ, 2019). The Australia and New Zealand Food Standards Code stipulates a ‘maximum permitted amount’ of vitamin D that may be added to a reference quantity of edible oil spreads, soy milks and drink powders (FSANZ, 2019). All measured vitamin D values were compared to this amount, which we expressed as μg/100 g, or μg/100 mL for soy milk products (Table 1), to allow comparison between products with differing reference sizes. Fortified breakfast cereals are not required to comply with a maximum permissible amount.

To compare the measured vitamin D content with that declared on the product label, overage/underage was calculated as the difference between the measured vitamin D content and that stated on the nutrition information panel, then expressed as a percentage of the vitamin D content on the nutrition information panel. In Europe and the US, guidelines are provided regarding the acceptable difference between declared vitamin D content data and analysed values for fortified foods (i.e. ‘tolerance values’). These are -35% to +50% for the European Union (EU) (European Commission, 2012) and ‘at least equal to the labelled value’ for fortified foods sold in the US (US Food and Drug Association, 2006). There are no similar nutrient tolerance values for fortified foods in Australia (Fabiansson, 2006); therefore, we compared percentage overage and underage to EU guidelines.

The range, standard deviation, %CV, and 95% CI were calculated to assess the variability of vitamin D content between batches of selected products (Table 2). For comparisons between different products within the same food category, and between all food products, the first replicate of product 1 was used. All comparisons between different products were done by comparing the range of percentage overage or underage (Figure 1a). In order to determine whether remaining shelf life was a predictor of the percentage overage or underage, the time between analysis date and best before date was calculated and a Pearson's correlation performed between this and percentage overage or underage. A P value of <0.05 was considered as statistically significant.

3. Results

3.1 Quality control results

The LOD for all vitamers was 0.2, 0.05, 0.02 and 0.05 µg/100 g for edible oil spread, drink powder, soy milk and breakfast cereal respectively. All RPDs and recoveries were within acceptable ranges. Mean RPDs between duplicate analyses were 5.35% and 5.29% for vitamin D_2 and vitamin D_3, respectively. In spiked samples, vitamin D recoveries were 86-113% for vitamin D_3, 80-93% for 25(OH)D_3, 101-115% for vitamin D_2 and 80-96% for 25(OH)D_2. Recoveries of vitamin D_3 from in-house control samples ranged between 100-105%.

3.2 Measured vitamin D content

Across all 14 fortified food products, the total content of vitamin D varied over 100-fold, ranging from 0.23 µg/100 g in soy milk (product 2 – the only low-fat soy milk analysed) to 27.0 µg/100 g in drink powder (product 2) (Table 1, Figure 2). Standardising vitamin D for serving size reduced this variability to range from 0.48 μg/serve in edible oil spreads (product 2) to 8.2 μg/serve in soy milk (average of product 1 replicates).

In addition to vitamin D_3, vitamin D_2 was found in all drink powder products (0.3-0.5 μg/100 g) and in some breakfast cereal products (0.1-0.2 μg/100 g) . Only vitamin D_2 was detected in soy milk products. In edible oil spreads, vitamin D was present as vitamin D_3 in all but one spread that was marketed as vegan, which was fortified with vitamin D_2. Neither 25(OH)D_3 nor 25(OH)D_2 was detected in any food sampled.

3.3 Measured vitamin D content compared to requirements of the Australia New Zealand Food Standards Code

One of four edible oil spreads tested did not meet the mandated minimum content of 5.5 μg/100 g containing 14% less than this minimum requirement. All edible oil spreads were compliant with the maximum permitted amounts in the Food Standards Code (Table 1, Figure 2). One drink powder product had a vitamin D content of 2 μg/100 g above the maximum permitted amount and two soy milk products had vitamin D contents that were more than three times higher than permitted (2.6 and 3.3 μg/100 g vs. the permitted amount of 0.8 μg/100 mL). The two soy milks that exceeded the maximum permitted amount in measured vitamin D also had declared amounts greater than the maximum permitted amount.

3.4 Measured vitamin D content compared to declared values (overage/underage)

Across all food categories the percentage overage/underage of vitamin D varied widely from -54% in soy milk product 2 to +190% in breakfast cereal product 2. Across all products, the median was an overage of more than one third higher than the declared value (median = 36.3 ± 60.6%).

There was also a wide variation in the percentage overage/underage across the products tested within each food category. The widest variation occurred in the breakfast cereal category, where all products had vitamin D contents greater than the declared value (range: +14.4% in the mean of product 1, +190% product 2). There were only two vitamin D-fortified drink powder products on the market, and both had a measured vitamin D content greater than the label value (4.0 % in the average of product 1 and 35.1% in product 2). Among edible oil spreads, products 1 and 2 had underages (-33.4 and -13.6%) whereas products 3 and 4 had overages (45% and 55%). In the soy milk category, the overage/underage ranged from -54% (product 2) to +64% (average of product 1 replicates).

3.4 Overage and underage compared to EU guidelines.

Most (three of four) breakfast cereals exceeded the EU tolerance limit of 50% over the declared value (Figure 1). The edible oil spreads and soy milk categories included products with overage and underage outside EU tolerance limits, whereas both fortified drink powder products complied with the overage/underage requirements of the EU.

3.5 Between-batch comparison

Data for product 1 replicates are shown in Table 2. The inter-sample variability is given as CV%, which ranged from 3.7% in replicates of the soy milk to 19.1% in replicates of the edible oil spreads. For the breakfast cereal and drink powder products, label values for vitamin D were captured within the 95% CI for the measured vitamin D content in the product replicates; however, label values for the soy milk and edible oil spreads were below and above the 95% confidence intervals, respectively (Table 2).

Figure 1B shows the overage/underage values of the replicates, for comparison with Figure 1A data showing overage/underage values of different products within each food category.

3.5 Association between vitamin D content with time to best before date

The number of days between the analysis date and best before date was determined for all 30 analytical samples (including replicate samples of the same product) with the range being from 2 and 417 days before their best before date (Table 1). Across all food products, there was no association between the number of days between the analysis date and best before date and the percentage overage/underage ($r = -0.13$, $p=0.492$).

4. Discussion

This study measured the vitamin D content in fortified foods using a validated LC-QQQ method and adds to the current literature on the vitamin D content of Australian foods (Dunlop et al., 2017; Dunlop et al., 2021; Hughes et al., 2018). The nutrition information panel proved an unreliable source of information for assessing the vitamin D content of fortified foods across all four food categories. In the products that we tested, the measured content of vitamin D varied substantially from that stated on the nutrition information panel, ranging from half to almost three times the label value. Only one product had a measured vitamin D content close to the nutrition information panel value (drink powder product 1), while the majority of products (11 of 14) had a measured vitamin D content in excess of the declared value. The vitamin D contents measured in three foods were lower than the declared value.

Discrepancies between the measured and declared values of vitamin D in a fortified food could be due to a number of reasons. The predominance of overages in our study suggests that food producers add more vitamin D than is stated on the nutrition information panel. This may be done to compensate for presumed degradation of vitamin D during processing and to maximise the likelihood that sufficient vitamin D remains until the end of a product's shelf life (WHO and FAO, 2006). In our study, the product with the largest underage was analysed just two days before the best before date; however, when we looked across all food products and within replicates of the same product, we found no association between days remaining before the best before date (from the analysis date) and the percentage overage or underage of vitamin D content. Although vitamin D is sensitive to oxygen and light, vitamin D fortificants are generally dried and include an antioxidant to promote stability (WHO and FAO, 2006), and vitamin D has been demonstrated as being stable across the shelf-life of various fortified foods (Hanson and Metzger, 2010; Indyk et al., 1996; Jafari et al., 2016; Wagner et al., 2008). For some products, failure to account for vitamin D that may be naturally present in the product's ingredients may also contribute to inadvertent addition of excess fortificant. This is a plausible scenario for malted chocolate drink powders where key ingredients include cocoa and milk solids, both of which contain naturally-occurring vitamin D (Dunlop et al., 2021).

Underage occurred in only three of 14 products included in our study. The largest underage was observed in a soy milk product, which was also the only low-fat soy milk product tested (<0.2 g fat /100 g). In two US studies that investigated the vitamin D content of dairy milks (Holick et al., 1992; Patterson et al., 2010), more low-fat milks contained vitamin D concentrations below the declared value than higher-fat milks; however, these differences could occur due to production and processing factors (Patterson et al., 2010), which would be expected to differ between soy and dairy milks. As vitamin D is a fat-soluble vitamin, the solubility of the fortificant could feasibly differ in food products according to their fat content; however, water-soluble vitamin D fortificants are available to prevent this issue

(WHO and FAO, 2006). Alternatively, inadequate homogenisation following addition of a vitamin D fortificant could contribute to differences in concentrations within and between batches. Overall, our findings suggest that manufacturers of the foods included in our study were more likely to err on the side of a vitamin D overage, and may be inadvertently compensating for presumed losses during processing and storage that do not eventuate.

The majority of products tested complied with the requirements of the Australia New Zealand Food Standards Code (FSANZ, 2019). However, of the edible oil spreads, which are the only foods to which vitamin D must be added, the vitamin D content measured in one product failed to meet the minimum requirement. Of the voluntarily-fortified foods, the vitamin D content of one malted chocolate drink powder product marginally exceeded the maximum permitted amount while two voluntarily-fortified soy milk products exceeded this limit by nearly two-fold. In Australia, fortified breakfast cereals are not subject to a maximum permitted amount of vitamin D. Rather, a maximum claim of 2.5 μg vitamin D per normal serving may be made by manufacturers on packaging of breakfast cereals that meet certain nutrient criteria. A maximum permitted amount was not proposed as modelling of vitamin D fortification in excess of the claimable amount indicated no risk to public health and safety (FSANZ, 2015). A maximum permitted amount of vitamin D in breakfast cereals is regulated in other countries such as Singapore (Singapore Government, 2005) and USA (US Food and Drug Association, 2021).

If the Australian products tested in this study were subject to EU nutrient tolerance values, half would be non-compliant. Similar studies conducted elsewhere in the world have had varied findings when comparing measured vitamin D to food label tolerance values. In a report commissioned by the New Zealand Food Safety Authority, one third of vitamin D-fortified foods (*n*=18) sampled (baby food, drinks, edible oil spreads and milk products) would have been non-compliant if tested against the EU tolerance limits (Thomson, 2006). Australia and New Zealand share a Food Standards Code (FSANZ, 2019), which does not include similar tolerance values for fortified foods. In the US, where the actual nutrient content of fortified foods must be "at least equal to" the declared value (US Food and Drug Association, 2006), a study found that 28% of 120 the vitamin D fortified dairy milk products sampled were over-fortified, where the analysed vitamin D_3 content was >125% of the declared value; 7% of the samples contained over 150% of declared values (Patterson et al., 2010). In contrast, a study conducted in The Netherlands, which is subject to EU tolerance limits, analysed vitamin D-fortified infant foods, showing that 93% of infant formulas, porridge and dessert (*n*=29) complied with EU vitamin D fortification requirements (Verkaik-Kloosterman et al., 2017). Enforceable tolerance limits, similar to those that apply to EU countries, may help to reduce the gap between declared and actual vitamin D content in Australian fortified foods.

When we investigated the variability in vitamin D content between batches of the same product, the edible oil spread product had the greatest between-batch variability (19% CV) compared to products from other food categories. In comparison, the aforementioned study commissioned by the New Zealand Food Safety Authority included eight between-batch analyses of margarine products, with %CVs ranging from ~3 to~35% (Thomson, 2006). In that study, the greatest %CV (46%) was observed in the category of 'food drinks' (Thomson, 2006). This 'food drinks' category included malted chocolate drink powders as well as liquid breakfast products, liquid meal replacements and other manufactured beverages; however, as products were deidentified, it is not possible to determine whether the higher variability of that product relates to a malted chocolate drink powder comparable to those included in our study, or another type of food drink. Nevertheless, between-batch variability appears to be common in vitamin D-fortified foods. In our study, the range of values for percentage overage or underage for different batches of the same product was narrower than that observed between different products in the same category. So, although batch to batch variability would contribute to some of the difference in vitamin D content compared to food labels, other factors such as inaccurate dosing and/or incomplete homogenisation of batches are likely to be more important contributors.

Our results suggest that the declared vitamin D content of fortified foods in Australia does not necessarily represent the actual vitamin D content. Discrepancies in the declared content versus actual content have wide-ranging implications for estimation of vitamin D intakes at the individual and population levels. The issue of underage is concerning for individuals who may use fortified foods to improve their dietary vitamin D intake, while overage may result in some believing that their vitamin D intakes are lower than they actually are. If fortified foods are primary contributors to vitamin D intakes in Australia as they are elsewhere (Ahmed et al., 2021; Herrick et al., 2019), large discrepancies in actual versus declared content of these foods may influence estimations of usual intakes at the population level. Hence, it is important that the declared content represents the actual vitamin D content. Manufacturers may require guidelines to achieve this. Internationally, the Codex Alimentarius recommendation is for the declared nutrient concentration on food labels to be based on analysed values rather than calculations (WHO and FAO, 2007). However, such requirement is not specified in the Australia New Zealand Food Standards Code and maximum permissible values are used in place of tolerance limits. More specific regulations in Australia around the vitamin D fortification of food may drive improved parity between declared and measured vitamin D concentrations in fortified foods.

A strength of our study is that all foods sampled were analysed for four forms of vitamin D using a LC-QQQ method with the ability to detect low vitamin D concentrations in food. We investigated the difference in vitamin D content between different food products and between batches of the same product. The products were all sampled in Perth, Australia; however, as these processed and packaged foods are generally produced at a central factory and transported around the country, our findings are expected to be representative of products available nationally. Although we selected the most popular brands in order to represent the most consumed products, limited products within each food category (14 of 61 identified brands/varieties) were analysed and we did not examine differences between products within the same batch. Our findings, therefore, support further investigation of a greater range of brands and varieties of fortified foods. A general limitation of food composition data is that they provide a snapshot of concentration values for a single time point based on resource-constrained sampling. We measured vitamin D content across multiple batches of the same product in order to capture any variation that occurs within products; however, the measured values reported may not represent the vitamin D content of specific product items available for purchase.

5. Conclusion

This was the first study in Australia to analyse the vitamin D content of fortified foods and compare them with the declared value on their nutrition information panels, along with comparing to the maximum permitted amounts allowed in Australia and to international tolerance limits. The analysed vitamin D content of the majority of the foods sampled deviated from the declared values, with almost half failing to meet international tolerance limits. Variation in the vitamin D content of different batches does not appear to relate to shelf life and rather appears to be due to variation in the amount of vitamin D added during food processing. Provision of guidelines to manufacturers and more specific tolerance values may help to reduce the difference between the declared and actual vitamin D content of fortified foods. In the meantime, health professionals and researchers should take the possibility of inaccurate label data into consideration when relying on declared vitamin D values of fortified foods.

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