Light Oxidation of Vitamin D in Different Containers

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INTRODUCTION

In recent years, there has been a growing concern regarding the amount of vitamin D people are getting. This vitamin is important for normal mineralization and growth of bones. Milk has commonly been fortified with vitamin D to decrease deficiencies, but studies have shown how excessive light exposure can decrease vitamin levels in milk Many studies have been done to test the stability of vitamins in milk. They have shown how excessive light exposure can decrease the vitamin levels in milk. Unlike vitamin A and riboflavin, the decay of vitamin D as a result of light exposure has not been as thoroughly studied. A major reason for this decay is the type of container used to hold the milk. Opaque, made of high density polyethylene, containers tend to better protect the fortified vitamins. Translucent, made of polyethylene terephthalate, containers are more susceptible to vitamin loss by light exposure but are more environmentally friendly. Another issue deals with the smaller bottles of milk often found in convenience stores. These small bottles are constantly exposed to the bright lights in the store's coolers. The loss of vitamin D because of too much light only adds to the already low amount in some milk as well as the growing number of deficiencies.

METHODS AND MATERIALS

Samples of Organic Valley whole milk were fortified with a designated amount of vitamin D_2 and D_3, and divided as shown in Table 1. Half were wrapped in foil and half were not. They were exposed for either 3, 5. or 7 days. The samples were placed on a rotating turntable in a refrigerator under a fluorescent light (See Photo 1). The strength of light exposure was measured with a light meter (ExTech Instruments easy view digital light meter, model EA31) and corresponded to that of a typical dairy cooler. The method of analysis involved precipitating protein in 10 ml of milk with 10 ml of ethanol, sonicating for 1 minute at 70% amplitude with a sonic horn immersed in the sample to break up complexes, and extracted with 2 liquid extractions of 15 ml each with hexane followed by 5 minutes centrifugation at 3500 rpm. The hexane layer was dried down under nitrogen and reconstituted in 1 ml hexane. Solid phase extraction with an adsorbent designed for fatty acid methyl esters (Ag-Ion SPE tube, Sigma-Aldrich, Bellefonte, PA), and reverse phase HPLC of the eluent. The levels of vitamin D before and after exposure were measured and compared using the HPLC method.

	Bottle 1 (OPAQUE)		Bottle 2 (TRANSLUCENT)	
DAY 1 (3 days)	FOIL	NO FOIL	FOIL	NO FOIL
DAY 2 (5 days)	FOIL	NO FOIL	FOIL	NO FOIL
DAY 3 (7 days)	FOIL	NO FOIL	FOIL	NO FOIL

Table 1. The experimental set-up.

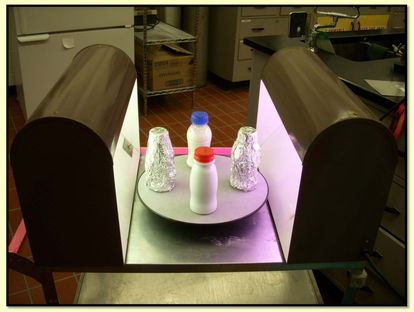
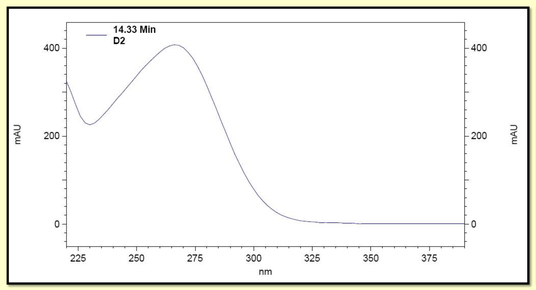


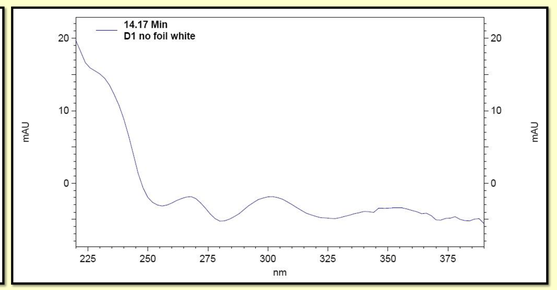
Photo 1. The rotating turntable with fluorescent light

	DAY 1		DAY 2		DAY 3	
	D_2	D_3	D_2	D_3	D_2	D_3
CLEAR						
Foil	621	482	648	513	670	588
No Foil	502	430	623	568	666	559
WHITE						
Foil	670	610	361	0	144	634
No Foil	123	31	431	530	318	507

Table 2. Concentrations of Vitamin D. .



Graph 2. The D_2 spectrum



Graph 3. The spectrum of day 1 white with no foil

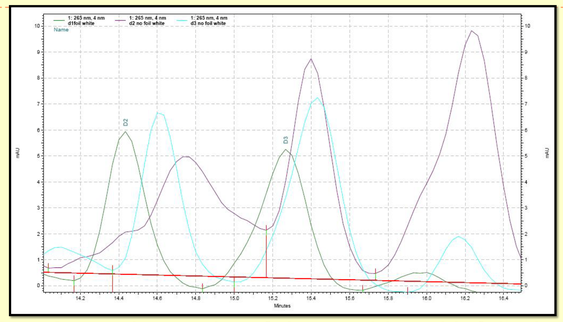
RESULTS & DISCUSSION

After analyzing the samples using the HPLC, the results were inconsistent. It appeared that the D2 and D3 peaks of the white bottle with no foil eluted later than the foil lined bottle(Fig. 1). This is a sign that the peaks picked up on the graph were something other than vitamin D. To further support this, the spectrum graph was analyzed (Graph 4). Knowing that vitamin D absorbs at 265 nanometers, the absorption spectrum should have been similar to that of Graph 2, but this was not the case. This type of result happened for all the values colored red in Table 2. It was concluded that these samples either underwent oxidation, or that other materials were formed during the process and eluted at a similar time. The differences of vitamin D concentrations seen between the clear (PETE) and opaque (HDPE) bottles were surprising. This could have been due to the type of plastic, it's oxygen permeability and light transmission.

Although the results were somewhat inconclusive, there were several points throughout this experiment at which vitamin D oxidation could have occurred:

- During storage of the sample before the extraction phase.
- During the N_2 dry-down within the extraction phase itself.
- With the SPE tubes used. The SPE tubes contained Ag-ions which bind to double bonds. These ions could have been absorbed along with the vitamin D during the extraction stage.
- The type of plastic bottles used. The oxygen permeability and light absorption spectrum of the PETE and HDPE containers are different.

A possible follow-up experiment could be to try and indentify the unknown peaks by doing liquid chromatography-mass spectrometry to see what their origin was.



Graph 1. The peaks of day 1 white with foil, day 2 white with no foil & day 3 white with no foil.

REFERENCES

1. King, Joan M., and David B. Min. "Riboflavin-Photosensitized Singlet Oxygen Oxidation Product of Vitamin D2." Journal of the American Oil Chemists' Society 79 (2002): 983-87.
2. "Milk, yes, but from what kind of container?" Tufts University Health & Nutrition Letter 16 (1999): 7.
3. Renken, Shelly A., and Joseph J. Warthensen. "Vitamin D Stability in Milk." Journal of Food Science 58 (1993): 552-55. Wiley Interscience.
4. "Vitamin D." Medical College of Wisconsin Healthlink. 14 Feb. 2001. National Institutes of Health.