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# PRODUCTION OF TURKISH NON-BRINED WHITE CHEESE FORTIFIED WITH VITAMIN D3

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**Abstract:** Vitamin D deficiency is a prevalent issue, particularly in developing countries, and fortifying foods with vitamin D is an essential strategy to enhance public health. Enriching white cheese with vitamin D3 is an appealing option for consumers, especially for infants who require vitamin D supplementation. This study aimed to optimize the white cheese production process by directly adding commercial non-emulsified vitamin D3 oil to pasteurized milk and quantifying the resulting vitamin D3 content in the cheese. Traditional white cheese was produced to achieve a final concentration of 10  $\mu$ g (400 IU) of vitamin D3. Using the HPLC-UV method, we compared the fortified cheese to a non-fortified control group regarding vitamin D3 content. Sensory analysis and microbiological analyses were also conducted. The study revealed that the fortified cheese contained 6.9  $\mu$ g (276 IU)/100g of vitamin D3, with no statistically significant differences observed in sensory evaluation or microbiological safety between the two groups. Consequently, our findings suggest that vitamin D3-fortified cheeses can effectively contribute to meeting daily vitamin D content and represents a significant advancement in functional food production, promoting public health.

**Keywords:** traditional food, functional food, dairy products, supplementation, microbiology, sensory quality

#### **INTRODUCTION**

Today, there is a growing awareness of nutrition, with individuals increasingly recognizing the importance of a healthy and balanced diet. In this context, many people strive to meet their body's basic nutrient needs by consuming nutritious foods. However, vitamin D deficiency, a crucial nutrient not abundantly present in many natural food sources, has emerged as a global health issue, particularly since it has become a recommended addition to infant nutrition (Basedow, 2022; Wu et al., 2023). Vitamin D is vital in bone health, immune system function, and overall well-being.

This deficiency is notably prevalent in developing countries, where various risk factors contribute to its occurrence, including malnutrition, advanced age, sun avoidance, sunscreen use, sedentary lifestyles, and obesity (Basedow, 2022; Płudowski et al., 2023). The prevalence of vitamin D deficiency has remained significant worldwide from 2000 to 2022 (Cui et al., 2023), with recent studies highlighting hypovitaminosis D among infants and children (Corsello, Spolidoro, Milani & Agostoni, 2023; Płudowski et al., 2023; Wu et al., 2023).

The implications of vitamin D deficiency are concerning, as it can lead to various health issues, particularly adverse effects on bone health and immune function. Research suggests that insufficient vitamin D levels during infancy and childhood may increase the risk of developing autoimmune diseases, cancer, and cardiovascular diseases later in life (Corsello et al., 2023; Cui et al., 2023). In response to this growing concern, fortifying foods with vitamin D has become a prominent strategy to combat vitamin D deficiency and enhance public health (Neill, Gill, McDonald, **McRoberts** & Pourshahidi et al., 2023). While the global consumption of dairy products is declining, cheese consumption remains high, with projections indicating a 30% increase in global dairy product consumption, particularly cheese, by 2032 (Sohail et al., 2023). This trend positions white cheese fortified with vitamin D3 as an appealing option for consumers looking to meet their vitamin D needs through a nutritious diet.

Numerous studies have shown that vitamin D3 is more effective than its D2 counterpart as a dietary supplement (Batacchi et al., 2017; Shieh et al., 2016; van den Heuvel, Lips, Schoonmade, Lanham-New & van Schoor, 2023). While research indicates no significant differences between the activities of D3 and D2 (Fisk, Theobald & Sanders, 2012; Nikooyeh and Neyestani, 2022), vitamin D3 is favoured for its higher bioavailability, greater shelf stability, and reliability in supply (Buttriss and Lanham-New, 2020). Our study opted for vitamin D3 due to the limited number of studies examining the effectiveness of D2 and its predominant use in dairy fortification studies.

White cheese, a semi-hard cheese widely produced in Mediterranean and Middle Eastern countries, is a popular dairy product consumed globally. It is known by various names across regions, including Feta, Bulgarian White, Brinza, Domiati, Danni, Jibnah, and Teleme. Turkish white cheese, specifically, is ripened in brine, resulting in a high salinity content, which is unsuitable for infant consumption. Mothers often introduce this calcium-rich food to their babies but typically soak the cheese in water to reduce its salt content. Although white cheese is rich in protein, calcium, and other essential nutrients, it lacks natural vitamin D. This gap has led cheese manufacturers to explore the fortification of white cheese with vitamin D, enabling consumers to fulfil their vitamin D needs while enjoying a healthy diet (Sohail et al., 2023).

Our study aimed to create a calcium- and vitamin D-rich white cheese without brine by incorporating vitamin D3 oil into pasteurized milk. We evaluated the effects of this addition on the final vitamin D3 content and optimized the production process accordingly. The findings provide valuable insights into the production and consumption of vitamin D3-fortified white cheese, highlighting its potential to shape market demand and trends.

# MATERIALS AND METHODS

# Material

Raw milk was obtained from the Ankara University Veterinary Medicine Faculty Farm. To produce white cheese enriched with vitamin D3 (Orzak Ocean ® 400 IU), 2 kg of raw milk was used, while another 2 kg was the control group for non-enriched cheese.

The experiments were conducted in triplicate. *Lactococcus lactis* subsp. *lactis* and *cremoris* starter cultures (CHR Hansen R-703, 2%) were used. A rennet strength of 1:18,000 was applied for the cheese-making process, using 20 mL of chymosin (Trakya, CHR Hansen, Istanbul, Turkey) and 20 g of CaCl<sub>2</sub> per 100 L of milk. HPLC grade solvents were purchased from Fisher Scientific (Fairlawn, NJ). The petroleum ether was low-boiling (30–60 °C, Fisher #E139-S4). USP vitamin D3 standard (USP #1711504) was used.

## **Cheese-making**

In the first step, 2 kg of raw milk with 3.5% protein and 3.5% fat was pasteurized at 75 °C for 5 min (Tunçtürk, Andiç & Ocak, 2010). Next, the pasteurized milk was cooled to 40 °C. Then, 12 drops of vitamin D3 (Orzak Ocean ® 400 IU) were added to the milk at 40 °C. After adding vitamin D3, *Lactococcus lactis* subsp. *lactis and Lactococcus lactis* subsp. *lactis* and *lactococcus lactis* subsp. *lactis lactis la* 



Figure 1. Some steps in cheese production

CHR Hansen, Istanbul, Turkey) and 20 g of  $CaCl_2$  per 100 L of milk, with 5-second intervals between each addition (Kasımoğlu et al., 2004).

The mixture was then stirred. To achieve coagulation, the milk mixture was placed in an incubator at 36.5 °C, and after 1 h, the resulting curd (pH 6.4) was transferred to a curd mesh to filter the whey. The titratable acidity of the whey was monitored during filtration. When the titratable acidity of the whey reached 12-13 SH (Soxhlet-Henkel) (after approximately 3 h), white cheese with the desired traditional titratable acidity was obtained. As a control group, 2 kg of raw milk was used to produce white cheese without vitamin D3, following the same process except for adding vitamin D3.

Fig. 1A illustrates how pasteurization is performed for both cheese samples. Fig. 1B, shows that both cheese groups appear as depicted after being removed from the incubator and cut with a knife. Fig. 1C, demonstrates the process of draining the cheese in the cheesecloth. Fig. 1D, shows that the cheese samples have the same appearance at the final stage.

#### **Calculation of vitamin D3 supplementation**

Tippetts, Martini, Brothersen and McMahon (2012) conducted a study where they fortified cheddar cheese with a commercial non-emulsified vitamin D3 preparation. Given our objecttive to fortify Turkish white cheese with a comparable non-emulsified vitamin D3, we used their findings as a foundational reference at the outset of our research. Tippetts et al. (2012) reported that in their study on enriching cheddar cheese with non-emulsified commercial vitamin D, 58% of the added vitamin D remained in the cheese.

#### Assumptions:

Based on Tippets et al.'s (2012) findings, we assumed that 58% of the D3 vitamin we added would remain in the cheese.

#### Target:

We aimed to achieve approximately 10  $\mu$ g (400 IU) of D3 vitamin per 100 g of cheese.

#### D3 Vitamin concentration:

The D3 vitamin (Orzak Ocean @ 400 IU) was such that 3 drops are equivalent to 10 µg (400 IU) of D3 vitamin. The vitamin was added in increments of 3 drops to ensure precise measurements. We used a simple proportional method to determine how many drops of D3 vitamin to add to reach our target (Toney-Butler, Nicolas & Wilcox, 2023).

#### Calculation of added D3 vitamin:

We added 12 drops of D3 vitamin to 2 kg of milk at  $40^{\circ}$ C.

Total D3 vitamin added:

12 drops  $\times$  (10  $\mu g$  (400 IU) /3) drops = 40  $\mu g$  (1600 IU)

D3 vitamin remaining in cheese:

Percentage of D3 vitamin remaining in cheese: 58%

Amount of D3 vitamin remaining in 200 grams of cheese:

Remaining D3 vitamin=40  $\mu$ g (1600 IU)  $\times 0.58=23.2 \mu$ g (928 IU)

D3 vitamin content per 100 grams of cheese: D3 vitamin per 100 grams= 23.2  $\mu$ g (928 IU) /2= 11.6  $\mu$ g (464 IU) Turkish white cheese is traditionally brined as the final step (Yaman, Aykas & Rodriguez-Saona, 2023). However, this step was omitted in our project to produce non-brined cheese suitable for infant nutrition. The cheese samples were stored in a refrigerator.

## Vitamin D analysis

The amounts of vitamin D3 retained in 100 g of white cheese samples enriched with vitamin D3 and control group cheese samples were analyzed and compared in terms of vitamin D3 amounts using High-Performance Liquid Chromatography coupled with Ultraviolet detection (HPLC–UV). The method for analyzing vitamin D3 involves saponification and extraction, after which the extract is transferred to an appropriate solvent. The vitamin D3 content was determined using normal and reverse-phase analytical HPLC techniques.

## Extraction

The extraction of samples was performed using the ethyl ether/petroleum ether method in accordance with AOAC Official Method 992.26 (AOAC, 1999). The sample was first weighed into a 250 mL Erlenmeyer flask, followed by the addition of 1.0 mL of internal standard and 400 mg of ascorbic acid as an antioxidant. The mixture was then combined with 15 mL of ethanol and thoroughly mixed before adding 135 mL of 1 M KOH solution. The flask was swirled to dissolve the KOH or suspend the sample, then refluxed at 75 °C for 30 min. After cooling the mixture in ice water, it was transferred to a separatory funnel with a 5 mL ethanol rinse.

The vitamin D3 was extracted by adding 130 mL of ethyl ether, followed by vigorous shaking, and then an additional 130 mL of petroleum ether was added with further shaking.

After phase separation, the aqueous layer was discarded, and the ether layer was sequentially washed with deionized water. The ether phase was collected in a 500 mL round-bottom flask, evaporated to dryness at 45 °C, and then redissolved in acetone before being evaporated again.

Finally, the dried sample was dissolved in 10 mL of ethyl ether, transferred to a centrifuge tube, rinsed with additional ethyl ether, and evaporated to dryness under nitrogen. The final extract was reconstituted in 1.0 mL of hexane for analysis.

# HPLC

Two chromatographic separations were conducted according to AOAC Official Method 2002.05 (AOAC, 2006). The first was a normalphase preparative HPLC using a 25.0 cm  $\times$  4.6 mm, 5 µm silica column on an Agilent 1200 system. Two solvent programs were applied: one without a column wash for pure vitamin D standards, and one with a column wash for food sample extracts. The isocratic mobile phase 1 (0.5% IPA, 2.0% MTBE, 48.75% cyclohexane, 48.75% n-heptane) was used for 25 min, followed by a gradient to mobile phase 2 (20% IPA, 80% n-heptane) for sample analysis. Vitamin D was eluted at around 17.5 min, and fractions were collected between 16 and 19 min. then dried and reconstituted in mobile phase 3 (20% MeOH, 80% ACN) for further analysis.

The second separation was a reversed-phase HPLC using a Thermo Separation Products chromatograph with an Inertsil ODS-2 column (25.0 cm  $\times$  4.6 mm, 5 µm). An isocratic mobile phase 3 was used at a flow rate of 1.3 mL/min, with detection at 265 nm on a UV6000 DAD. Quantification was achieved by integrating the UV 265 nm chromatogram.

# Sensory analysis

Hedonic rating scales quantify the emotional aspects of consumers' perceptions of food. A 9point scoring system is commonly used, though a 5-point scoring system can also be applied (Tuorila et al., 2008). To evaluate the sensory differences between the two cheese groups, a 5point scoring test (5 - like very much, 4 - like moderately, 3 - like slightly, 2 - neither like nor dislike, 1 - dislike) was applied to 20 consumers. The consumers included 10 males and 10 females, aged 18 to 65 years. Consumers were untrained individuals who frequently consumed dairy products as a part of regular diet. Sensory evalua-tions were conducted in a sensory analysis labo-ratory under controlled lighting and temperature conditions (22 °C).

Cheese samples were cut into 2x2 cm cubes and served at room tempe-rature (22 °C). The first group consisted of cheese with added vitamin D3 that was not brined, while the second group comprised cheese without added vitamin D3 that was also not brined. Consumers evaluated the cheeses' colour, consistency, aroma, taste, mouthfeel, smell, and general impression.

### Microbiological analysis

Initially, a microbiological analysis of the raw milk from which cheese would be made was carried out. On the other hand, after cheese production, both vitamin D-fortified and nonfortified white cheese samples were microbiologically analyzed. Samples were examined regarding the enumeration of total mesophilic aerobic bacteria, *Enterobacteriaceae* spp., coliform group bacteria, and yeasts and mould counts. It aimed to compare the microbiological counts of commercial non-emulsified vitamin D-enriched cheese and that of unenriched cheese.

Principally, 10 g of samples were taken at 0 (production day), 1, 3, 5, and 7 days and placed in two separate sterile sample bags, and 90 ml of sterile 0.1% peptone water solution was added. Then, the samples were homo-genized in a stomacher device (AES, France) for 1 min, 1 ml of homogenized sample was mixed with 9 ml sterile Peptone Water (Peptone water, Oxoid CM0009B) solution to obtain se-rial dilutions.

Microbial analyses were carried out using the classic culture method on selective media in triplicate. Cheese samples were stored in the refrigerator at 4-6 °C during this period. Plate Count Agar (PCA, Oxoid CM0325) was used to enumerate aerobic bacteria count and the plates were incubated at 30 °C for 48 h (Carrillo-Lopez et al., 2020). *Enterobacteriaceae* spp. and coliform group bacteria were pour-plated on Violet Red Bile Glucose Agar (Oxoid CM0485) and Violet Red Bile Lactose Agar (Oxoid CM0107), respectively.

These plates were incubated at 37 °C for 48 h (Kačániová et al., 2018). Rose Bengal Chloramphenicol Agar (Oxoid PO0214) was used for the enumeration of yeasts and moulds at 25 °C for 3-5 days (Mureşan et al., 2021). The colonies were counted after incubation, and the results were stated as log cfu/ml. Microbiological analysis results were shown as log cfu/ml-g in Table 1.

## Statistical analysis

Sensory and microbiological analysis results were expressed in terms of mean and standard deviations using Microsoft Office Excel 2019. T-test (SPSS for Windows 11; One-way ANOVA) determined significant differences between means.

## **RESULTS AND DISCUSSION**

## Vitamin D analysis

According to the analysis report, there was no vitamin D3 in the control cheese sample that was not enriched with vitamin D3. This analysis showed that Turkish white cheese does not naturally contain vitamin D3. On the other hand, in the cheese sample fortified with vitamin D3, the content of 0.69  $\mu$ g/100g was observed to be equal to 6.9  $\mu$ g (276 IU) /100g. The analysis shows that 34.5% of the vitamin D3 we added during cheese-making was re-tained in the chees

The literature shows numerous studies on fortifying various cheeses with vitamin D, commonly using its emulsion form. Researchers either used pre-made emulsions or created emulsions through different methods (Crevier, Bélanger, Vuillemard & St-Gelais, 2017).

Various studies have explored fortifying cheese with vitamin D using different methods. Crevier et al. (2017) enriched cottage cheese by adding emulsified vitamin D to milk cream. Ganesan, Brothersen and McMahon (2011) fortified cheddar cheese by directly adding vitamin D emulsion to milk. Banville, Vuillemard and Lacroix (2000) used water-soluble vitamin D for cheddar cheese. Stratulat et al (2015) used encapsulated vitamin D3 from flaxseed oil in cheddar cheese. Flaxseed oil is a rich source of omega-3 fatty acids, particularly alpha-linolenic acid (ALA). However, it does not naturally contain vitamin D3.

These studies highlight the effectiveness of using emulsified vitamin D for cheese fortification.

Tippetts et al. (2012) used commercial nonemulsified vitamin D oil to fortify cheddar cheese, retaining 58% of the vitamin D in the final product.

In our study, Turkish white cheese was fortified with commercial non-emulsified vitamin D oil, resulting in a 34.5% retention rate of vitamin D. Various factors could explain the difference from similar studies. To properly analyze this data, it was necessary to compare the methods used in the production of two different types of cheese. For instance, the milk used in the Tippetts et al. (2012) study, was pasteurized at 72 °C for 15 seconds, was cooled to 5 °C before being used in cheese making, and then the milk

was heated to 39 °C and cooled to 31 °C to prevent coagulation problems that may arise from cooling. Commercial non-emulsified vitamin D oil was added to milk cooled to 31 °C. On the other hand, in our study, after raw milk was obtained, it was kept in the refrigerator (4 °C) for approximately 20 h and then heated at 75 °C for 5 min for pasteurization. The pasteurized milk was cooled to 40 °C, and at this temperature, the commercial non-emulsified vitamin D oil we used for enrichment was added to the milk. Factors such as differences in pasteurization temperature-time parameters and vitamin D addition temperatures in the studies may have caused different vitamin D retention rates in both cheeses. It is reported that the stability of vitamin D3 may vary depending on exposure to heat, light, moisture, and oxygen (Maurya, Bashir & Aggarwal, 2020), cheese production conditions, and cheese type (Ganesan et al., 2011).

Hendy, Gamal El Din and Awad (2023) produced cheese using a process similar to ours, using the non-emulsified form of vitamin D3 to enrich Karish cheese, Egypt's traditional cheese. In their study, non-emulsified vitamin D3 was added directly to pasteurized and cooled milk, as in our study. However, unlike the cow milk we use to enrich white cheese, fat-free buffalo milk makes Karish cheese. Similar to our study, after the pasteurization process was carried out using temperature and time parameters, it was observed that the vitamin D3 retention rate in Karish cheese (33.3%) was very close to ours (34.5%). Since vitamin D is a fat-soluble vitamin, it is thought that the difference in the amount of fat in milk may have affected the retention of vitamin D3 in cheese.

Many commercially available baby foods meet the infant's daily vitamin D requirement of 10  $\mu$ g (400 IU), obviating the need for additional vitamin D supplementation for infants consuming vitamin D-fortified formula (Abrams, 2020).

However, upon weaning from breastfeeding and/or formula, it is recommended that infants consume milk enriched with vitamin D to ensure an intake of at least 10 µg (400 IU) per day (Wagner and Greer, 2008). In our study, 34.5% of the added vitamin D3 (6.9 µg /12 drops) was retained in the cheese. Adding 19 drops of vitamin D3 to cheese milk using this fortification method would result in  $\approx$  10.9 µg (437 IU) in the final product. Consequently, vitamin D3fortified cheeses could effectively contribute to meeting the daily vitamin D requirements of infants and children who do not receive vitamin D supplements.

## Sensory analysis

The arithmetic mean results obtained from the scoring test applied to 20 consumers are presented in the graph. Consumers reported no noticeable difference between the cheese samples regarding colour, consistency, and odour characteristics. While no statistically significant differences were found in the aroma and taste attributes, a preference for the cheese without vitamin D enrichment was observed. This suggests that other factors, such as texture or aftertaste, might influence consumer preference, even when sensory attributes appear similar. Although these findings provide valuable insights, instrumental analyses conducted in future studies could provide more detailed data, enhancing the validity of the sensory results and offering a more comprehensive understanding of the cheese samples' characteristics. Moreover, it is important to recognize that sensory preferences can be subjective and influenced by various external factors, such as individual taste preferences, cultural backgrounds, and previous experiences with fortified products. As a result, larger sample sizes and a more diverse group of participants may be needed to capture a broader range of consumer perceptions. Additionally, exploring the long-term effects of vitamin D fortification on consumer acceptance could provide useful information on how fortification might affect product perception over time. Fig. 2 presents the arithmetic mean values based on the scoring test.

In their study, Hendy et al. (2023) stated that in their sensory analysis on Karish cheese, to which they added non-emulsified vitamin D directly to milk, the samples with added vitamin D received lower scores than the other cheese groups they compared, similarly to our study.

## Microbiological analysis

According to the results of microbiological analysis, microbiological counts of two groups of cheeses are given in Table 1. Microbial counts of raw milk at day 0 are shown in Fig. 3. According to the microbiological analysis results, it was determined that there was no differrence in terms of total mesophilic aerobic bacteria, *Enterobacteriaceae* spp., coliform group bacteria, yeast, and moulds in the cheese

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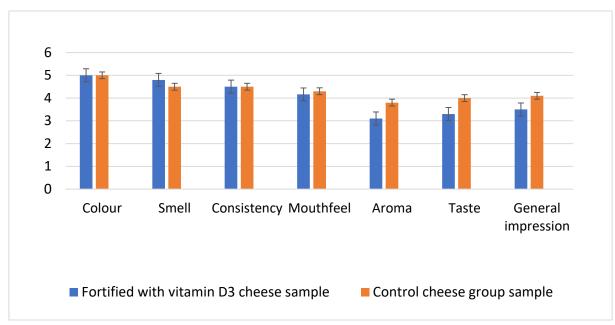


Figure 2. Arithmetic mean of the 5-point scale scoring test

Microbiological counts of cheese groups (log cfu/g)

Parameters	Examined cheese samples	Day 0	Day 1	Day 3	Day 5	Day 7
Total mesophilic aerobic bacteria	D+	2.78	<2.0	<2.0	<2.0	<2.0
	D-	2.7	<2.0	<2.0	<2.0	<2.0
Enterobacteriaceae spp.	D+	<2.0	<2.0	<2.0	<2.0	<2.0
	D-	<2.0	<2.0	<2.0	<2.0	<2.0
Coliform group bacteria	D+	<2.0	<2.0	<2.0	<2.0	<2.0
	D-	<2.0	<2.0	<2.0	<2.0	<2.0
Yeasts and molds	D+	2.7	<2.0	<2.0	<2.0	3.14
	D-	2.78	<2.0	<2.0	2.78	3.11

D+: Sample of cheese enriched with vitamin D; D-: Sample of cheese not enriched with vitamin D

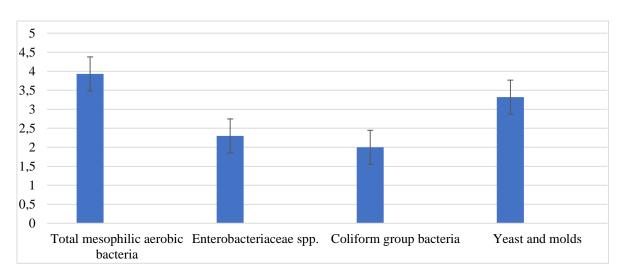


Figure 3. Microbiological counts of raw milk at day 0 (log cfu/ml).

samples with and without vitamin D added. When the microbiological analysis results were examined and total mesophilic bacteria, *Enterobacteriaceae* spp., coliform bacteria, yeast, and mould counts in raw milk and cheeses on day 0 were compared, it was seen that the bacterial load decreased after cheese making in both cheese groups. These data show that the pasteurization process applied during cheese making plays a role in reducing bacterial counts, highlighting its importance in producing a hygienic and safe product.

This effect is particularly relevant for cheeses intended for vulnerable groups, such as infants, where microbial safety is paramount. The fact that there is no statistical difference between the bacterial, yeast, and mould loads of the control group cheeses enriched with vitamin D3 and the unenriched control group cheeses shows that vitamin D3 enrichment does not have a significant effect on the change in the microbiological load in the cheese with the methods we used.

Although the total number of live aerobic bacteria as well as yeast and mould counts were 2.7 cfu log/g in the first (Day 0) cheese analysis, the decrease in bacterial growth on Day 1 is noticeable. This suggests that the amount of organic acid released due to fermentation during cheese production suppresses the total number of aerobic bacteria, yeast, and moulds in the cheese. On the other hand, the fact that there was no statistical difference in terms of total mesophilic aerobic bacteria, Enterobacteriaceae spp., coliform group bacteria, yeast and mould counts in cheese samples with and without vitamin D supports that consumers will not be prejudiced about consuming cheese enriched with vitamin D. In addition, unlike the traditional method, cheeses produced without brining process were detected below the detection limit in terms of total mesophilic aerobic bacteria, Enterobacteriaceae spp., and coliform group bacteria during the 3-day storage period, which provides information about the shelf life of the products.

# CONCLUSIONS

Vitamin D3-fortified cheeses have been found to help meet the daily vitamin D requirements of individuals who do not take vitamin D supplements. By furthering this research, manufacturers could develop cheeses with specified amounts of vitamin D in the final product. Such cheeses would contribute to public health and represent an advancement in the production of functional foods.

## **AUTHOR CONTRIBUTIONS**

Conceptualization, B.O.A., A.K.; Design, B.O.A.; Supervision, B.O.A.; Materials, B.O.A.; Literature research: BOA, AK; Data collection and processing, B.O.A., A.K.; Conducting experiments, B.O.A., A.K.; Analysis and interpretation, B.O.A., A.K.; Writing manuscript, B.O.A., A.K.; Critical review, B.O.A., A.K.

### DATA AVAILABILITY STATEMENT

Researchers who provide a methodologically sound proposal.

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## **CONFLICT OF INTEREST**

The authors have declared that no competing interests exist.

## REFERENCES

- Abrams, S. A. (2020). Vitamin D in preterm and full-term infants. *Annals of Nutrition and Metabolism*, 76(Suppl. 2), 6-14. https://doi.org/10.1159/000508421
- AOAC. (1999). Official methods of analysis (16<sup>th</sup> ed.).
   Gaithersburg, MD: AOAC International. Method 992.26, Vitamin D3 (cholecalciferol) in ready-to-feed milk-based infant formula, section 50.1.05.
- AOAC. (2006). Official methods of analysis (18<sup>th</sup> ed.). Gaithersburg, MD: AOAC International. Method 2002.05, Determination of vitamin D3 (cholecalciferol) in selected foods, section 45.1.22A.
- Banville, C., Vuillemard, J., & Lacroix, C. (2000). Comparison of different methods for fortifying Cheddar cheese with vitamin D. *International Dairy Journal*, *10*(5-6), 375-382. https://doi.org/10.1016/S0958-6946(00)00054-6
- Basedow, K. S. (2022). Knowledge, attitudes, and practices of US-based registered dietitians regarding vitamin D as a key nutrient in public health (Doctoral dissertation). University of North Florida, Brooks College of Health, Jacksonville, FL.
- Batacchi, Z., Robinson-Cohen, C., Hoofnagle, A. N., Isakova, T., Kestenbaum, B., Martin, K. J., Wolf, M. S., & De Boer, I. H. (2017). Effects of vitamin D2 supplementation on vitamin D3 metabolism in health and CKD. *Clinical Journal of the American Society* of Nephrology, 12(9), 1498-1506.

https://doi.org/10.2215/CJN.00530117

- Buttriss, J., & Lanham-New, S. (2020). Is a vitamin D fortification strategy needed? *Nutrition Bulletin*, 45(2), 115-130. https://doi.org/10.1111/nbu.12430
- Carrillo-Lopez, L. M., Juarez-Morales, M. G., Garcia-Galicia, I. A., Alarcon-Rojo, A. D., & Huerta-Jimenez, M. (2020). The effect of high-intensity ultrasound on the physicochemical and microbiological properties of Mexican panela cheese. *Foods*, 9(3), 313. https://doi.org/10.3390/foods9030313
- Corsello, A., Spolidoro, G. C., Milani, G. P., & Agostoni, C. (2023). Vitamin D in pediatric age: Current evidence, recommendations, and misunderstandings. *Frontiers in Medicine*, 10, 1107855. https://doi.org/10.3389/fmed.2023.1107855
- Crevier, B., Bélanger, G., Vuillemard, J.-C., & St-Gelais, D. (2017). Production of cottage cheese fortified with vitamin D. *Journal of Dairy Science*, 100(7), 5212-5216. https://doi.org/10.3168/jds.2016-12308
- Cui, A., Zhang, T., Xiao, P., Fan, Z., Wang, H., & Zhuang, Y. (2023). Global and regional prevalence of vitamin D deficiency in population-based studies from 2000 to 2022: A pooled analysis of 7.9 million participants. *Frontiers in Nutrition*, 10, 1070808. https://doi.org/10.3389/fnut.2023.1070808
- Fisk, C. M., Theobald, H. E., & Sanders, T. A. (2012). Fortified malted milk drinks containing low-dose ergocalciferol and cholecalciferol do not differ in their capacity to raise serum 25-hydroxyvitamin D concentrations in healthy men and women not exposed to UV-B. *The Journal of Nutrition*, 142(7), 1286-1290. https://doi.org/10.3945/jn.111.156166
- Ganesan, B., Brothersen, C., & McMahon, D. J. (2011). Fortification of Cheddar cheese with vitamin D does not alter cheese flavor perception. *Journal of Dairy Science*, 94(7), 3708-3714. https://doi.org/10.3168/jds.2010-4020
- Hendy, S. A., Gamal El Din, A. M., & Awad, R. A. (2023). Production of functional Karish cheese fortified with vitamin D3 in nanoemulsion. *Al-Azhar Journal of Agricultural Research*, 48(1), 31-40. https://doi.org/10.21608/ajar.2023.165487.1095
- Kačániová, M., Terentjeva, M., Kunová, S., Nagyová, L., Horská, E., Haščík, P., Kluz, M., & Puchalski, C. (2018). Identification of the Slovak traditional cheese "Parenica" microflora. Acta Agraria Debreceniensis, 150, 227-239.
  - https://doi.org/10.34101/actaagrar/150/1719
- Kasımoğlu, A., Göncüoğlu, M., & Akgün, S. (2004). Probiotic white cheese with *Lactobacillus acidophilus*. *International Dairy Journal*, 14(12), 1067-1073. https://doi.org/10.1016/j.idairyj.2004.04.006
- Maurya, V. K., Bashir, K., & Aggarwal, M. (2020). Vitamin D microencapsulation and fortification: Trends and technologies. *The Journal of Steroid Biochemistry and Molecular Biology*, 196, 105489. https://doi.org/10.1016/j.jsbmb.2019.105489
- Mureşan, C. C., Marc, R. A., Semeniuc, C. A., Socaci, A. N., Fărcaş, A., Fracisc, D., Rodica Pop, C., Rotar, A., Dodan, A., & Mureşan, V. (2021). Changes in physicochemical and microbiological properties, fatty acid, and volatile compound profiles of Apuseni cheese during ripening. *Foods*, 10(2), 258. https://doi.org/10.3390/foods10020258
- Neill, H. R., Gill, C. I., McDonald, E. J., McRoberts, W. C., & Pourshahidi, L. K. (2023). The future is bright:

Biofortification of common foods can improve vitamin D status. *Critical Reviews in Food Science and Nutrition*, *63*(4), 505-521. https://doi.org/10.1080/10408398.2021.1950609

- Nikooyeh, B., & Neyestani, T. R. (2022). The effects of vitamin D-fortified foods on circulating 25(OH)D concentrations in adults: A systematic review and
- concentrations in adults: A systematic review and meta-analysis. *British Journal of Nutrition, 127*(12), 1821-1838. https://doi.org/10.1017/S0007114521002816
- Płudowski, P., Kos-Kudła, B., Walczak, M., Fal, A., Zozuliska-Ziółkiewicz, D., Sieroszewski, P., Peregud-Po-
- IISKa-Złotkiewicz, D., Steroszewski, P., Peregud-Pogorzelski, J., Lauterbach, R., Targowski, T., & Lewiski, A. (2023). Guidelines for preventing and treating vitamin D deficiency: A 2023 update in Poland. *Nutrients*, 15(3), 695. https://doi.org/10.3390/nu15030695
- Shieh, A., Chun, R. F., Ma, C., Witzel, S., Meyer, B., Rafison, B., Swinkels, L., Huijs, T., Pepkowitz, S., & Holmquist, B. (2016). Effects of high-dose vitamin D2 versus D3 on total and free 25-hydroxyvitamin D and markers of calcium balance. *The Journal of Clinical Endocrinology & Metabolism*, 101(8), 3070-3078. https://doi.org/10.1210/jc.2016-1871
- Sohail, Z., Khan, N., Moazzam, M., Mujahid, S., Sindhu, A., Khan, H., Sohail, N., Afzal, M., Aleena, Zaheer, A., & Ishaq, I. (2023). Perspective chapter: Beyond delicious – The hidden functional benefits of cheese. In A. M. Abdou (Ed.) Recent trends on cheese as functional food with great nutritive and health benefits. IntechOpen.
  - https://doi.org/10.5772/intechopen.113047
- Stratulat, I., Britten, M., Salmieri, S., Fustier, P., St-Gelais, D., Champagne, C. P., & Lacroix, M. (2015). Enrichment of cheese with vitamin D3 and vegetable omega-3. *Journal of Functional Foods*, 13, 300-307. https://doi.org/10.1016/j.jff.2015.01.004
- Tippetts, M., Martini, S., Brothersen, C., & McMahon, D. (2012). Fortification of cheese with vitamin D3 using dairy protein emulsions as delivery systems. *Journal of Dairy Science*, 95(9), 4768-4774. https://doi.org/10.3168/jds.2011-5134
- Toney-Butler, T. J., Nicolas, S., & Wilcox, L. (2023). *Dose* calculation ratio and proportion method. Treasure Island, FL: StatPearls.
- Tunçtürk, Y., Andiç, S., & Ocak, E. (2010). The effect of homogenization and pasteurization on white cheese and whey composition. *GIDA - Journal of Food*, 35(5), 339-345.
- https://doi.org/10.29133/yyutbd.235918 Tuorila, H., Huotilainen, A., Lähteenmäki, L., Ollila, S.,
- Tuoma, H., Huomanen, A., Lanteenmark, L., Onna, S., Tuomi-Nurmi, S., & Urala, N. (2008). Comparison of affective rating scales and their relationship to variables reflecting food consumption. *Food Quality* and Preference, 19(1), 51-61. https://doi.org/10.1016/j.foodqual.2007.06.007
- van den Heuvel, E. G., Lips, P., Schoonmade, L. J., Lanham-New, S. A., & van Schoor, N. M. (2023). Comparison of the effect of daily vitamin D2 and vitamin D3 supplementation on serum 25-hydroxyvitamin D concentration (total 25(OH)D, 25(OH)D2, and 25(OH)D3) and importance of body mass index: A systematic review and meta-analysis. Advances in Nutrition, 15, 100133.
  - https://doi.org/10.1016/j.advnut.2023.09.016

- Wagner, C. L., & Greer, F. R. (2008). Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics*, 122(5), 1142-1152. https://doi.org/10.1542/peds.2008-1862
- Wu, Y., Wang, F., Li, A., Gao, J., Li, B., Sheng, H., Ma, J.,
  & Liao, X. P. (2023). Vitamin D status among infants and children in Shanghai, China: A hospital-

based study. *Food Science & Nutrition*, 11(6), 3111–3120. https://doi.org/10.1002/fsn3.3292

Yaman, H., Aykas, D. P., & Rodriguez-Saona, L. E. (2023). Monitoring Turkish white cheese ripening by portable FT-IR spectroscopy. *Frontiers in Nutrition*, 10, 1107491.

https://doi.org/10.3389/fnut.2023.1107491

#### PROIZVODNJA TURSKOG BELOG SIRA BEZ SALAMURE OBOGAĆENOG VITAMINOM D3

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Sažetak: Nedostatak vitamina D je rasprostranjen problem, posebno u zemljama u razvoju, i obogaćivanje hrane vitaminom D je ključna strategija za unapređenje javnog zdravlja. Obogaćivanje belog sira vitaminom D3 je privlačna opcija za potrošače, posebno za dojenčad koja zahtevaju suplementaciju vitaminom D. Ova studija je imala za cilj optimizaciju procesa proizvodnje belog sira direktnim dodavanjem komercijalnog neemulzifikovanog uljnog preparata sa vitaminom D3 u pasterizovano mleko i kvantifikaciju rezultujućeg sadržaja vitamina D3 u siru. Tradicionalni beli sir je proizveden tako da se postigne konačna koncentracija od 10 µg (400 IU) vitamina D3. Korišćenjem HPLC-UV metoda, uporedili smo obogaćeni sir sa neobogaćenom kontrolnom grupom u pogledu sadržaja vitamina D3. Takođe su sprovedene senzorske i mikrobiološke analize. Studija je pokazala da obogaćeni sir sadrži 6,9 µg (276 IU)/100g vitamina D3, bez statistički značajnih razlika u senzornoj evaluaciji ili mikrobiološkoj bezbednosti između dve grupe. Shodno tome, naši nalazi sugerišu da sirevi obogaćeni vitaminom D3 mogu efikasno doprineti zadovoljenju dnevnih potreba za vitaminom D. Ovo istraživanje ističe potencijal za razvoj sireva sa specificiranim sadržajem vitamina D i predstavlja značajan napredak u proizvodnji funkcionalne hrane, promovišući javno zdravlje.

**Ključne reči:** tradicionalna hrana, funkcionalna hrana, mlečni proizvodi, suplementacija, mikrobiološki kvalitet, senzorski kvalitet

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