Fortification of cheese with vitamin D₃ using dairy protein emulsions as delivery systems

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ABSTRACT

Vitamin D is an essential vitamin that is synthesized when the body is exposed to sunlight or after the consumption of fortified foods and supplements. The purpose of this research was to increase the retention of vitamin D₃ in Cheddar cheese by incorporating it as part of an oil-in-water emulsion using a milk protein emulsifier to obtain a fortification level of 280 IU/serving. Four oil-in-water vitamin D emulsions were made using sodium caseinate, calcium caseinate, nonfat dry milk (NDM), or whey protein. These emulsions were used to fortify milk, and the retention of vitamin D₃ in cheese curd in a model cheesemaking system was calculated. A nonemulsified vitamin D₃ oil was used as a control to fortify milk. Significantly more vitamin D₃ was retained in the curd when using the emulsified vitamin D₃ than the nonemulsified vitamin D₃ oil (control). No significant differences were observed in the retention of vitamin D₃ when emulsions were formulated with different emulsifiers. Mean vitamin D₃ retention in the model system cheese curd was 96% when the emulsions were added to either whole or skim milk compared with using the nonemulsified oil, which gave mean retentions of only 71% and 64% when added to whole and skim milk, respectively. A similar improvement in retention was achieved when cheese was made from whole and reduced-fat milk using standard manufacturing procedures on a small scale. When sufficient vitamin D₃ was added to produce cheese containing a target level of approximately 280 IU per 28-g serving, retention was greater when the vitamin D₃ was emulsified with NDM than when using nonemulsified vitamin D₃ oil. Only 58 ± 3% of the nonemulsified vitamin D₃ oil was retained in full-fat Cheddar cheese, whereas 78 ± 8% and 74 ± 1% were retained when using the vitamin D₃ emulsion in full-fat and reduced-fat Cheddar cheese, respectively. **Key words:** vitamin D, oil-in-water emulsion, Cheddar cheese, emulsifier

INTRODUCTION

Vitamin D₃ is an essential vitamin that is synthesized in the skin from 7-dehydrocholesterol when the skin is exposed to sunlight. However, the major source of vitamin D₃ in modern society is through the consumption of fortified foods and supplements. In today's environment, health organizations such as the US Food and Drug Administration are becoming more aware of the link between vitamin D deficiency and the increased risk of chronic diseases, such as diabetes mellitus, cancer, autoimmune disorders, and osteoporosis (Calvo et al., 2004). Recent studies in the United States and United Kingdom (Pal and Shaw, 2001; Tomashek et al., 2001) have shown an increase in childhood rickets due to vitamin D deficiency, suggesting that insufficient vitamin D is being ingested or synthesized.

In certain areas and cultures, the population may not be exposed to sufficient sunlight, resulting in vitamin D deficiency. For example, among veiled Arab women in Denmark, 85% of women have severe vitamin D deficiencies (Weaver and Fleet, 2004). Other sectors of the population that have limited capacity of vitamin D synthesis through cutaneous production are those who live in northern latitudes during winter, those who protect their skin from UV-B rays, elderly subjects, and dark-skinned individuals (Calvo et al., 2004; Weaver and Fleet, 2004). The current recommended dietary allowance (**RDA**) of vitamin D, as defined by the Food and Nutrition Board of the Institute of Medicine, is between 600 and 800 IU/d (depending on age, and assuming no vitamin D synthesis due to sun exposure), the estimated average requirement is 400 IU (Institute of Medicine, 2011), and the tolerable upper limit (**UL**) intake is 4,000 IU/d for all ages (National Institute of Health, 2011).

United States and Canadian populations are dependent on fortified foods and supplementation for their vitamin D needs during times when sunlight and subsequent UV-B absorption are lacking (Calvo et al., 2004). Vitamin D is present in some food sources such as eggs, mushrooms, and fish. Naturally occurring vitamin D levels in egg yolks and mushrooms are inconsistent. Fatty fish is also a natural source of vitamin D, with

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salmon being the most commonly consumed in the United States. Other sources, such as liver and other organ meats, have high vitamin D levels but are not consumed regularly. For many people, milk fortified with vitamin D has been their major source of dietary vitamin D (Banville et al., 2000) but milk consumption has declined in recent years. In contrast, cheese consumption has increased, suggesting that cheese may be a good alternative for vitamin D fortification. It is important, therefore, to evaluate the amount of vitamin D that can be retained in cheese during manufacture and aging (Banville et al., 2000).

Banville et al. (2000) studied the effect of vitamin D₃ delivery matrix on its retention in Cheddar cheese, with levels of 1 to 3 IU/g of cheese being achieved. Entrapment of vitamin D₃ in multilamellar liposomes increased retention by approximately 20% (to 62% retention) compared with a water-soluble emulsion or when crystallized vitamin D was mixed in the cream before addition to the milk (~41% retention). When much higher levels of vitamin D were added (500 to 1,000 IU/g of cheese), using either a pre-dissolved crystalline or pre-emulsified form of vitamin D₃, Kazmi et al. (2007) reported retention levels of 87 and 90% for the crystalline and the pre-emulsified forms of vitamin D₃, respectively. Finally, Wagner et al. (2008) studied the fortification of full-fat and low-fat cheeses (1,000 IU/g of cheese) and observed retention levels similar to that found by Kazmi et al. (2007) for full-fat cheese (91%) but lower retention in low-fat cheese (<55% retention). These studies provide an excellent background on the fortification of cheese and dairy products with pre-emulsified vitamin D; however, little information on the type of emulsion used is reported and the use of label-friendly ingredients, such as dairy proteins, as emulsifiers was never reported. Moreover, the fortification levels used in the Kazmi et al. (2007) and Wagner et al. (2008) studies are well above the RDA values (and a single serving of such vitamin D-fortified cheese would far exceed the UL) and may not reflect retention of vitamin D when used at more realistic levels. Therefore, there is a need to explore retention of vitamin D in cheeses at levels that would supply about 25 to 30% of the vitamin D RDA (i.e., ~200 IU/28-g serving of cheese).

The objective of this study was to evaluate the efficiency of different dairy-based delivery matrices on the retention of vitamin D₃ in full-fat and low-fat curd model systems and in small-scale production using standard manufacturing procedures. Oil-in-water emulsions formulated with different dairy protein powders as emulsifiers (sodium and calcium caseinates, NDM, and whey protein concentrate) were used as delivery matrices. Results obtained from the model systems

were verified using a small-scale Cheddar cheese system with the target standard serving of vitamin D of 200 to 400 IU/28-g serving of cheese. In addition, we compared the retention of emulsified vitamin D₃ in full-fat and reduced-fat cheese with that of a commercially available nonemulsified vitamin D₃-fortified oil.

MATERIALS AND METHODS

Emulsion Formulation

Oil-in-water (o/w) emulsions were made in triplicate using 4 protein powders: NDM (36% protein, Foster Farms Dairy, Modesto, CA); whey protein concentrate (WPC, 80% protein, Grande CIG, Lomira, WI); calcium caseinate (CaCN, 94.2% protein, Erie Foods International Inc., Rochelle, IL); and sodium caseinate (NaCN, 94% protein, Erie Foods International Inc.). The oil phase was a 1:1 (by wt) mixture of vitamin D₃ (10⁶ IU/g of oil; BASF Corp., Florham Park, NJ) and soybean oil (Bunge Limited, White Plains, NY). The oil phase in the o/w emulsions consisted of 25,000 IU vitamin D₃/g and 50 g/kg oil. The water phase consisted of a 0.01 M Na₂HPO₄ solution (pH = 7) containing 20 g/kg protein and 2 g/kg Microgard 730 (cultured dextrose, Danisco, Bakersfield, CA) as an antimicrobial. Protein content of the emulsifiers used was determined from the ingredient sheet provided by the company. Emulsions were blended using high shear force (Ultra Turrax IKA T18 basic, IKA, Wilmington, NC) for 1 min at 18,000 rpm and then homogenized with a microfluidizer (model M-110S, Microfluidics, Newton, MA; 17.4 ± 1.6 MPa) for 1 pass. A commercial, non-emulsified vitamin D₃ oil, a standard ingredient used in the dairy industry to fortify milk (VS-AD200, Tate & Lyle, Sycamore, IL), containing 40,000 IU/g, was used as a control. The amount of vitamin D₃ in the fortified emulsions, control, fortified cheese-milk, cheese, whey, and curd was quantified as described below.

Emulsion Physicochemical Stability

Each emulsion (5 to 7 mL) was placed into individual flat-bottomed test tubes (Sci Tec Inc., Sandyhook, CT), and stability was measured daily for a week using a TurbiScan 2000 (Sci Tec Inc.) in backscattering mode (Mengual et al., 1999). Readings were obtained every 40 µm of the length of the tube to characterize emulsion homogeneity. Repeating the measurement of backscattered light along the sample length over time produces a superimposable emulsion fingerprint, which characterizes the stability or instability of the sample (i.e., the more identical the readings over time, the more stable the system; Mengual et al., 1999; Tippetts

and Martini 2009). Because emulsions were used immediately after preparation, emulsions with destabilization rates of less than 1 mm/d were considered stable (McClements, 2004).

Model Cheesemaking

Pasteurized (72°C for 15 s) skim and whole (nonhomogenized) milks were obtained from the Gary Haight Richardson Dairy Products Laboratory (Utah State University, Logan). Milk was stored at 5°C before use. The milk was heated to 39°C and cooled to 31°C to restore coagulation properties inhibited by cooling (Qvist, 1979). Four hundred milliliters of milk was fortified with 400 µL of one of the experimental emulsions, or 250 µL of nonemulsified vitamin D₃ oil as a control treatment, and homogenized with an Omni International 5000 homogenizer (Omni International, Kennesaw, GA) for 30 s. The target level of vitamin D₃ was 5,000 IU/200 mL of milk to gain a good understanding of the difference between emulsifiers, whereas lower concentrations of vitamin D₃ were used during the small-scale cheesemaking as described below, to more closely represent an industrial process. The concentrations used for the small-scale cheesemaking were close to the RDA (200 to 400 IU), which is ≤10% of the UL.

Two hundred-milliliter portions of fortified milk were placed in 250-mL polycarbonate Nalgene centrifuge bottles (VWR, West Chester, PA). Then, 4 g of glucono-δ-lactone and 0.4 mL of double-strength (~650 international milk clotting units/mL) chymosin rennet (Maxiren DS, DSM Food Specialties, Parsippany, NJ) were added, mixed well, and incubated for 30 min at 31°C to allow coagulum formation. The coagulum was manually cut into 16 segments using a metal spatula and then centrifuged at 1,000 × *g* at 25°C for 30 min. After centrifuging, the supernatant whey was decanted and weighed, and the curd was weighed. Approximately 100 g of whey and more than 5 g of curd were retained and frozen for vitamin D₃ analysis using liquid chromatography (AOAC, 2007; official method 2002.05) as recommended by the Food and Drug Administration and analyzed by a certified laboratory (O'Neal Scientific Services Inc., St. Louis, MO). Each treatment was performed in triplicate.

Small-Scale Cheesemaking

The NDM emulsifier was chosen to evaluate the retention of vitamin D₃ in full- and reduced-fat Cheddar cheese using fortified o/w emulsions and compare it to the retention of a nonemulsified commercial vitamin D₃ source (control-whole milk). Pasteurized (72°C for 15 s), nonhomogenized, and cooled milk (whole and

2% reduced-fat) were obtained from the Gary Haight Richardson Dairy Products Laboratory and made into cheese over 2 d (in triplicate) using 13.6 kg of milk per vat using a completely randomized design. Milk was preheated to 39°C and then cooled to 31°C after which 400 µL of NDM vitamin D₃ emulsion, formulated as described above, was added. The target concentration of vitamin D₃ was 400 IU per serving (28g of cheese), which is within current guidelines (National Institutes of Health, 2011). Whole milk was fortified with 250 µL of nonemulsified vitamin D₃ oil as a control comparison. Milk samples were taken at this point for vitamin D₃ quantification. After 5 min, 2.0 g of frozen pellets of *Lactococcus lactis* ssp. *lactis/cremoris* starter culture (DVS 850, Chr. Hansen, Milwaukee, WI) was added as starter, and then 1.6 mL of CaCl₂ (35% solution) and 0.7 mL of single-strength annatto color (Nelson-Jameson, Mashfield, WI) were added. After 30 min of ripening, double-strength (~650 international milk clotting units/mL) chymosin rennet diluted 1:20 in cold water (Maxiren DS, DSM Food Specialties) was added and mixed well. The milk was allowed to coagulate and the curd was cut after 30 min using wire harps (0.64-cm spacing). The curd was allowed to heal for 5 min and then stirred for 25 min. The curd was then heated to 39°C over 25 min and stirred for an additional 35 min. The whey was drained when the curd reached a pH of 6.3. The curd was dry stirred until it reached pH 5.5. A whey sample was taken for analysis before salting. Then, the curd was salted (40 g of NaCl), hooped, and pressed at 241 kPa overnight, vacuum packaged, and stored at 4°C. A portion of each cheese sample was grated with a microplane grater, vacuum packed, frozen, and sent for vitamin D quantification in duplicate. The percentage of vitamin D₃ (in IU) in the cheese was calculated as in equation 1:

$$\frac{\text{IU}_{\text{curd}}}{\text{IU}_{\text{m}}} \times 100 = \% \text{ IU}_{\text{curd/m}}, \quad [1]$$

where IU_{curd} is the amount of vitamin D₃ in the curd, IU_m is the amount of vitamin D₃ in the milk, and % IU_{curd/m} is the ratio of vitamin D₃ obtained in the curd with respect to the milk, expressed as a percentage.

Statistical Analysis

Cheesemaking trials were performed in triplicate. Data reported are the mean and standard error values calculated from the replicates. Significant differences were analyzed using a 2- or 1-way ANOVA test, as appropriate, and Bonferroni and least significant difference post-tests (α = 0.05). Statistical analysis was

performed using Graph Pad software (GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego, CA) and SAS 9.1 TS Level 1M3 XP_PRO platform (SAS Institute Inc., Cary, NC). All emulsion treatments were compared with a control (nonemulsified vitamin D₃ oil).

RESULTS AND DISCUSSION

Emulsion Stability

The stability of o/w emulsions formulated with the 4 different emulsifiers (NDM, WPC, CaCN and NaCN) is shown in Figure 1. Emulsions had a rate of destabilization of approximately 0.6 to 0.7 mm/d and, because they were used immediately after formulation, were considered stable (McClements, 2004). Emulsions formulated with WPC as emulsifier had a significantly ($P < 0.05$) higher phase separation at d 7 than emulsions formulated with NDM or NaCN as emulsifiers. This slightly higher destabilization rate might be caused by mineral impurities and partial denaturation of the proteins during WPC processing (McClements, 2004). The NDM emulsifier was initially more stable compared with the other emulsifiers; however, it was not significantly more stable than NaCN by d 7.

Model Cheesemaking

The amounts of whey and curd obtained during the model process were, on average, 162 ± 2 g and 42 ± 1 g for full-fat curd, and 185 ± 1 g and 20 ± 1 g for low-fat curd. The yield of curd in the model cheesemaking

using whole milk (~20%) was higher than the amount obtained during a standard cheesemaking process (~10%) because of the higher moisture content of the curd. The higher moisture content in the model system was due to lack of pressing of the curd, which occurs during a standard cheesemake process. The lower curd yield observed for the skim milk samples was due to the lack of fat (Mistry, 2001). No significant differences were found between treatments in the amount of whey and curd within a given milk type. Therefore, the type of emulsifier used did not affect curd yield.

Vitamin D Retention in the Model System

Mean vitamin D₃ content of the milk and the amount of vitamin D₃ obtained in the whey and curd are shown in Table 1. Significantly less ($P < 0.05$) vitamin D₃ was lost in the whey when using the emulsified treatments compared with that lost in the whey using the nonemulsified vitamin D₃ oil. Curds obtained with the emulsified vitamin D₃ contained approximately 50% more vitamin D₃ than the curd obtained using the nonemulsified oil. The retention of vitamin D₃ in curds formulated with whole and skim milks fortified with the emulsified vitamin ranged from 96 to 98%, whereas the retention of vitamin D₃ in curds formulated with the whole and skim milk fortified with the nonemulsified vitamin were 71 and 62% for whole and skim milks, respectively. Vitamin D₃ retention was not significantly different ($\alpha = 0.05$) between curd obtained from whole and skim milks for any of the emulsion treatments. The retention levels of vitamin achieved in this model system are slightly higher than those obtained by Kazmi et al. (2007), who reached retention levels of 87 and 90% vitamin D₃ when it was incorporated into the cheese-milk in crystalline or emulsified forms, respectively.

Vitamin D Retention in Cheddar Cheese

Nonfat dry milk was used as the emulsifier for this experiment because the NDM emulsion had the best stability over the first 48 h and no significant differences in vitamin D₃ retention were observed as a function of emulsifier used, as previously discussed (Table 1). The objective of the small-scale cheesemaking was to use vitamin D₃ levels at concentrations near the RDA (600 to 800 IU), but still under the UL, which would allow the consumer to safely consume more than one serving per day. The proximate analyses of the cheeses obtained are summarized in Table 2. The yields obtained for the whole and reduced-fat cheeses were approximately 9 and 7%, respectively.

Retention of vitamin D₃ was not significantly different ($P < 0.05$) between cheeses made from whole (78

Figure 1. Effect of emulsifier on the stability [change in thickness (mm) of serum level] of oil-in-water emulsions over 1 wk: NDM (▼), whey protein concentrate (WPC, ◆), sodium caseinate (NaCN, ▲), or calcium caseinate (CaCN, ■).

Table 1. Amount of vitamin D₃ (expressed as mean IU $\pm$ SE) added to 200 mL of whole and skim milk as a nonemulsified oil (control) or an oil emulsified using NDM, whey protein concentrate (WPC), sodium caseinate (NaCN), or calcium caseinate (CaCN) and its partitioning between whey and curd produced using model cheesemaking (n = 3)

Emulsion	Vitamin D ₃				Recovery ¹ (%)
	Milk	Whey	Curd	Whey + Curd	
Whole milk					
Control	5,025 ± 451 ^a	1,152 ± 190 ^b	2,914 ± 286 ^b	4,066 ± 311 ^a	71.5 ± 2.9 ^b
NDM	4,771 ± 304 ^a	151 ± 51 ^a	4,418 ± 462 ^a	4,569 ± 448 ^a	96.6 ± 1.2 ^a
WPC	5,659 ± 552 ^a	164 ± 13 ^a	4,496 ± 205 ^a	4,660 ± 192 ^a	96.4 ± 0.4 ^a
NaCN	5,173 ± 527 ^a	110 ± 22 ^a	4,180 ± 300 ^a	4,290 ± 288 ^a	97.4 ± 0.6 ^a
CaCN	4,680 ± 192 ^a	113 ± 8 ^a	4,294 ± 252 ^a	4,407 ± 245 ^a	97.4 ± 0.3 ^a
Skim milk					
Control	5,219 ± 611 ^a	1,349 ± 214 ^b	2,214 ± 171 ^b	3,562 ± 302 ^a	62.4 ± 3.6 ^b
NDM	5,992 ± 187 ^a	99 ± 13 ^a	4,252 ± 140 ^a	4,351 ± 127 ^a	97.7 ± 0.4 ^a
WPC	5,292 ± 653 ^a	152 ± 27 ^a	3,948 ± 548 ^a	4,100 ± 574 ^a	96.3 ± 0.2 ^a
NaCN	5,096 ± 361 ^a	172 ± 21 ^a	4,589 ± 76 ^a	4,761 ± 61 ^a	96.4 ± 0.5 ^a
CaCN	4,967 ± 456 ^a	140 ± 7 ^a	4,398 ± 139 ^a	4,538 ± 146 ^a	96.9 ± 0.1 ^a

^{a,b}Means with a common letter in the same column were not significantly different ($\alpha = 0.05$).

¹Recovery = curd/(whey + curd) $\times$ 100.

$\pm$ 8%) or reduced-fat (74 $\pm$ 1%) milk (Table 3), which had 7,887 $\pm$ 450 (6.4 IU/g), and 7,580 $\pm$ 93 IU (7.8 IU/g), respectively. Retention of vitamin D₃ in the curd using the nonemulsified oil was significantly less than that made with the NDM emulsion (58%). Greater retention using the NDM emulsion might be attributed to hydrophobic interactions between the protein emulsifier and the casein matrix, which holds the emulsion in the curd. In contrast, only physical entrapment is responsible for the retention of the nonemulsified vitamin D₃ in the curd, resulting in a greater loss of vitamin D₃ in the whey during cheesemaking and therefore a lower retention of this compound in the curd. The retention of vitamin D₃ observed in the small-scale cheesemaking is lower than that reported for the model system. The main difference between these 2 experiments was the target concentration of vitamin D₃ in the curd. A high concentration of vitamin D₃ was targeted for the model cheesemaking (250 IU/g), whereas a lower concentration of vitamin D₃ (200 to 400 IU/28-g serving) was targeted in the small-scale cheesemaking to make it more comparable to a real cheesemaking process. In addition, it is interesting to note that the ratio of fortified emulsion to milk used in the model cheesemaking

was 1:1,000, whereas the ratio used in the small-scale cheesemaking was 1:34,000. This means that more emulsion was present in the model system cheesemaking that could interact with casein and improve the retention of the vitamin D₃ in the cheese curd. The loss of vitamin D in whey during cheesemaking is attributed to the affinity of vitamin D to whey proteins. When lower amounts of vitamin D are added to the system, the ratio of whey protein to vitamin D is dramatically increased, which would therefore increase the amount of vitamin D bound to whey proteins and increase its loss in the whey, with an expected lower recovery in the curd. The effect of higher recovery of vitamin D in a cheese matrix as a function of vitamin D₃ concentration was also shown by Ganesan et al. (2011), where 89% of vitamin D₃ was recovered in a cheese with 400 IU/serving, whereas only 87% was obtained in a cheese with 200 IU/serving. High fortification levels of vitamin D₃ (500–1,000 IU/g) were also evaluated by Kazmi et al. (2007), with recovery values of 87 and 91% in Cheddar cheese made with crystalline and emulsified vitamin D, respectively. However, specific characteristics of the emulsified vitamin D₃ used were not reported in that study and therefore it is difficult to compare these

Table 2. Proximate analysis of Cheddar cheese made with whole and reduced-fat milk and a whole milk control in a small-scale setting (n = 3)

Milk ¹	Mass (kg)	Fat (%)	pH	Moisture (%)	Salt (%)
Whole	1.23 $\pm$ 0.04 ^a	33.3 $\pm$ 2.9 ^a	5.45 $\pm$ 0.06 ^{ab}	36.2 $\pm$ 1.1 ^a	2.00 $\pm$ 0.28 ^a
Reduced	0.97 $\pm$ 0.01 ^b	23.0 $\pm$ 1.8 ^b	5.50 $\pm$ 0.10 ^b	36.8 $\pm$ 1.8 ^a	2.46 $\pm$ 0.57 ^a
Control	1.23 $\pm$ 0.02 ^a	33.7 $\pm$ 0.6 ^a	5.27 $\pm$ 0.10 ^a	36.1 $\pm$ 0.6 ^a	1.54 $\pm$ 0.28 ^b

^{a,b}Means within a column with different superscripts are significantly different ($\alpha = 0.05$).

¹Whole and reduced-fat milks were fortified with an emulsion that had NDM as the emulsifier; control milk was whole fat and fortified with vitamin D₃ oil.

results in terms of the effect of the delivery matrix on the retention of vitamin D₃ in the cheese. Wagner et al. (2008) fortified full- and low-fat Cheddar cheese with 900 IU of vitamin D₃ per gram of cheese. These authors reported recoveries of 91 and 55% of vitamin D for the full- and low-fat cheeses, respectively. A water-soluble vitamin D₃ premix was used by these authors, but the specific characteristics of the fortification source, such as type of emulsions (oil-in-water vs. water-in-oil), emulsifier used, and droplet size, were not provided. The low retention of vitamin D₃ in low-fat cheese reported by Wagner et al. (2008) suggests that during vitamin D fortification most of the vitamin is retained in the curd matrix due to its high solubility in the oil phase and its entrapment in the curd matrix. When the fat content in the milk is reduced, less lipid is available for the vitamin D to dissolve in, and therefore more vitamin D₃ will interact with whey proteins (Forrest et al., 2005), resulting in the loss of vitamin with the whey. It is interesting to note that the retention of vitamin D₃ obtained in our study for reduced-fat cheese (74%) was significantly higher than that obtained by Wagner's group (55%; Wagner et al., 2008). These results suggest that indeed, the incorporation of vitamin D₃ in the form of an oil-in-water emulsion using dairy proteins as emulsifiers increases the retention of vitamin D₃ in the curd matrix, probably because of lower availability of this compound to interact with whey proteins.

In summary, the slight differences in retention of vitamin D reported by the different studies mentioned above can be attributed to different factors: (1) the type of delivery matrix used to deliver the vitamin D₃, (2) the target concentration of vitamin D in the cheese, and (3) the cheesemaking procedure used. In general, these studies agree on the fact that the delivery matrix used to incorporate vitamin D₃ in a cheesemaking process affects its retention in the cheese matrix and that some of the vitamin D₃ is lost in the whey. The loss of vitamin D₃ in the whey can be explained by the

ability of vitamin D₃ to interact with β -lactoglobulin and β -casein (Forrest et al., 2005). When vitamin D₃ is incorporated in the milk as a nonemulsified oil source, lower retention is expected because some of the vitamin D₃ will be eliminated with the whey by interacting with the β -lactoglobulin and by being dissolved in the lipid and aqueous phases in the whey. When vitamin D₃ is delivered as an oil-in-water emulsion, retention in the curd is improved because of increased interactions between the emulsifier and the curd matrix. In addition, if the emulsions are formulated with dairy proteins, retention is improved even further due to the ability of the dairy proteins to interact with the casein matrix in the curd through hydrophobic interactions. Finally, when lower amounts of vitamin D are used to fortify the cheese, the whey protein: vitamin D ratio is higher, which results in increased loss of the vitamin in the whey.

CONCLUSIONS

The retention of vitamin D₃ in the cheese curd is improved by incorporating vitamin D₃ as an emulsion using milk proteins as emulsifier. The retention of vitamin D₃ in a model cheese system formulated using these emulsions was 96 to 98%, whereas the retention in small-scale cheesemaking was 74 to 78% for reduced-fat and full-fat cheeses, respectively. Although the type of protein powder (NDM, WPC, or caseinate) used to formulate the emulsion did not affect the retention of vitamin D₃ in the curd, the use of a dairy protein emulsifier was better than use of nonemulsified vitamin D₃ in an oil carrier. We also demonstrated that the target concentration of vitamin D₃ in the cheese might affect retention of the compound. Therefore, for this information to be useful in an industrial setting, we recommend evaluating fortification at levels commonly used in commercial production rather than higher levels. It is important to note that this research was performed in a small-scale cheesemaking setting and needs to be scaled up to be used in an industrial process.

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Table 3. Initial amount of vitamin D₃ in milk (IU_m), amount of vitamin D₃ retained in curd (IU_{curd}), and percentage of vitamin D₃ retained in the curd (calculated using equation 1)¹

Milk ²	IU _m	IU _{curd}	% IU _{curd/m}
Whole	10,147 ± 67 ^a	7,887 ± 450 ^a	78 ± 8 ^a
Reduced	10,213 ± 67 ^a	7,580 ± 93 ^a	74 ± 1 ^a
Control	10,175 ± 0 ^a	5,902 ± 192 ^b	58 ± 3 ^b

^{a,b}Means within a column with different superscripts are significantly different ($\alpha = 0.05$).

¹Values are expressed in mean international units (IU) ± SE (n = 3).

²Whole and reduced-fat milks were fortified with an emulsion that had NDM as the emulsifier; control milk was whole fat and fortified with vitamin D₃ oil.

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