

# Vitamin Fortification of Fluid Milk

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**Abstract:** Vitamin concentrates with vitamins A and D are used for fortification of fluid milk. Although many of the degradation components of vitamins A and D have an important role in flavor/fragrance applications, they may also be source(s) of off-flavor(s) in vitamin fortified milk due to their heat, oxygen, and the light sensitivity. It is very important for the dairy industry to understand how vitamin concentrates can impact flavor and flavor stability of fluid milk. Currently, little research on vitamin degradation products can be found with respect to flavor contributions. In this review, the history, regulations, processing, and storage stability of vitamins in fluid milk are addressed along with some hypotheses for the role of vitamin A and D fortification on flavor and stability of fluid milk.

**Keywords:** fluid milk, light oxidation, vitamin A, vitamin D, vitamin fortification

**Practical Application:** Many of the degradation components of vitamins A and D have flavor/fragrance applications, but there is little published research on their possible flavor contributions to fluid milk. Vitamin concentrates can impact flavor and flavor stability of fluid milk. Proposed mechanisms of off-flavor development and changes in flavor stability of fluid milk are discussed in this review.

## Introduction

Current fluid white milk consumption in the United States has steadily declined in the last 50 years (International Dairy Foods Association 2008; USDA 2014). As a result, the level of dietary vitamin D provided by fluid milk in the U.S. diet has also declined (Moore and others 2004; Whiting and Calvo 2011; Looker and others 2011). Increased fortification of vitamin D in fluid milk and fortification of a larger variety of products with vitamin D have been recommended (Calvo and Whiting 2003; Dietary Guidelines Advisory Committee 2015). Milk is commonly displayed under fluorescent or light-emitting diode (LED) lighting in retail dairy cases. Longer storage periods increase the risk of light exposure, which increase the risk of altering milk quality before purchase (Johnson and others 2015). Both fluorescent and LED lighting sources deliver light energy in UV and visible wavelengths regions that cause light oxidation of photosensitive molecules such as riboflavin in milk. Light oxidation is the most common source of flavor and nutritional problems in milk. Another possible source of flavor variability in fluid milk is vitamin fortification.

Vitamin fortification has a long history in fluid milk in the United States to reduce rickets in children, and the FDA mandated in the 1990s that fortified fluid milks must be within 100% to 150% of label claims to address documented variability in vitamin amounts (Public Health Service 1940). Reduced fat and skim milks are required to be fortified with vitamin A at a minimum of 2000 International Unit (IU) and this is optional for whole milk. All fluid pasteurized milk must be fortified with vitamin D at a minimum of 400 IU. Vitamin fortification is a standard procedure for pasteurized fluid milks in the United States and vitamin concentrates are added to milk before pasteurization (PMO 2015). There are 2 types of vitamin concentrates: oil soluble and water

dispersible formulations (Murphy and Newcomer 2001). Recent work has addressed vitamin D fortification of cheesemilk (Wagner and others 2008; Ganesan and others 2011; Tippetts and others 2012), processed cheese (Upreti and others 2002), and yogurt (Hanson and Metzger 2010) but the role of vitamin concentrate on flavor and flavor stability of fluid milk has not been addressed. Vitamin A is sensitive to light exposure and rapidly degrades. This degradation process occurs more quickly in skim milk compared to milks with fat in them (Senyk and Shipe 1981; Whited and others 2002). The 2 previous studies suggested that vitamin A concentrate imparted an off flavor to skim milk, but the specific role of the actual vitamin source and the carrier on off flavor or light oxidized flavor was not evaluated. It is of key importance for the dairy industry to strategically position vitamin fortification and enhance fluid milk quality. Understanding the possible role of vitamin fortification on off flavor and light oxidized flavor in fluid milk is of great importance.

The hypotheses of this review are as follows: (1) vitamin concentrates can contribute flavor to fluid milk, and (2) vitamin concentrates can increase light oxidized off flavors in fluid milk and/or contribute off flavor(s) to fluid milk.

## Origin of Vitamin Fortification in Fluid Milk

Fortification is defined as the process of adding micronutrients such as essential vitamins to food (Alvarez 2009). The fortification of food products has been practiced for more than 80 y. Vitamin deficiencies that lead to rickets, ariboflavinosis, and pellagra in the U.S. are reduced by the consumption of foods fortified with vitamin D, vitamin B<sub>2</sub>, and niacin, respectively (West and others 2002; Bishai and Nalubola 2002). Vitamin D fortification of milk and milk products began in the 1930s. This practice was recommended by the American Medical Association Council on Foods and Nutrition to assist in reducing rickets in children (Stevenson 1955). Various methods such as animal feed supplementation and direct irradiation were applied to increase vitamin D content in milk, but the direct addition of vitamin concentrates proved to be most reliable and has become the accepted industry practice (Roadhouse and Henderson 1950). The wide acceptance of milk fortification with vitamin D led to the fortification of milk with

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vitamin A, which was initiated in the 1940s (Public Health Service 1940). The goal of fortifying milk with vitamin A was to ensure that Americans have a constant source of vitamin A because milk provided 10% of American consumers' food energy.

## The Need for Vitamin Fortification

### Vitamin D

Vitamin D is essential for calcium absorption and is involved in the mineralization process required for bone growth. Deficiency of vitamin D causes rickets (softening of bones) in children and osteomalacia in adults (Ceglia 2009). Recent studies also suggest that vitamin D plays a role in prevention of prostate, breast, and colorectal cancers (Grant and others 2007; Schwartz and Skinner 2007; Garland and others 2006; Bouillon and others 2006). The major vitamin D forms responsible for human health benefits are ergocalciferol (D<sub>2</sub>; Figure 1) and cholecalciferol (D<sub>3</sub>; Figure 2). These vitamin D forms are considered to be inactive until they are converted to their biologically active form, 1,25-dihydroxy vitamin D<sub>3</sub>, in the liver or kidney (Holick 1995).

Vitamin D is an essential vitamin that is made when the body is exposed to sunlight (Holick 1994). However, no photosynthesized vitamin D is produced in the skin for several months of the year for those who live in northern latitudes during winter, and supplementation of vitamin D is required to prevent deficiency (Calvo and others 2004; Weaver and Fleet 2004). Vitamin D<sub>3</sub> is found mainly in fish products and fish liver oils (Kutsky 1981). However, most foods typically consumed by humans are low in vitamin D content. Fish contains approximately 120 to 500 IU of vitamin D<sub>3</sub> per 3-oz serving, which is 50% to 200% of the recommended daily intake level as opposed to less than 25% in unfortified grains, meats, vegetables, and breakfast cereal (Holden 2009). Vitamin D<sub>3</sub> can also be produced synthetically by purification of 7-dehydrocholesterol from animal products and converted to vitamin D<sub>3</sub> by irradiation (Kutsky 1981). This synthetic form of vitamin D<sub>3</sub> is added to many foods, particularly milk products.

### Vitamin A

Vitamin A is needed for normal growth, vision, reproduction, and differentiation of epithelial cells. Vitamin A deficiency results in night blindness, xerophthalmia (progressive blindness caused by drying of the cornea of the eye), keratinization (accumulation of keratin in digestive, respiratory and urinary-genital tract tissues) and finally exhaustion and death (Zile and Cullum 1983). Vitamin A is a group of compounds that includes retinoids and carotenoids (Zile and Cullum 1983). Vitamin A from animal sources is already in a form of retinol that can be easily absorbed by the human body, whereas vitamin A from plant sources is a carotenoid that the body can transform into a retinol (Zile and Cullum 1983). Vitamin A palmitate (Figure 3) is a form of vitamin A found naturally in animal sources and also produced synthetically. Whole milk, butter, cheese, and eggs are also important dietary sources of vitamin A palmitate (Zile and Cullum 1983). Vitamin A fortification in reduced and fat-free milks is required because whole milk contains some vitamin A palmitate; however, vitamin A levels in reduced and fat free milks are much lower because fat soluble vitamin A palmitate is removed with fat. Therefore, fortification of vitamins in dairy products has been one of the approaches to address vitamin A deficiencies (Parish and Richter 1979).

### Vitamin Regulations in Fluid Milk

In 1935, vitamin fortification of milk was addressed in the U.S. Public Health Service Milk Ordinance and Code, and in 1939, Vitamin D Milk was defined as milk in which the vitamin D content was increased by a method and in an amount approved by the regulating health official (Public Health Service 1940). The Milk Ordinance and Code did not specify any fortification levels, but did recommend monitoring vitamin D concentrations by bioassays in a laboratory approved setting as required by the health officer. In 1953, the Milk Ordinance and Code established a level of at least 400 IU per quart (qt) for vitamin D milk fortification. This document also addressed the option of fortifying milk with other vitamins and minerals under "Fortified Milk and Milk

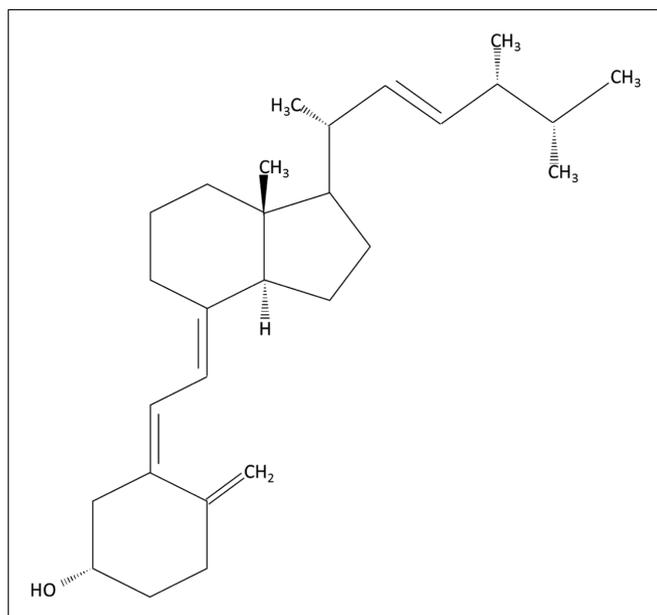


Figure 1—Chemical structure of ergocalciferol or vitamin D<sub>2</sub>.

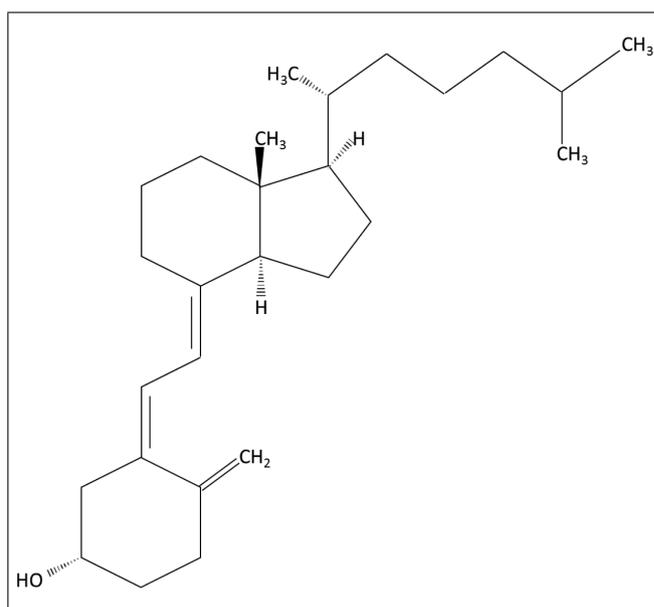


Figure 2—Chemical structure of cholecalciferol or vitamin D<sub>3</sub>.

Products,” although no concentrations were specified for other nutrients. The Milk Ordinance and Code continued to be revised and became the Grade “A” Pasteurized Milk Ordinance (PMO) in 1965 (Public Health Service 1965).

According to the U.S. Food and Drug Administration (FDA) regulations as specified in the PMO, the acceptable fortification concentrations were 80 to 120% of the label claims for both vitamins A and D. Over fortification of fluid milk with vitamin A and D can cause intoxication, soft tissue damage, and kidney failure (Jacobus and others 1992; Blank and others 1995). Based on FDA regulatory mandate (Public Health Service 1965), fluid milk products with levels of vitamin D over 800 IU and vitamin A over 6000 IU in fluid milk are considered a public health threat and should be prohibited from sale and distribution. In 1978, the PMO required that each processor monitor vitamin concentrations in fortified products at least once a year in a FDA certified laboratory using standard test methods such as high performance liquid chromatography (HPLC) methods (Public Health Service 1978).

New regulatory standards for vitamin fortification in the 1990s arose due to issues with variability in the vitamin content of retail milk. Over fortification of milk with vitamin D in one processing plant resulted in human illness from consumption of these products (Jacobus and others 1992). Several studies also found that numerous fortified milk products failed to meet label claims (Brown and others 1992; Holick and others 1992; Nichols 1991). Murphy and others (2001) reported that 53% to 55% of fortified milk products were out of compliance with the label contents for vitamin A and for vitamin D. Milk fortification practices were not entirely consistent with the Nutritional Labeling and Education Act of 1990. As a result, the FDA revised the accepted fortification levels and mandated that vitamin concentrations for fortified fluid products must be within 100% to 150% of the label claims (Nichols 1992), which equates to 400 to 600 IU per quart for vitamin D and 2000 to 3000 IU per quart for vitamin A (PMO 2015). If fluid milk products are found below 100% or above 150% of the required values or label claims, they should be resampled and the source of the problem determined (PMO 2015).

Fortification was not specified for low-fat or skim milks. Low-fat milk was first defined in the 1965 PMO as containing not less than 0.5% and not more than 2% fat. Skim milk was defined as milk with fat removed to a content of less than 0.5%. In the 2015 PMO, 21 CFR 130.10 states

“That nutrients must be added to the food to restore nutrient levels so that the product is not nutritionally inferior to the standardized food for products which combine a nutrient content claim, i.e., low-fat, non-fat, or reduced fat, with a standardized term, i.e., milk, sour cream, eggnog.”

Therefore, vitamin A should be added to dairy products from which fat has been removed, in an amount necessary to replace the amount of these vitamins lost in the removal of fat. Unfortified whole milk is not considered to be a significant source of vitamin D because natural vitamin D concentrations in whole milk range from 0.34 to 0.84 IU per gram of fat (McBean and Speckmann 1988). Thus, removal of fat does not render milk nutritionally inferior for vitamin D, and therefore fortification with vitamin D is optional for all milks. If added, vitamin D must be present at 400 IU per quart (PMO 2015). Most fluid milk in the United States is fortified with vitamin D due to the importance of vitamin D in human nutrition (Holick and others 1992; McBean and Speckmann 1988; Miller and others 2000).

The fortification of milk with vitamin D almost eliminated the public health concern of rickets in the 19th century. However, vitamin D deficiency has reemerged as a global health concern. Current low intakes of vitamin D and reduced time in sunlight exposure (increased time spent indoors) have resulted in inadequate vitamin D status (Dietary Guidelines Advisory Committee 2015). There has been interest in fortifying dairy products with higher levels of vitamin D. Patterson and others (2010) found that on average, vitamin D<sub>3</sub> in 2% milk was higher in 2007 compared with the vitamin D<sub>3</sub> levels in 2001, with a trend toward more samples of whole milk having greater than 150% of the labeled content. Hanson and Metzger (2010) reported that increasing the fortification of vitamin D from 100 to 250 IU per serving was stable over the shelf lives of HTST-processed 2% milk, UHT-processed 2% chocolate milk, and low-fat yogurt, and no change in sensory characteristics was found in these products. They concluded that increasing the fortification of vitamin D in milk was a feasible strategy to increase vitamin D supplementation.

Fortifying products with higher amounts of calcium or vitamin D is permitted by FDA provided that the product and any label claims comply with FDA regulations including standards of identity (U.S. Food and Drug Administration, 2013). The addition of vitamin D to milk and yogurt is optional. Unfortified cheese and cultured dairy products are not considered good sources of vitamin D. If added, the minimum amount of vitamin D in each quart of the product must not be less than 400 IU (PMO 2015). The acceptable range for vitamin D is 400 IU to 600 IU per quart of milk (PMO 2015).

Vitamin A can be found in significant amounts in unfortified whole milk because it is primarily associated with the fat phase of the milk at 37.7 IU per gram of fat (McBean and Speckmann 1988). However, milk fat removal results in vitamin A reduction in low-fat and skim milks. As demand for low-fat and skim milk products increased in the United States, there was a nutritional

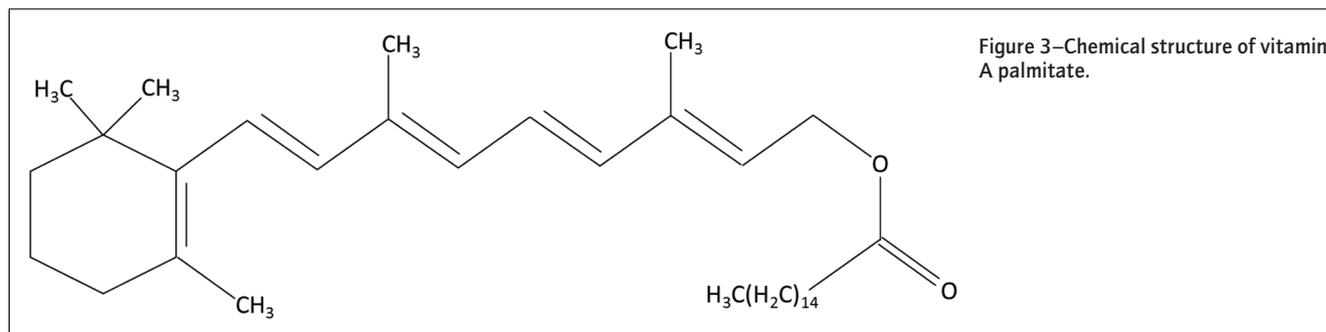


Figure 3—Chemical structure of vitamin A palmitate.

concern for reduction of vitamin A present in these products. This concern was addressed in the 1978 PMO (Public Health Service 1978), which required low-fat and skim milks to be fortified with vitamin A to the nutritional equivalence of the general milk standard—containing not less than 2000 IU per quart. Vitamin A fortification is optional for whole milk, but if added, the concentration must not be less than 2000 IU per quart.

To ensure proper fortification of dairy products, it is important to effectively monitor product concentrations. The latest revision of the PMO (2015) mandated that each manufacturer have representative milk products assayed at least annually in a laboratory certified by the FDA with methods that are acceptable to the FDA. Currently HPLC methods are the testing procedures of choice for vitamins A and D in milk. FDA is involved in the standardization of testing procedures among laboratories and has implemented a certification program for vitamin testing laboratories. Current listings of certified laboratories can be found in the IMS Quarterly Report. Samples for vitamin testing should be sent to an approved laboratory within a few days of processing, kept cold ( $\leq 4$  °C) without freezing and protected from light. Once received at the laboratory, analyses should begin as soon as possible.

### Vitamin Premixes and Addition in Fluid Milk Processing

In the United States, vitamin D is generally added to fluid milk as synthetic vitamin D<sub>3</sub>, whereas vitamin A is added as synthetic retinyl palmitate (Public Health Service 1965). Synthetic vitamin D<sub>3</sub> is made from irradiation of animal fat, usually from lanolin, the waxy secretions from sheep skin (Budvari 1996; Holick 1999; Smith 2016). Retinyl palmitate is made from combining vitamin A acetate with methyl palmitate in the presence of sodium hydroxide (Budvari 1996; Smith 2016). There are 2 different forms of vitamin premix: oil based and water dispersible formulations. These are available for the various dairy processing systems (van Deutekom 2015). In general, oil-based vitamin premix has to be added into the flow of milk after the cream separator. The water dispersible vitamin premix can be added to the flow of milk before the separator, or anywhere in the milk flow (van Deutekom 2015). Water dispersible vitamins are not water soluble, only oil soluble. An emulsifier (polysorbate) is added into the vitamin premix to make it water dispersible. Water dispersible vitamin premix (water as the main ingredient) has a specific gravity  $>1.00$ , whereas oil-based premix (oil as the main ingredient) has a specific gravity  $<1.00$  (van Deutekom 2015). Most vitamin premix neither contains nor is manufactured from any genetically modified materials (van Deutekom 2015). Oil-based vitamin premix with corn oil as the carrier may be manufactured with commodity corn oil and therefore cannot be certified as GMO free (van Deutekom 2015). Vitamin premixes contain vitamin D<sub>3</sub> and/or vitamin A palmitate in a carrier generally consisting of a combination of any of the following: sunflower oil, corn oil, water, polysorbate 80, propylene glycol, and glycerol monooleate. Antioxidants and/or preservatives may also be added (Murphy and Newcomer 2001).

It is also important to note that vitamin concentrate potency will degrade with time. Therefore, concentrates should be stored in accordance with manufacturer's recommendation to maintain label potency (PMO 2015). According to Sensory Effects (Bridgeton, Mo., U.S.A.) and International Food Products (IFP; Fenton, Mo., U.S.A.), 2 commercial suppliers, vitamin premix product should be stored at room temperature (10 to 27 °C) in a dry, dark place. Product should be used within 1 year from date of manufacture. IFP does not make any natural claims for their vitamin premix products (van Deutekom 2015). Sensory effects has an organic

label claim on some of their vitamin premix products that contain sunflower oil as the vitamin carrier (Payne 2015).

Vitamins can be added into the pasteurizing vat, the HTST balance tank, or on a continuous basis into the pipeline after standardization. The addition of vitamins usually occur after separation and fat standardization, and before pasteurization. Homogenization will then take place after pasteurization to allow the vitamins to be distributed evenly throughout the milk. Two vitamin addition procedures can be used: the batch addition or addition with metering pumps. The batch procedure requires accurate measurement of the fortified milk volume, accurate measurement of the vitamin concentrate, and proper mixing. The metering pump procedure requires the pumps in the HTST unit to be activated only when the unit is in forward-flow (PMO 2015).

Under or over fortification can occur when vitamins are added before separation and standardization, resulting in low fat product being under fortified and high fat product being over fortified. This occurs because vitamin A and D are fat-soluble, they will gradually become more concentrated in the milk fat portion of the milk. Therefore, it is recommended to add the vitamins after separation and standardization (PMO 2015).

### Methods of Vitamins A and D Analysis

A list of the official AOAC (2007), CEN (2000), and ISO (2000) methods available for determining fat soluble vitamins has been reported (Blake 2007). These procedures involve mostly liquid chromatography (LC), but also include spectrophotometric and gas chromatographic (GC) techniques. In the various official procedures for fat soluble vitamins, extractions are usually made either by saponification or by direct solvent extraction. Saponification removes the fat portion of milk and facilitates extraction by releasing carotenoids, retinoids, tocopherols, and vitamin D compounds from the matrix. Saponification is generally performed with the addition of antioxidants such as ascorbic acid, pyrogallol, butylated hydroxyl toluene, or hydroquinone to reduce oxidation losses, along with nitrogen flushing (Blake 2007). After saponification, extraction takes place with organic solvents such as hexane, petroleum ether, ethyl ether or mixtures of these substances (Table 1). Vitamin D can also be directly extracted with organic solvents without saponification process. These organic solvents are methyl dichloride alone, or mixed with methanol and hexane alone, or mixed with ethyl ether, or chloroform (Kazmi and others 2007). However, vitamin D recoveries from direct extraction were lower than those obtained by saponification (Delgado and others 1992; Hagar and others 1994). Vitamin D<sub>3</sub> is insoluble in water, but soluble in 95% ethanol, acetone, fats, and oils, and readily soluble in chloroform and ether. Retinyl palmitate is also insoluble in water, but soluble in chloroform, ether, and vegetable oil (corn oil), and slightly soluble in alcohol.

For over 20 years, HPLC has been the method of choice for the determination of total fat-soluble vitamins such as vitamins A and D. However, HPLC methods raise environmental and economic concerns due to the large amount of organic solvents used. Thus, the continued search to improve HPLC further have led to ultra-high performance liquid chromatography (UHPLC). In comparison with HPLC, UHPLC uses narrow bore, shorter columns, less run time, lower flow rate, lower injection volume and solvent volume/sample, smaller particle size, and much higher back pressure (Bohoyo-Gil and others 2012; Rivera and Canela-Garayoa 2012). Thus, the UHPLC method has several advantages over conventional chromatography, which include faster analyses due to shorter retention times, narrower peaks giving increased

**Table 1–Vitamin analysis methods.**

Author name	Extraction method	Detection method	Product evaluated	Compounds separated	Sensitivity
Salo-Vaananen and others 2000 (Food Chemistry)	Saponification follow by extraction with hexane-ethyl acetate (8:2), stream N2	HPLC-UV	Fluid milk and fish	Cholecalciferol (vitamin D3), tocopherols, beta-carotene, all-trans-retinol	Detection limits: 0.003 $\mu\text{g}/100\text{ mL}$ for vitamin D3, <1 $\mu\text{g}/100\text{ mL}$ for beta-carotene, and 2 $\mu\text{g}/100\text{ mL}$ for tocopherols and all-trans-retinol
Upreti and others 2002 (Journal of Dairy Science)	Saponification followed by extraction with petroleum ether/diethyl ether, stream N2	HPLC-UV	Processed cheese	Cholecalciferol (vitamin D3)	Vitamin D3 content of 2.5 $\mu\text{g}/100\text{ mL}$ for processed cheese
Staffas and Nyman 2003 (Journal of AOAC International)	saponification followed by extraction with heptane, stream N2	HPLC-UV	Milk, liquid infant formula, cooking oil, margarine, infant formula, fish oil	Cholecalciferol (vitamin D3)	Vitamin D3 content reported in milk: 0.4 to 0.418 $\mu\text{g}/100\text{ mL}$
Kazmi and others 2007 (International Dairy Journal)	Direct extraction with methanol/chloroform, stream N2	HPLC-UV	Cheese, yogurt, ice cream	Cholecalciferol (vitamin D3)	95% to 97% recovery for cheese, 99% to 102% for yogurt, 98% to 99% for ice cream
Phillips and others 2008 (Journal of Food Composition and Analysis)	Extraction with ethyl ether/petroleum ether and separatory funnel, stream N2	HPLC-UV and MS	Canned salmon, vitamin D3 fortified skim milk, orange juice, ready-to-eat breakfast cereal, processed cheese	Cholecalciferol (vitamin D3)	Vitamin D content reported in skim milk: 1.03 $\mu\text{g}/100\text{ mL}$ to 1.15 $\mu\text{g}/100\text{ mL}$
Byrdwell 2009 (Journal of Agricultural and Food Chemistry)	Extraction with ethyl ether/petroleum ether and separatory funnel, stream N2	HPLC-UV and MS	Skim milk, orange juice, cereal, salmon, spiked peanut oil, processed cheese	Cholecalciferol (vitamin D3)	Vitamin D content reported in skim milk: 1.08 $\mu\text{g}/100\text{ mL}$ to 1.14 $\mu\text{g}/100\text{ mL}$
Chen and Reddy 2015 (Journal of AOAC International)	Saponification followed by extraction with diethyl ether in hexane and separating funnel, stream N2	HPLC-UV	Skim, 2% fat, and whole milk	All-trans-retinyl palmitate and cholecalciferol (vitamin D3)	Detection limits: 0.0022 $\mu\text{g}/\text{mL}$ for vitamin A and 0.0008 $\mu\text{g}/\text{mL}$ for vitamin D3
Chauveau-Duriot and others 2010 (Analytical Bioanal Chem)	Saponification follow by extraction with hexane-ethyl acetate (8:2), stream N2	Comparison between HPLC & UPLC	Forages, bovine plasma, and milk	Carotenoids, retinol, tocopherols	Sensitivity was higher for UPLC than for HPLC (that is for retinol: 0.8 and 1.4 ng per injection vs. 1.3 and 2.0 ng per injection, respectively)
AOAC Official method 2002.06	Extraction with hexane, stream N2	LC-UV	Skim, 1% fat milk, 2% fat milk, and 1% fat chocolate milk	All-trans-retinyl palmitate	
AOAC Official method 2002.05	Saponification followed by extraction with heptane and separatory funnel, stream N2	LC-UV	Fluid milk, infant formula, gruel, margarine, cooking oil, and fish oil	Cholecalciferol (vitamin D3)	

signal-to-noise ratio, higher resolution and sensitivity (Rivera and Canela-Garayoa 2012). UHPLC also saves at least 80% of mobile phase compared to HPLC (Chen and Kord 2009). Similar to the HPLC methods, UHPLC methods also have to undergo special attention to method standardization, validation, sampling, and sample preparation to guarantee data reliability, because the smaller the analytical sample, the more difficult it is to guarantee a representative sample. In milk, the UHPLC method provided similar concentrations of vitamin A to that obtained by an HPLC method (Chauveau-Duriot and others 2010).

In all of these procedures, it is important to avoid vitamin losses due to light oxidation. Significant errors may result from poor use of glassware in vitamins analysis. Therefore, the use of fluorescent lighting fitted with an appropriate UV filter in the laboratory

is recommended. Low-actinic glassware (amber colored glassware which is used to protect contents from light) can also be used to reduce vitamin losses (Castanheira and others 2006).

### Vitamin Destruction by Photooxidation

The light visible spectrum is the portion of the range of all possible frequencies of electromagnetic radiation that is visible to the human eye. The human eye can typically respond to wavelengths from 390 to 700 nm (Starr 2005). Light exposure at wavelengths below 500 nm causes the destruction of light-sensitive vitamins such as riboflavin, vitamin A, and vitamin C, induces chemical reactions that affect milk proteins and lipids, and results in the development of unpleasant flavors in fluid milk (Fanelli and others 1985; Sattar and others 1977; Schroder and others 1985). Dairy

products are very sensitive to light oxidation because of the presence of riboflavin (vitamin B<sub>2</sub>). Milk contains large amounts of riboflavin, a water-soluble vitamin with an average concentration between 1.36 and 1.75 mg/L (Dimick 1982; Zygoura and others 2004). Light oxidation dramatically alters the structure of riboflavin and reduces its level in milk (Bekbolet 1990). Riboflavin is the most studied photosensitizer in milk (Sattar and others 1977; Webster and others 2009), and this strong photosensitizer is able to absorb visible and UV light and transfer this energy into highly reactive forms of oxygen known as singlet oxygen (Boff and Min 2002). Excitation of riboflavin occurs when exposed to light at 250, 270, 370, 400, 446, and 570 nm wavelengths (Kyte 1995). The cascade of oxidation reactions lead to significant losses of vitamins (vitamin A, B<sub>2</sub>, C, D, and E) and amino acids, and also leads to lipid oxidation and formation of strong off-flavors (Borle and others 2001). As a result, off-flavors may be linked to the decrease in nutritional value of milk. Therefore, light protection of photosensitive molecules in milk is needed to protect milk flavor and nutrient quality. Despite vitamin loss and established changes in milk flavor which are unpleasant to consumers, HTST milk in the U.S. is still routinely packaged in clear HDPE jugs (Brotherson and others 2016; Potts and others 2017).

Studies have shown that whole milk products have slower rates of vitamin A losses compared to low-fat or skim milk products (Senyk and Shipe 1981; deMan 1981; Gaylord and others 1986; Whited and others 2002). Senyk and Shipe (1981) indicated that light at wavelengths of 400 to 500 nm penetrated 40% to 50% deeper into skim milk than into whole milk. Measureable vitamin A losses occurred at 2, 4, and 16 hours at 2000 1× fluorescent light for nonfat, reduced fat, and whole milk, respectively (Whited and others 2002). Vitamin A losses were also distinct between fluorescent light and LED light exposure (Brotherson and others 2016).

Natural vitamin A in whole milk was more stable to light than added vitamin A due to natural vitamin A is found in milk fat globules whereas added retinyl palmitate is dispersed in the water phase of milk, which was more prone to oxidation due to greater contact with oxygen (Thompson and Erdody 1974). Vitamin D loss occurred at a rate much slower than other vitamins in milk. Using the same system of exposing milk samples in test tubes at the same light intensity of 300 ft-c, Gaylord and others (1986) reported the rate constant for riboflavin loss was 0.0616 per hour and retinyl palmitate was 0.0298 per hour, whereas Renken and Warthesen (1993) reported the rate constant for vitamin D loss was 0.0009 per hour. Chocolate milk products also have reduced vitamin A degradation, either due to protection by carrageenan alone or in combination with chocolate color and/or flavor. Vitamin A protection in chocolate milk is due to increased light scattering by the additional particles. Chocolate flavor components of chocolate milk can also reduce development of light-oxidized off-flavors (Chapman and others 1998). These findings demonstrate that exposure of fluid milk to light can adversely affect both flavor quality and nutritional value of fluid milk products.

## Vitamin Stability and Storage

### Vitamin D

Several studies have been conducted on the stability of vitamin D in milk and other dairy products (Banville and others 2000; Kazmi and others 2007; Wagner and others 2008; Hanson and Metzger 2010; Tippetts and others 2012). These studies have all indicated that vitamin D is stable during processing and storage.

Vitamin D in fortified homogenized whole milk is very stable and is not affected by pasteurization or other processing procedures (PMO 2015). Vitamin D<sub>3</sub> appears to be stable in cheese during both short-term (Banville and others 2000) and long-term storage (Kazmi and others 2007; Wagner and others 2008). Vitamin D was also stable over the shelf life of HTST-processed 2% fat milk, UHT-processed 2% fat chocolate milk, and low-fat strawberry yogurt (Hanson and Metzger 2010). Tippetts and others (2012) suggested incorporation of vitamin D<sub>3</sub> as an emulsion using milk proteins as emulsifier to improve retention of vitamin D<sub>3</sub> in the cheese curd. Vitamin D<sub>3</sub> is also stable in yogurt and ice cream stored for 4 weeks, with high retention of 95% to 100% and 98% to 100%, respectively (Kazmi and others 2007).

Vitamin D itself is susceptible to degradation by oxygen, heat, and light once freed from the protection of food matrix. Degradation products resulting from UV light reactions include lumisterol, tachysterol, isotachysterol, and suprasterols (Bouillon and others 1998; Grady and Thakker 1980). Other degradation products of vitamin D also include trans-vitamin D (from oxidative reactions), pyrovitamin D, and isopyrovitamin D (from thermal reactions; Grady and Thakker 1980). Octanoate and decanoate ester of vitamin D<sub>3</sub> were also identified as degradation products of vitamin D<sub>3</sub> in a thermally stressed tablet containing vitamin D<sub>3</sub> (Ballard and others 2007). Disagreement with respect to vitamin D stability due to other environmental factors has been reported. Cremin and Power (1985) stated that vitamin D was unstable to oxidation, light, and acid. Kutsky (1981) reported that vitamin D was unstable to oxidation and light but stable to acid and alkali, whereas Kreutler (1980) reported that vitamin D was remarkably stable to light, heat, and oxygen, and these factors did not affect its activity.

### Vitamin A

As stated previously, vitamin A is generally added to fluid milk as retinyl palmitate (Public Health Service 1965). Retinyl palmitate is the ester of retinol and palmitic acid. The stability of added retinyl palmitate may be affected by heat, light, or the presence of acids which may cause degradation or conversion of 11-*cis*-retinal to all-*trans*-retinal, resulting in lowered biological activity (Mousseron-Cadet 1971). Ultraviolet light causes isomerization and degradation of retinoid compounds in solution. Under more intense light, other transformations can take place such as dimerization or chemical reaction between 2 monomers of retinyl esters (Mousseron-Cadet 1971). In addition, large losses of vitamin A activity can occur during processing, transportation and storage of fortified foods (Dary and Mora 2002). Hartman and Dryden (1974) reported that procedures such as pasteurization, sterilization, spray and roller drying or evaporation caused little loss of vitamin A in milk products. However, prolonged heating of milk, butter, or butterfat at high temperatures in the presence of oxygen can decrease vitamin A activity.

Despite reported vitamin A off-flavor in fluid milk products, degradation products of vitamin A palmitate in fluid milk have not been previously reported in the literature. However, in nonfat dry milk, a distinct hay-like flavor was detected from oxidation products of vitamin A palmitate, the major oxidation products attributed to the off flavor were beta-ionone (Figure 4) and dihydroactinidiolide (Suyama and others 1983). In another study conducted with corn flakes fortified with vitamin A palmitate, 2 vitamin A palmitate isomers, 9-*cis* and 13-*cis* were found from degradation of vitamin A palmitate during elevated (45 °C) temperature storage (Kim and others 2000). This study also showed that the loss of vitamin A was reduced in the presence of other

vitamins including B<sub>1</sub>, B<sub>6</sub>, B<sub>12</sub>, C, and D (Kim and others 2000). Different forms of vitamin premix also have an effect on vitamin A stability when exposed to light. Triangle test results indicated that light-induced off flavor could be distinguished in skim milks with water based vitamin A at 2000 IU after 6 h of light exposure, while off flavor in skim milks with oil based vitamin A at 2000 IU could not be distinguished until after 24 h of light exposure (Fellman and others 1991). Furthermore, oil matrices have been shown to have protective effects on vitamin A stability by delaying its oxidative degradation (Dary and Mora 2002; Loveday and Singh 2008).

### Effect of Vitamin Addition on Fluid Milk Flavor

There are many factors that can influence milk flavor, including milk handling, processing, and storing (Strobel and others 1953; Nursten 1997). Other factors that may contribute to off-flavors in milk are enzymatic degradation of milk fat and protein related to increasing milk somatic cell count, bacterial growth, chemical composition of milk, chemical changes, and addition of foreign material (Bodyfelt and others 1988; Barbano and others 2006). Pasteurization of milk imparts a cooked flavor, especially immediately after processing (Badings and others 1981; Boelrijk and others 2003). In general, proteins, lipids and carbohydrates are the precursors of the aroma compounds that contribute these milk flavors. However, vitamin degradation may also contribute aroma-active compounds. Several studies have suggested that added vitamin A concentrate imparted a detectable off-flavor, particularly in skim and low-fat milk, and occasionally in whole milk products (Weckel and Chicoye 1954; Whited and others 2002). Consumers have reported that vitamin A fortified milk has an oily, haylike flavor (Fellman and others 1991). However, there are no published studies, to our knowledge, that have directly evaluated the role of vitamin preparation addition on flavor and flavor stability of fluid milk.

### Future Work

Vitamin fortification has an established history and role in fluid milk. Ongoing interest in maximizing milk flavor and appeal and interest in increasing vitamin D fortification levels demands that the role of vitamin fortification on fluid milk flavor and flavor stability be clarified. Sensory experiments need to be conducted on vitamin fortified skim and 2% fat milk because they represent the majority of vitamin A and D fortified milks purchased and fat plays a protective role on vitamin degradation, light oxidation, and sensory thresholds of off flavors. If vitamin fortification results in off flavors, it will be more likely detected in reduced fat or skim milks where sensory thresholds for nonpolar compounds are reduced compared to whole milk or higher fat products. Aroma

active compounds in pure vitamin concentrates need to be identified with gas chromatography-mass spectrometry (GC-MS) and gas chromatography-olfactometry (GC-O) to pinpoint if vitamin premixes are the source. Representative vitamin concentrates can then be selected to fortify skim and 2% fat milk for sensory testing. Additional experiments with skim and 2% fat milk also need to be conducted with light exposure to determine if vitamin concentrates accelerate or contribute light oxidized flavors in fluid milk.

### Conclusions

Off-flavors in fluid milk can negatively impact milk consumption and consumer product acceptability. Established sources of off-flavor in fluid milk are thermal degradation, enzymes associated with high milk somatic cell count, microbial contamination, and exposure to light. The most common source of flavor problems encountered in milk is exposure to light. Milk exposure to light can result in vitamin destruction. Another possible source of off-flavor in fluid milk is vitamin fortification. Several studies have addressed the influence of light oxidation on fluid milk flavor and stability, and the effect of light exposure on vitamin stability. No studies have directly addressed the effect of vitamin addition on flavor and flavor stability of fluid milk. Understanding the impact of vitamin addition and degradation in fluid milk will help the dairy industry to better position vitamin fortification and enhance fluid milk quality. Identifying the aroma-active compounds in milk resulting from vitamin fortification can also help identify sources of off flavors in fluid milk and milk products that may negatively impact milk flavor quality and consumer product acceptability.

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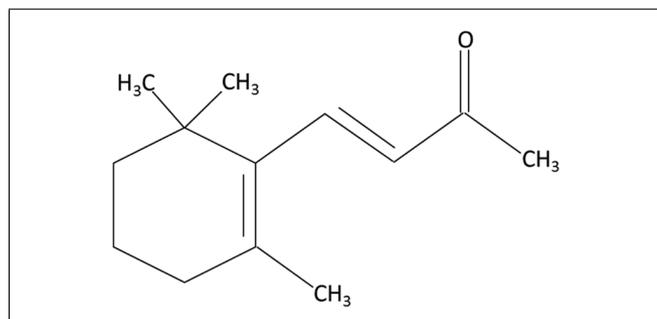


Figure 4—Chemical structure of beta-ionone.

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