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**H Riar**
Department of Dairy Technology, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

**N Goel**
Department of Dairy Technology, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

**PK Singh**
Department of Dairy Technology, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

**S Sivakumar**
Department of Dairy Technology, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

**SK Mishra**
Department of Dairy Microbiology, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

**R Chawla**
Department of Dairy Technology, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

**Correspondence**
**N Goel**
Department of Dairy Technology, College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

# Changes in instrumental color and proximate parameters in yoghurt fortified with vitamin a and d nanoemulsion during storage

**H Riar, N Goel, PK Singh, S Siva Kumar, SK Mishra and R Chawla**

**Abstract**
Micronutrients are an important dietary component required in small amount and are essential for the overall growth and development of human body. Micronutrient malnutrition is a major public health concern and globally more than 2 billion people suffer from this. The most common form of MNM is Iron, Vitamin A & D deficiency and folic acid. The vitamin A & D deficiency is prevalent in all the age groups. To curb the deficiency, fortification of vitamins in yoghurt was carried out as it is a wholesome nutritious product liked by people across the globe as well as in India. The nanotechnology based approach using micro fluidisation was used to encapsulate and protect the degradation of vitamins. The vitamin fortified yoghurt was prepared by addition of vitamin A & D fortified nanoemulsion. The effect of storage on the acidity, pH and instrumental colour of vitamin fortified yoghurt was studied. It was observed that vitamin fortified yoghurt had better physico-chemical properties as compared to control. The storage study depicted that the product had acceptable quality till 14$^{th}$ day of the storage.

**Keywords:** Vitamin a, vitamin d, yoghurt, nano emulsion, micro fludisation

## 1. Introduction

Functional Food is defined as "A food, which beneficially affects one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease" (EFSA 2018)[1].Considering growing consumer awareness about the direct correlation between diet and its possible effect on the human health, in recent years, demand for the functional foods has increased manifolds (Singh and Singh 2016) [2]

Nowadays, various functional ingredients are booming into the market viz. prebiotics, phytochemicals, micronutrients etc. Micronutrients are an important dietary component, required in small amount but are essential for the overall growth and development of body. Micronutrient malnutrition (MNM) is a major public health concern and globally more than 2 billion people suffer from this. Most common forms of MNM are Iron, Vitamin A & D, Folic acid and Iodine deficiency (WHO 2016). This study addressed the problems associated with Vitamin A and D deficiency.

Vitamin A plays an essential role in our body especially for maintaining healthy eyes. According to WHO 2016, more than 3 million pre-school children are deficient in vitamin A. Nearly, 44-50% preschool children in South Asian regions were affected by severe Vitamin A deficiency (VAD) and 85% of these children with xerophthalmia reside in India (WHO 2015). A significant increase in the magnitude of Vitamin A deficiency among Indian women from 2001 (5.9%) to 2011 (30.3%) was observed (Akhtar *et al.* 2013) [3].NSSO reported that 62% of the Indian pre-school children suffer from Vitamin A deficiency (Suri *et al.* 2017) [4].Vitamin D deficiency is also prevalent in all the age groups including toddlers, school children, pregnant women and adult males and females residing in urban and rural India (Go swami R. *et al*. 2008 [5], Aparna *et al*. 2018[6]).

Fortification of dairy products viz yoghurt with vitamins could be a holistic approach to address the problem of vitamins deficiency. Yoghurt, a fermented product has excellent health benefits and its market grew globally at 5-10% across the globe (International Dairy Federation 2016). In India, it was reported to grow at a CAGR of 28.9% during 2011-2015 and is projected to touch US$1 billion by the end of 2021.The main limitations faced in fortification of vitamins in dairy products like yoghurt were poor dispersibility in aqueous medium and instability due to environmental stress. Therefore, an effort was carried out to

Overcome these challenges using an advanced technology i.e. nanotechnology that has provided the opportunities to improve the functionality of food products without compromising its traditional taste and texture.

Vitamin A & D fortified yoghurt was prepared by addition of vitamin A & D fortified nano emulsion in the yoghurt. This paper discusses the effect on physico-chemical properties of vitamin A and D nano emulsion fortified yoghurt during storage studies.

## 2. Materials and methods

### 2.1 Raw materials

Freeze dried yoghurt culture (NCDC 144) was procured from National Collection of Dairy Cultures, NDRI, Karnal**.** Fresh and clean raw milk was procured from Experimental Dairy Plant, GADVASU, and Ludhiana. The skim milk powder was purchased from Verka Milk Plant, Ludhiana, India. Instant zed WPI (instant zed Bi pro) was purchased from Davisco Food International Inc, Geneva, Switzerland. The chemicals used in this study were of analytical grade and all aqueous solutions were made using Milli-Q distilled water. Clarified butter fat (*Ghee*) of Verka® brand was purchased from MILKFED, Chandigarh, India.

For fortification Vitamin A in form of Retinyl acetate and Vitamin D in form of Cholecalciferol were procured from Sigma-Aldrich (Merck), Darmstadt, Germany.

### 2.2 Preparation of Vitamin A & D fortified yoghurt

The control and vitamin A & D fortified yoghurt was prepared using method described by NP Shah 2017 [7] with slight modifications. For vitamin A and D fortified yoghurt raw milk was standardised to 2.5% fat and 10% SNF using skim milk powder. Vitamin A & D nano emulsion was prepared by using oil phase (10% w/v) containing ghee, vitamin A (100% RDA value) and vitamin D (100 % RDA value) and aqueous phase (90% w/v) containing 3% WPI as an emulsifier. Both the phases were mixed using Ultra Turrax T-25 disperser (IKA-Werke GmbH and Co. KG, Staufen, Germany) at 12000 rpm for 5 min and converted into nano emulsions using micro fluidiser® (M-110P, Microfluidics, newton, MA, USA) at pressure 15000 psi/3$^{rd}$ pass. Vitamin A and D nano emulsion was added into the raw milk. The raw milk along with the nano emulsion was heat treated at 90ºC for 5-10 min holding. The heat treated milk was cooled to 42ºC and inoculated with yoghurt starter culture at 42ºC @ 3%. Both vitamin fortified yoghurt and control samples were filled in 100 ml polystyrene cups and incubated for 4-5 hours till the acidity reaches 0.6%. The set yoghurt samples were stored at 5ºC refrigeration temperature till spoilage.

### 2.3 Determination of acidity and pH

The titratable acidity was determined as per method of AOAC (2006). 10 g of sample was taken in a porcelain dish and mixed homogenously by adding 10 ml hot distilled water (65°C). This was followed by addition of 1 ml of 0.5 percent phenolphthalein indicator. The mixture was titrated against 0.1N sodium hydroxide with continuous stirring till the pink colour appeared. Acidity was expressed as percent lactic acid $= 9xNxV_1/V_2$ Where, N= Normality of NaOH, $V_1$= Volume of NaOH used and $V_2$= Volume of sample taken.

PH readings were taken by calibrated hand pH meter pHep+, maker: Hanna instruments, Model No: HI98108. The readings were taken in triplicates at 10ºC.

### 2.4 Determination of instrumental colour of yoghurt

Surface colour of yoghurt samples were measured using Colour Flex Colorimeter (Hunterlab, Reston, Virginia) supplied along with the universal software Easy Match QC (version 4.62) and the results were expressed in terms of CIE-LAB system. The colorimeter was equipped with dual beam xenon flash lamp as source of light. Prior to analysing the samples, the instrument was calibrated with standard black glass and white tile as specified by the manufacturer of the equipment. Data was received through the software in terms of values for L* (lightness, 0 (black) to 100 (white); a* (redness, +60 (red) to -60 (green)) and b* (yellowness, +60 (yellow) to -60 (blue)) (Hunter 1975) [8].The sample of yoghurt was placed in the transmission port of the optical unit of a color difference meter. Light energy from a controlled source was directed through the specimen into an integrating sphere. Phototubes are positioned at the top of the sphere to view the interior of the sphere. Electrical signals proportional to light quantities present are directed by cable to the measuring unit, where they are read directly as L*, a* and b* colour values.

## 3. Results and Discussion

### 3.1 Effect of storage on the acidity and pH of vitamin fortified yoghurt

It was observed that the acidity of vitamin A & D fortified yoghurt increased significantly ($p<0.05$) from 0.60% lactic acid on 0$^{th}$ day to 0.80 % on 7$^{th}$ day and increased remarkably to 1.10% on the 14$^{th}$ day (Fig. 1). Although the control sample with no nano emulsion added also showed a significant increase ($p<0.05$) in acidity but the increase was less as compared to optimised product as on14$^{th}$ day the maximum acidity reached was around 0.98percent lactic acid.

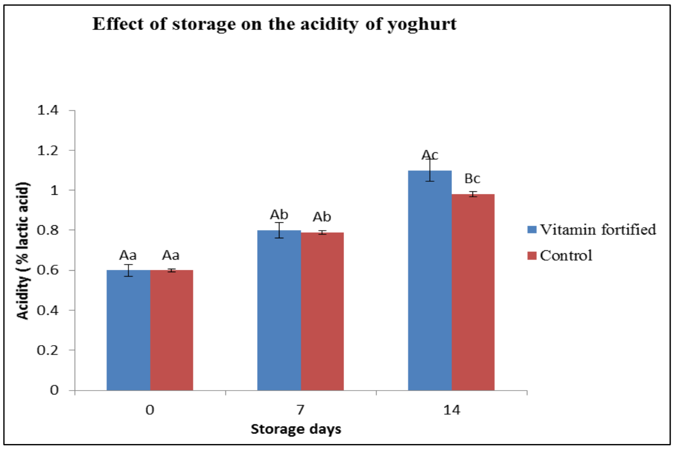


**Fig 1:** Effect of storage on the acidity of yoghurt (Values bearing Mean± S.E.) where, $^{A, B, C}$ superscripts represent significant difference due to storage days, $^{a, b, c}$ superscripts represent significant difference between the samples ($p<0.05$)

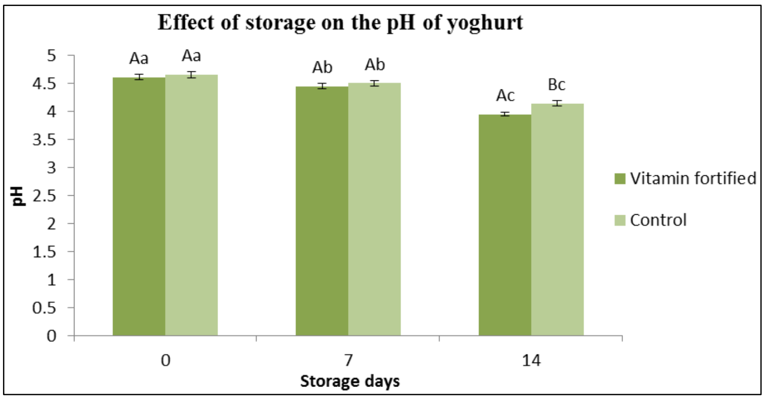


**Fig 2:** Effect of storage on the pH of yoghurt (Values bearing Mean± S.E.) where, A, B, C superscripts represent significant difference due to storage days, a, b, c superscripts represent significant difference between the samples ($p<0.05$)

The pH value also showed a significant decrease ($p<0.05$) from 4.61 ($0^{th}$ day) to 3.95 ($14^{th}$ day) for the vitamin fortified product and for control the pH value decreased from 4.65 ($0^{th}$ day) to 4.14 ($14^{th}$ day) (Fig. 2). This increase in acidity and decrease in pH could be due to addition of nanoemulsion containing protein and fat. However, change in acidity and pH is mainly attributed to formation of lactic acid by lactic acid bacteria. Therefore, increase in acidity and decrease in pH may be attributed to the higher number of microbial count in vitamin fortified yoghurt due to the presence of fat, proteins and vitamins. It has been reported by some researchers that the higher content of nutrients and vitamins leads to faster growth of the microbes (Abrahamsen and Rystaad 1991[9], Salvador and Fiszman 2004 [10]).

### 3.2 Effect of storage on the instrumental colour of vitamin fortified yoghurt

**L* value**: L* value for fortified yoghurt and control on 0, 7 and $14^{th}$ day were 90.22, 90.01, 89.49 and 90.11, 89.99, 89.77 respectively (Fig. 3). It was observed that with the increase in the storage days, there was no significant difference ($p>0.05$) obtained in the L* value for both vitamin fortified as well as the control sample. Also, no significant difference ($p>0.05$) in L* value was observed between the fortified sample and control. This showed that the storage as well as fortification had no effect on the brightness of the product when compared with the control.

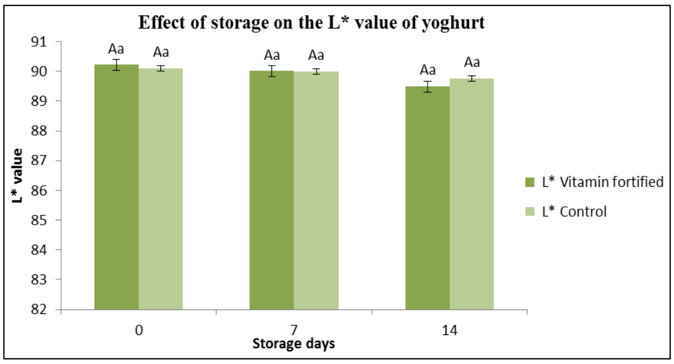


**Fig 3:** Figure represents the effect of storage on the L* value of yoghurt (Values bearing Mean± SE) where, a, b superscripts represent significant difference due to storage days and A, B superscripts represent significant difference due to different samples.

**B* value**: a* value for fortified yoghurt and control on 0, 7 and $14^{th}$ day were -1.67, -1.65, -1.63and -1.62, -1.59, -1.51 respectively (Fig. 4).There was no significant difference obtained in the a* value for the vitamin fortified product and control with the increase in the storage days except on $14^{th}$ day. The shift in colour was observed which might be due to the microbial growth and degradation of the components during storage.

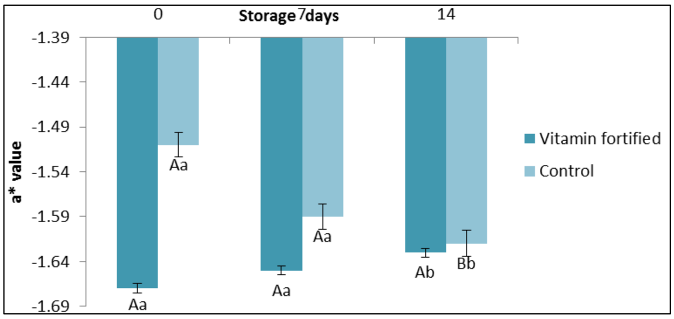


**Fig 4:** Figure representing the effect of storage on thea* value of yoghurt (Values bearing Mean± SE) where, a, b superscripts represent significant difference due to storage days and A, B superscripts represent significant difference due to different samples.

**B* value:** b* value for fortified yoghurt and control on 0, 7 and $14^{th}$ day were 10.98, 11.22, 11.38 and 11.73, 12.02, 12.12 respectively (Fig. 5). The b* value showed that there was a significant shift towards yellowish side in both vitamin fortified as well as control sample. This may be due to the increase in the microbial growth as well as degradation of different components of yoghurt which led to increase in the yellowness in both the samples.

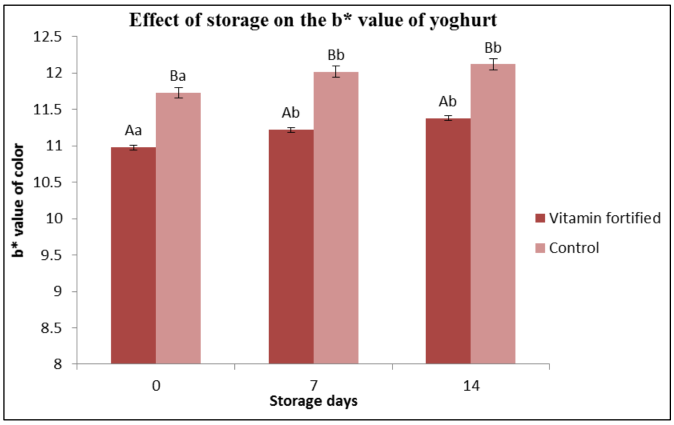


**Fig 5:** Figure representing the effect of storage on the b* value of yoghurt (Values bearing Mean± SE) where, a, b superscripts represent significant difference due to storage days and A, B superscripts represent significant difference due to different samples

## 4. Conclusion

The micronutrient deficiency is one of the prevailing issues across the world especially in children and women. Vitamin fortification in the products like yoghurt can be a lucrative idea to address this problem. Vitamin A and D fortified yoghurt was prepared using a novel approach of nanotechnology. During storage, the vitamin fortified yoghurt showed increase in acidity and decrease in pH. The acidity development in vitamin fortified sample was more as compared to the control sample which was attributed to increased microbial growth. However the color values (L*, a* and b*) in fortified samples were found comparable with the control sample except on the 14th day. Vitamin A and D fortified yoghurt prepared using nanotechnology was found comparable with the control.

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