

Production of functional karish cheese fortified with vitamin D₃ in Nanoemulsion

S. A. Hendy^{1,*}, A. H. Gamal El Din¹, and R. A. Awad²

¹Dairy Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.

²Food Science Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

* Corresponding author E-mail: samehhendy@azahar.edu.eg (S. Hendy)

ABSTRACT:

Karish Cheese is one of the most Egyptian low fat dairy products consumed. The deficiency of vitamin D is a major global public health problem especially in Middle East. The aim of this research is to develop fortification methods to improve the vitamin retention and Karish cheese properties. VD₃ nanoemulsion was formulated using maltodextrin (MD) and modified starch (MS) to achieve a fortification level 600 IU/100g of VD₃ in cheese. The vitamin was added to cheese with two different addition methods either direct in skim milk after heat treatment or by spraying into the scooped curd before the whey draining. Four treatments were made to investigate the effect of the fortification methods on vitamin retention and the properties of cheese. T1 and T2 were the treatments of cheese fortified with nanoemulsion with two addition methods direct and spraying respectively. T3 and T4 represent the treatments of cheese fortified with VD₃ emulsion under the same two methods. However, the most of VD₃ was lost for T1, T3 and T4 in the drained whey. Treatment T2 has a significant ($P < 0.05$) difference exposed in vitamin retention by 87 % in comparison with T1, T3 and T4 which retain only 33.3, 6.66 and 40.25 % respectively. Slight changes were observed in pH and titratable acidity and no changes were reported in fat and salt contents. Moisture content of T1 was about 1.3 - 2.3% higher than other treatments and control in zero time and was achieved 1.9-2.9% after 28 days. T1 was significantly ($P < 0.05$) lower in protein and ash contents than control and other treatments. There were no differences between the sensory properties of T2, T4 and control. The lowest score of sensory properties was observed for T1 and T3. Karish cheese fortified by spraying with VD₃ nanoemulsion could be a suitable strategy for production of functional free fat Karish cheese for those suffering from vitamin D deficiency.

Keywords: Karish cheese; Vitamin; VD₃; nanoemulsion; fortification; functional.

INTRODUCTION

The dairy industry is one of the most traditional industries in the world. In the past three decades, the introduction of the concept "functionality" has produced important changes in the type of dairy products that the consumers expect and demand. Driven by important socioeconomic changes around the world, and the ever-rising cost of health services, which makes it necessary to find less expensive ways to recover health. Among these approaches, the addition of probiotic bacteria, prebiotic, omega-3 fatty acids, and the addition of vitamins are without a doubt some of the most broadly disseminated strategies (Ortiz *et al.*, 2017).

Karish cheese is one of the most popular skimmed milk acids coagulated consumed cheese in Egypt. It is a promising food particularly for old people and those suffering from blood pressure and obesity related diseases due to its characterized high protein, low fat and low salt contents (Allam *et al.*, 2017; Youssef *et al.*, 2018). Despite the high nutritional value of Karish cheese, it is

considered poor source of fat-soluble vitamins content, especially VD.

Vitamin D (also referred to as "calciferol") is a fat-soluble vitamin that is naturally present in a few foods, added to others, and available as a dietary supplement. Vitamin D obtained from sun exposure, foods, and supplements is biologically inert and must undergo two hydroxylations in the body for activation. Recommended Dietary Allowance (RDA) of vitamin D is around 400-600 IU. 1 mg of vitamin D is equal to 40 IU NIH (2022). Vitamin D deficiency can result from inadequate exposure to sunlight, not consuming the recommended levels of the vitamin Wimalawansa *et al.*, (2018); Lips *et al.*, (2019). In foods and dietary supplements, vitamin D has two main forms, Vitamin D₂ (VD₂) which called (ergocalciferol) and Vitamin D₃ (VD₃) which called (cholecalciferol), that differ chemically only in their side-chain structures Silva and Furlanetto (2018). Sufficient vitamin D levels are necessary, not only for normal growth and development of bones, but also for the prevention of fatal chronic diseases. Vitamin D₃ fortification of free fat dairy products has

received considerable attention because of their ability to provide rich sources of calcium, in addition to reducing the risk of Vitamin D deficiency (Kazmi and Rousseau 2007; Cashman and Kiely 2016; Leskauskaite 2016; Itkonen 2018).

The term 'nano' is a Greek word, means dwarf and signifying 1 billionth of a meter (1 nm = 10^{-9} m). Nanoparticles are very useful as delivery vehicle of Functional ingredients Chavada (2016). The different delivery systems used in dairy nanotechnology include the association colloids, biopolymeric nanoparticles, nanoemulsions, nanofibers, nanocapsules etc. These systems will serve as a vehicle for carrying functional ingredients, protect functional ingredient from degradation and control the release of functional ingredients Weiss *et al.*, (2006).

Fortification of VD₃ nanoparticles in dairy products is widely evaluated in recent years. The research mentioned that reducing the size of the particles during the synthesis of VD₃ nanoparticles lead to increase their bioavailability and bioactivity. Moreover, the ability of nanocapsules to protect the vitamin from damage during the production process. The in vivo studies also explained the opportunity of using VD₃ nanoparticles to reduce the risk of VD₃ deficiency.

From these points, the objective of this study was to investigate the effect of using different methods of fortifying VD₃ nanoemulsion during manufacturing processes of Karish cheese and the ability to retain the vitamin, to find out to what extent it could be suitable for use as fortifiers to innovate functional low fat dairy products.

MATERIAL AND METHODS:

Material:

Vitamin D₃ (cholecalciferol) powder was purchased from Fermenta Biotech Limited Co. Maltodextrin (MD) and Succinic anhydride modified starches (MS) were purchased from Cargill Egypt. Sunflower oil was purchased from Savola Egypt. Buffalo milk and salt that were used for Karish cheese manufacture were provided by Friends for dairy products company (6th October, industrial zone, Egypt). Starter YCX-11 was purchased from Chr. Hansen (Denmark). Tween 80, acetic acid, ethanol, NaOH and other chemicals used for our experiments in present work was obtained from Bio. Co.

Preparation and Estimation of VD₃

VD₃ stock solution was prepared by dissolving VD₃ powder in ethanol, the mixtures were stirred magnetically at 1400 rpm and 40 °C for 60 min and then filtered using Watman filter paper No.1. Dissolved VD₃ was mixed with sunflower oil and the solution was stirred for a further 30 min. then, the organic solvent was removed by rotary evaporation under nitrogen at 25°C. VD₃ concentration was determined using high performance liquid chromatography (HPLC).

Preparation of VD₃ in emulsion Form

Tween 80 (1% w/w) was added to the distilled water and the solution was stirred for 30 min. Then, the organic phase (1% w/w), composed of a lipophilic (VD₃) was incorporated drop by drop into the aqueous phase, while it was homogenized using Unidrive X1000 CAT operating at 10000 rpm for 15 min in order to obtain a coarse emulsion of oil in water (o/w).

Preparation of VD₃ in Nanoemulsion Form

Nanoemulsion of VD₃ prepared according to Mujica-Álvarez *et al.*, (2020). The aqueous phase was prepared by adding the encapsulated agents to distilled water. Maltodextrin (MD) was combined with modified starch (MS) at a 70:30% (w/w). Mixtures were stirred magnetically at 650 rpm and 50 °C for 90 min. After that, Tween 80 (1% w/w) was added to the aqueous phase and the solution was stirred for a further 30 min. Then, the organic phase (1% w/w), composed of a lipophilic (VD₃) was incorporated drop by drop into the aqueous phase, while it was homogenized at 10000 rpm for 15 min in order to obtain a coarse emulsion of oil in water (o/w). Finally, in order to obtain nanoemulsions, the coarse emulsion was homogenized by ultrasound treatment (Sonics vibra cell VCX 750). Ultrasound homogenization was performed at 80% of the amplitude and 20 kHz of frequency for during 4 min.

Nanoemulsions Characterization

Morphology Characterization

The morphology of VD₃ nanoemulsion was observed by Transmission electron microscopy (TEM) (JEOL JEM 1400 USA). One drop was added to a copper grid and allowed to dry for 2 minutes. A drop of sodium phosphotungstate (2%, w/v) was set over the nanoemulsion droplet as a negative stain and

was allowed to dry before analysis (Yang *et al.* 2014).

Zeta Potential

The surface charge of VD₃ nanoemulsion were measured by laser zeta meter (Zetasizer Ver. 7.04 Malvern Instruments, UK).

The encapsulation efficiency (EE%)

The Encapsulation efficiency (EE%) was defined as the ratio of encapsulated VD₃ (VD_{3E}) to total VD₃ (VD_{3T}) expressed as percentage. VD₃ nanoemulsion solutions centrifuged at 30,000 rpm for 15 min followed by withdrawal clear solution to determine (VD_{3E}) after excluded precipitated amount which is estimated by using high performance liquid chromatography (HPLC). The Encapsulation efficiency of VD₃ nanoemulsion was indirectly determined using the following equation according to Jafari, *et al.*, (2007).

$$EE\% = VD_{3E} / VD_{3T} \times 100$$

Karish Cheese Manufacture

Karish cheese was made as mentioned by Ahmed *et al.* (2005) in Friends for dairy products factory using a typical industrial procedure. Skimmed buffalo's milk (0.15% acidity, 4.2% protein, 0.1 % fat, 8.9 % TS) was divided into five equal portions, the first part was used to make unfortified cheese was pasteurized at 80°C for 20s in a plate pasteurizer heat exchanger and then cooled to 37°C. Lactic acid starter was added to skimmed milk at 37°C. After complete coagulation (pH 4.5–4.6), the curd was scooped into food grade plastic mat for draining, dry salt (0.5 g/100 g cheese), was sprinkled on the surface of curd layers and left to drain over night at 4°C. The resultant cheese was then cut into blocks. Cheese enveloped in polyethylene film, vacuumed and stored at 4°C for 28 days. The four other portions of skimmed milk were used to produce the fortification Karish cheese with VD₃ emulsion and VD₃ nanoemulsion with two different fortification methods as follow:

Direct in skim milk cheese: The vitamin was added to two portions of skimmed milk before acidification conducted by lactic acid bacteria (T1 and T3 treatments of cheese fortified with VD₃ nanoemulsion and VD₃ emulsion respectively).

Sprayed into the scooped cheese curd before the end of whey draining: The vitamin was added to two portions by spying into the scooped cheese curd (T2 and T4 treatments of

cheese fortified with VD₃ nanoemulsion and VD₃ emulsion respectively).

Cheese Yield

Cheese yield was calculated as described by Fox, *et al.*, (2017).

Chemical Analysis

The pH, titratable acidity, total protein, ash, moisture and salt contents were determined according to AOAC, (2019). Fat content was determined using Gerber tube for milk and cheese according ISO 19662 (2018). Duplicate samples were analyzed for each trial.

Sensory Properties

Karish cheese Organoleptic evaluation carried out by staff member of dairy dept. (Fac. Agric., Al-Azhar Univ., Cairo, Egypt) and factory technical staff who had several years' experience in cheese manufacturing and analysis using a score card. Cheese samples were organoleptically scored for flavour (50 points), body and texture (35 points) and appearance (15 points). According to the score card suggested by Ismail (2004) with some modifications.

Statistical analysis:

The data was analyzed by ANOVA according to the appropriate experimental designs and expressed as means ± standard deviation, which were then statistically compared by Duncan test at the confidence level of 0.05 using SPSS program, version 20.0 (IBMSPPSSSTATISTICS20). All experiments were repeated in triplicates according to SAS (1996).

Statistical Analysis System (SAS), (1996). SAS user's guide. Statistics. SAS Inst. Inc. Ed., Cary, NC, USA.

RESULT AND DISCUSSION

Concentration of VD₃ in stock solution

High-performance liquid chromatography (HPLC) was used to determine the VD₃ concentration and calculated by peak area integration at 254 nm, Fig.1. The result showed that the total VD₃ concentration detected was 1896.61(ug/g) WHICH equal 75864.41 (IU).

Nanoemulsions Characterization

Morphology and particle size characteristics of VD₃ nanoemulsion

Morphology and particle size characteristic of VD₃ nanoemulsion were examined using transmission electron microscopy (TEM) with

direct magnifications (40000 ×). In Fig. (2) the mixtures were an opalescent solution, indicating the formation of VD₃ nanoparticles. The interior structure of nanoparticles demonstrated a circular shape consisting of a dark core.

The image analysis of VD₃ Nanoemulsion complies with the data obtained by Hasanvand *et al.*, (2015) who reported that high amylose starch nanocarriers of VD₃ had a granular shape.

Image analysis of TEM photographs shows that the particle size ranged between 19.2 to 36.1 nm, with an average size of 27.65 nm. The results indicated that the nanoparticles were formed in nanoscale particles. The result was confirmed with Meghani, *et al.*, (2019) who reported that the hydrodynamic size of vitamin D encapsulated cinnamon oil nanoemulsion was 48.96 nm. Hasanvand *et al.*, (2015) also reported that high amylose starch nanocarriers of VD₃ had a particle size ranging from 14.2 to 31.8 nm. The obtained results were better than the results observed by Park *et al.*, (2017) who demonstrated that the optimum particle size of VD₃ nanoparticle was 132.9 nm. It could be noted that the modification conducted during nanoemulsion preparation using both homogenization and sonication techniques lowers the particle size to nanoscale.

Surface charge:

The ζ-potential of the VD₃ nanoemulsion was highly negatively charged (-24.03 mV), which indicates a good stability of nanoemulsion due to the high repulsive force resulting from the presence of the negative charge on the nanoparticles' surface. This result is better than Teng, and Wang, (2013) who demonstrated that the Zeta potential of vitamin D-loaded nanoparticles developed from carboxymethyl chitosan (CMCS) were negatively charged. Zeta values ranging from (-10 to -20 mV) at pH 7.5. High negatively charged VD₃ nanoemulsions prepared using long chain triglycerides (corn oil) could be due to the fact that the electrical characteristics of the droplets were dominated by the presence of the adsorbed surfactant layer (Ozturk *et al.*, 2015).

Encapsulation efficiency (EE):

The calculated EE% of VD₃ nanoemulsions was 100%. The obtained result showed high effective loads of VD₃ obtained in comparison to other studies. The result was confirmed with Zhang *et al.*, (2022) who reported that the

encapsulation efficiency of VD₃ in resultant oil-in-water nanoemulsions reached 99.9%. The observed result was also agreed with Teng, and Wang, (2013) who reported that the complex vitamin D-loaded nanoparticle achieved significantly higher encapsulation efficiency (up to 96.8%).

Karish cheese Properties:

Cheese Yield:

The obtained data from Table (1) exposed that the yield of T1 treatment was about 09 to 2.4% higher than control and other treatments, which may cause an increase in the moisture content as a result of the mechanism of nano-coating polysaccharides agent. Aminifar and Emam (2016) found that polysaccharides led to enclosing water and reducing syneresis from cheese curd, which increases the yield and moisture. These results are similar to those reported by Stratulat *et al.*, (2015) who reported that the yield of cheese fortified with VD₃ was increased 1 to 1.5% compared to control cheese. The result obtained also agreed with Ahmed *et al.*, (2004) which stated that the yield of Karish cheese produced by Exopolysaccharide (EPS) was about 4% higher in cheese than typical traditional Karish cheese (control).

Titrate acidity and pH value

There were slight differences in the acidity and pH values of control and four different Karish cheese treatments as explained in Table (2). The results ranged between (92. - 0.94%) for acidity with pH values (4.46 to 4.51) at zero time. Slight changes were observed in the values of pH and acidity during the storage period. The acidity showed a slight increase, and pH decreased at 5 °C in all treatments including control. The decrease in pH during storage could be referred to the converting of residual lactose in cheese to lactic acid, which is developed in the cheese by lactic acid bacteria at the end of the storage period. This result agreed with Awad *et al.*, (2015) who reported that the decrease in pH values during storage may be due to the hydrolysis occurring in protein contents and lactose. No significant effects were noted about the influence of VD₃ fortification on the acidity development in Karish cheese by lactic acid bacteria. These results were confirmed with Leskauskaitė *et al.*, (2016) who reported that fresh yogurt fortified by VD nanoemulsion has a pH of 4.6. The result does not agree with Jalal *et al.*, (2022) who observed that the fortification of yogurt with 1000 IU of VD had led to an increase in acidity and a decrease in pH values of the treatments.

Moisture content

The data represented in Table (2) indicated that the moisture content of fresh fortified Karish cheese treatments T1 and T3 were 77.8% and 76.5% respectively were being significantly ($P < 0.05$) higher than T2, T4 and control (75.8, 75.5 and 75.6 %) respectively. During storage period, cheese moisture gradually decreased in all treatment accompanied by an increase in total solids. The moisture content after 28 days was about (75.4 and 74.70%) for T1 and T3 and (73.5, 73.7 and 73.5%) for T2, T4 and control respectively. It could be observed that the moisture content was decreased during storage which may occur due to the acidity development and its effect on the increase of whey syneresis over time. The obtained result agreed with Hussein and Shalaby (2014) & Awad *et al.*, (2015) who explained that the development of acidity in Karish cheese is accompanied by an increase in moisture loss.

The higher moisture content of treatment (T1) at zero time could be due to the ability of polysaccharide used in nanoemulsion formulation (modified starch and maltodextrin) to reduce the whey syneresis through the effects of water binding. This result agreed with Pang *et al.*, (2019) who stated that the addition of starch could reduce syneresis by partial gelatinization and water binding effects. Larsen, (2009) stated that starch increase the moisture content of low-fat cheeses significantly. Maltodextrin led to increase moisture content of low-fat cheese when compared to cheese made without maltodextrin addition (Iakovchenko and Arseneva, 2016).

Protein content

The protein contents in cheese can be influenced by different factors such as, milk composition, addition of protein sources in the formula and moisture contents of cheese. The protein content was significantly ($P < 0.05$) affected by the moisture contents of final products. It could be seen from the data in Table. (2) that the total protein of all fresh treatments ranged between 14.00-15.5 % in comparison with control which was about 15.2 at zero time. The highest protein value (15.5%) was observed for T4 fortified VD₃ emulsion by spraying in the curd. On the other hand, the lowest amount of protein (14.00 %) was recorded for T1 respectively which is directly fortified with VD₃ nanoemulsion in skimmed milk. The obtained results were complied with Awad *et al.*, (2015) who mentioned that the

protein content of Karish cheese was decreased with increasing the added ratio of stabilizer due to the increase in moisture contents of treatments. The total protein in all treatment increased steadily during storage period at 5 °C which could be related to the decrease in moisture content.

Fat content

The results obtained from Table (2) explained that the fat content of control and experiments Karish cheese was not changed at zero time and during the storage period. The low-fat content of skimmed buffalo milk (0.1 % fat) used to manufacture Karish cheese played the key roles in the absence of significant differences. This result was complied with Ahmed *et al.*, (2005) who mentioned that slight changes were observed in fat content of Karish cheese produced using skimmed milk (0.1 g fat/100 ml milk).

Salt content

It could be seen from the data obtained from Table (2) that low salt content was observed in control and experiments Karish cheese due to low percentage of salt used and the partially losses during the whey syneresis step. Slight increase in the salt content occurred during the storage period related to the decrease in cheese moisture content.

Ash content

The results in Table (2) explained that the ash content of T1 treatment was significantly ($P < 0.05$) lower than control and T2 treatment. The low ash content of T1 could be due the higher content of moisture in cheese. Slight increase in the ash content occurred during the storage period related to the increase in cheese total solids.

Retention of VD₃ in Karish cheese treatments

The highest concentration of VD₃ in fortified cheese was retained in T2 treatment with 87% compared to 33.3, 6.66 and 40.25% for T1, T3 and T4 respectively. It could be observed from the obtained results that the retention of VD₃ can be increased by the addition of vitamin by spraying into the curd at the end of draining process which help to prevent the vitamin losing as compared to vitamin added directly in the milk before coagulation. This result was confirmed with Crevier *et al.*, (2017) who reported that the addition of VD₃ after the draining step by mixing fortified cream with the fresh cheese curds of cottage cheese led to retain the vitamin without loss. Nzekoue, *et al.*, (2021)

observed that the fortification of ricotta cheese with VD₃ added after the draining phase avoid any loss of the vitamin in whey. Banville *et al.*, (2000) mentioned that there are different factors such as fermentation by acidification, lactic acid bacteria and oxygen may be involved in destabilizing of VD₃ during cheese making. Kazmi (1993) reported that the loss of VD of fortified fermented dairy products might have occurred due to the rapid decrease of pH from 6.7 to 4.3 during fermentation. It could be concluded that the addition of VD₃ by spraying into the scooped curd resulted in a higher retention of VD in cheese, compared to that added directly in skimmed milk before acidification due to the manufacturing process factors which might lead to excessive vitamin loss.

Karish Cheese Sensory Properties

The sensory properties of fortified fresh and stored Karish cheese treatments in comparison with control are listed in Table (3). All fortified treatments did not impart an off flavor as determined by sensory analysis. T2 treatment showed a high level of acceptability than other treatments. T1 and T3 treatments described by panelists as very moist and soft in comparison with control and other treatments that were described as dry and granular, in softness and lowest moist. Storage fortified Karish up to 28 days decreased the quality of sensory properties of all treatments including the control.

Score for appearance (cracks), texture (granular) and flavor (acid) were also affected by the moisture content. These descriptions are consistent with the obtained chemical composition. T1 treatment received lower scores than control and other treatments. Nevertheless, these results are consistent with those of other workers who used lactic starter cultures and the same production condition to produce free fat Karish cheese.

In general, the average score of organoleptic tests of Karish cheese fortified with VD₃ nanoemulsion support its suitability for fortification of cheese products.

CONCLUSION

The forgoing results led satisfactorily to conclude that, it could successfully produce functional free fat Karish cheese fortified with VD₃ nanoemulsion by spraying the vitamin into the scooped curd before the end of whey draining. This ensures that Karish cheese could be a new source of vitamin D as a possible health-promoting nutraceutical free fat dairy

product and elevate the vitamin physiological benefits.

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Table.1. Yield of Karish cheese treatments fortified with VD₃

Karish Cheese Treatments				
(Control)	T1	T2	T3	T4
30.15 %	32.40 %	30.12 %	31.50	30.00

(C) Unfortified Karish Cheese (Control) –(T1) Fortified with VD₃ Nanoemulsion added direct in skim milk – (T2) Fortified with VD₃ Nanoemulsion added by spraying on the curd. (T3) Fortified with VD₃ emulsion added direct in skim milk - (T4) Fortified with VD₃ emulsion added by spraying on the curd.

Table 2. Chemical Composition of fortified Karish cheese treatments with VD₃ when fresh and during storage period.

Cheese* Treatments	Storage Period at 4 °C (day)	Acidity%	pH value	Moisture %	Protein %	Fat %	Salt %	Ash %
(Control) (C)	Zero	0.93 ^a	4.48 ^{ab}	75.60 ^{bcd}	15.20 ^{cde}	0.3 ^a	0.50 ^{abcd}	1.87 ^b
	14	1.12 ^a	4.26 ^{de}	74.70 ^{de}	15.60 ^{ab}	0.3 ^a	0.60 ^{abc}	1.93 ^{ab}
	28	1.21 ^a	4.12 ^{de}	73.50 ^e	16.18 ^{ab}	0.3 ^a	0.68 ^a	2.10 ^a
(T1)	Zero	0.92 ^a	4.48 ^{ab}	77.80 ^a	14.00 ^g	0.3 ^a	0.38 ^d	1.25 ^c
	14	1.08 ^a	4.31 ^{bcd}	76.00 ^{ab}	14.35 ^{fg}	0.3 ^a	0.47 ^{cd}	1.28 ^c
	28	1.14 ^a	4.20 ^{de}	75.40 ^{abc}	14.62 ^{efg}	0.3 ^a	0.55 ^{abcd}	1.33 ^c
(T2)	Zero	0.93 ^a	4.46 ^{abc}	75.80 ^{bcd}	15.10 ^{cde}	0.3 ^a	0.50 ^{abcd}	1.84 ^b
	14	1.13 ^a	4.28 ^{cde}	74.00 ^{cde}	15.30 ^{cde}	0.3 ^a	0.61 ^{abc}	1.89 ^{ab}
	28	1.18 ^a	4.10 ^e	73.50 ^{de}	16.10 ^{ab}	0.3 ^a	0.67 ^a	1.99 ^{ab}
(T3)	Zero	0.92 ^a	4.51 ^a	76.50 ^{abc}	14.40 ^{fg}	0.3 ^a	0.40 ^d	1.36 ^c
	14	1.10 ^a	4.31 ^{bcd}	75.80 ^{bcd}	14.71 ^{def}	0.3 ^a	0.48 ^{bcd}	1.40 ^c
	28	1.16 ^a	4.16 ^{de}	74.70 ^{de}	15.33 ^{cd}	0.3 ^a	0.55 ^{abcd}	1.45 ^c
(T4)	Zero	0.94 ^a	4.47 ^{ab}	75.50 ^{bcd}	15.50 ^{bc}	0.3 ^a	0.50 ^{abcd}	1.85 ^b
	14	1.14 ^a	4.30 ^{bcd}	74.80 ^{de}	15.80 ^{abc}	0.3 ^a	0.62 ^{abc}	1.91 ^{ab}
	28	1.19 ^a	4.12 ^{de}	73.70 ^e	16.20 ^a	0.3 ^a	0.66 ^{ab}	2.00 ^{ab}

*(C) Unfortified Karish Cheese (Control) –(T1) Fortified with VD₃ Nanoemulsion added direct in skim milk – (T2) Fortified with VD₃ Nanoemulsion added by spraying on the curd. (T3) Fortified with VD₃ emulsion added direct in skim milk - (T4) Fortified with VD₃ emulsion added by spraying on the curd.

Means ± Standard deviation with different small letters within each row are significant at 5 % level.

Table 3. Organoleptic test of Karish Cheese fortified with VD₃ Nanoemulsion

Storage Period	Parameters	Karish Cheese Treatments				
		C	T1	T2	T3	T4
Fresh	Appearance (15%)	14.40	11.66	14.3	12.77	14.20
	Body & Texture (35%)	33.11	29.22	33.22	31.33	32.9
	Flavor (50%)	44.44	43.77	44.47	44.11	44.51
	Total Score (100)	91.95	84.65	91.99	88.21	91.66
After 14 days	Appearance (15%)	13.30	10.66	13.10	11.71	13.15
	Body & Texture (35%)	32.11	28.18	32.22	30.33	31.9
	Flavor (50%)	43.35	42.65	43.45	43.10	43.47
	Total Score (100)	88.76	81.49	88.77	85.14	88.52
After 28 days	Appearance (15%)	12.10	09.68	12.10	10.71	12.20
	Body & Texture (35%)	31.10	27.17	31.20	29.33	30.10
	Flavor (50%)	42.52	41.63	42.44	42.11	42.74
	Total Score (100)	85.72	78.48	85.74	82.15	85.04

(C) Unfortified Karish Cheese (Control) –(T1) Fortified with VD₃ Nanoemulsion added direct in skim milk – (T2) Fortified with VD₃ Nanoemulsion added by spraying on the curd. (T3) Fortified with VD₃ emulsion added direct in skim milk - (T4) Fortified with VD₃ emulsion added by spraying on the curd

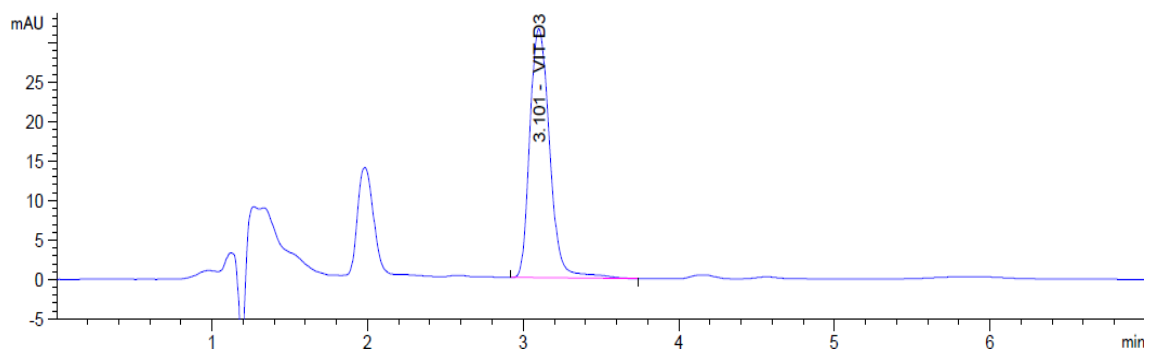


Figure 1. RP-HPLC chromatograms of the concentration of VD₃ stock solution.

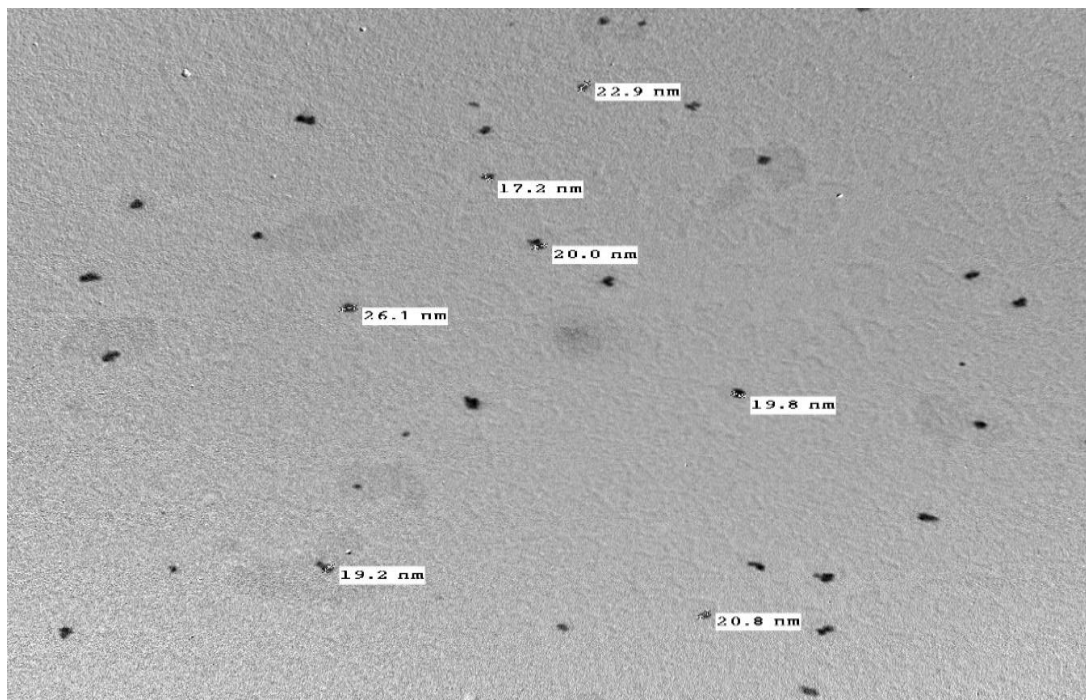


Figure 2. TEM photographs of VD₃ nanoemulsion using 40000 × magnifications showing nanoparticles shapes and size.

إنتاج جبن قريش وظيفي مدعم بمستحلب فيتامين د3 نانوي

سامح عبدالله أحمد هندی^{1*}، عبدالحكم محمد جمال الدين¹، رزق عزب عواد²¹ قسم الألبان، كلية الزراعة بالقاهرة، جامعة الأزهر، القاهرة، مصر.² قسم علوم الأغذية، كلية الزراعة، جامعة عين شمس، القاهرة، مصر.البريد الإلكتروني للباحث الرئيسي: samehhendy@azahar.edu.eg

الملخص العربي

يعتبر الجبن القريش أحد منتجات الألبان قليلة الدسم الأكثر استهلاكاً في مصر. يعد نقص فيتامين د مشكلة صحية عالمية. الهدف من هذا البحث هو تطوير طريقة لتحسين عملية الاحتفاظ بالفيتامين وخصائص الجبن القريش المدعم. تم إعداد مستحلب نانوميتر من VD3 باستخدام المالتوديكسترين (MD) والنشا المعدل (MS) للوصول إلى مستويات 600 وحدة دولية / 100 جم من VD3 في الجبن. تمت إضافة الفيتامين إلى الجبن بطريقتين مختلفتين (الإضافة مباشرة في اللبن الحالي من الدسم أو من خلال الرش على الحثرة). تم إعداد أربعة معاملات لدراسة تأثير طرق التدعيم على الاحتفاظ بالفيتامين وخصائص الجبن. تمثل المعاملات T1 و T2 الجبن المدعم بمستحلب النانو بطريقتين بالإضافة على التوالي. بينما تمثل T3 و T4 معاملات الجبن المدعم بمستحلب VD3 بنفس الطريقتين. حدث فقد لمعظم VD3 في المعاملات T1 و T3 و T4 أثناء تصفية الشرش. احتفظت المعاملة T2 بصورة معنوية بنسبة 87٪ من فيتامين د3 المضاف بالمقارنة مع T1 و T3 و T4. لوحظت تغيرات طفيفة في رقم pH وحموضة التعادل ولم يتم ملاحظة أي تغييرات في كل من نسبة الدهن والملح. كان محتوى الرطوبة في T1 أعلى بنحو 1.3 - 2.3٪ من المعاملات الأخرى بالإضافة إلى العينة المرجعية للجبن الطازجة، وتراوح الفرق بين 1.9-2.9٪ بعد 28 يوماً. كانت المعاملة T1 أقل بصورة معنوية في محتوى البروتين والرماد من العينة المرجعية والمعاملات الأخرى. لا توجد فروق بين الخصائص الحسية لـ T2 و T4 والعينة المرجعية بالإضافة إلى المعاملة T1، يمكن إنتاج جبن قريش مدعم بطريقة الرش بمستحلب نانوميتر من VD3 كاستراتيجية مناسبة لإنتاج جبنه وظيفي خالي من الدسم.

الكلمات الاسترشادية: الجبن القريش، فيتامين د3، مستحلب نانوي، التدعيم، وظيفي.