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Shelf life of Vitamin D and carotene enriched yoghurt

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Abstract
Yoghurt is a fermented dairy product consumed by billions of people around the world. Food enrichment is thought to be a highly effective solution and among the most cost effective public health interventions currently available. The crystalline vitamin D the carotene were enriched in yoghurt. The optimized level of enrichment of vitamin D at the concentration of 1500 IU per litre of milk and carotene at the concentration of 15% per litre of milk were incorporated in yoghurt and subjected to sensory evaluation, texture analysis and microbial profile during storage up to 14 days at 5 °C. Further, the vitamin D and carotene enriched yoghurt samples were subjected to High Performance Liquid Chromatography (HPLC) to assess the retention of vitamin D and carotene in the enriched product during storage and found satisfactory. Hence, the developed Vitamin D and carotene enriched yoghurt will address the nutritional deficiency prevalent among the public and thereby improves the nutritional status.

Keywords: Yoghurt, Vitamin D, carotene enrichment, sensory evaluation, texture analysis microbial profile, shelf life

Introduction
Vitamin D deficiency is pandemic, yet it is the most under diagnosed and under treated nutritional deficiency in the world. Indian socio-religious and cultural practices do not facilitate adequate sun exposure, thereby negating potential benefits of plentiful sunshine. Consequently, subclinical Vitamin D deficiency prevails in epidemic proportions all over the Indian subcontinent, with a prevalence rate of 70-100 per cent in the general population (Ritu, and Gupta, 2014) [1].

Vitamin D promotes calcium absorption in the gut and maintains adequate serum calcium and phosphate concentrations to enable normal mineralization of bone and to prevent hypocalcaemia. It is also needed for bone growth and bone remodeling by osteoblasts and osteoclasts. Without sufficient Vitamin D, bones can become thin, brittle, or misshapen. Vitamin D sufficiency prevents rickets in children and osteomalacia in adults. Vitamin D is crucial for calcium homeostasis and musculoskeletal health. Adequate vitamin D status during adolescence might help to reduce the risk of osteoporotic fractures in later life. There is also finding of evidences linking vitamin D status with non-skeletal disorders including autoimmune disorders (Crohn's disease, multiple sclerosis, rheumatoid arthritis, and type 1 diabetes), infections, and risk of developing cancers of the breast, colon, prostate and ovaries (Holick, 2007) [2].

Vitamin A is essential for sight and cell differentiation. Deficiency of vitamin A results in night blindness and ultimately blindness, growth retardation, damage of mucous membrane, and reproductive disorders. Carotene supplementation of milk is very simple and easy to incorporate. The supplementation like iodine in salt, vitamin A in milk is being done in routine practice (Petrogianni *et al.*, 2014) [3].

Food enrichment is thought to be a highly effective solution and among the most cost effective public health interventions currently available. Yoghurt is a favorite dairy product for billions of people around the world and the producers constantly seek out ways of bringing new varieties for new eating occasions to be enjoyed anywhere and anytime. In order to redress the above mentioned issue, yoghurt is a logical vehicle for enrichment of vitamin D and Carotene.

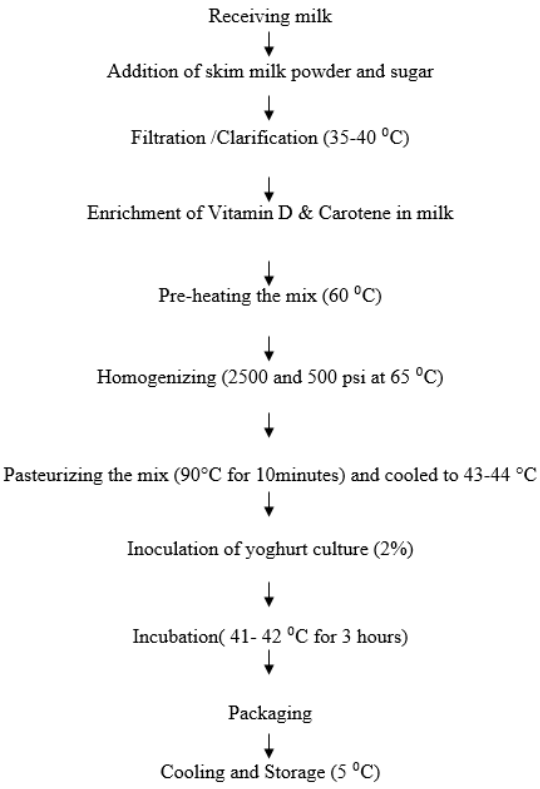
Hence, the resultant product will be immensely helpful in ensuring nutritional adequacy, bone health and optimal overall health of the consumers at large.

Materials and Methods

The study was carried out at the Department of Livestock Products Technology, Veterinary College and Research Institute, Orathanadu, Thanjavur District, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS). Fresh milk obtained from milking cross bred cows maintained at the Instructional Livestock Farm Complex, Veterinary College and Research Institute, Orathanadu was used for this study. Skim milk powder testing 5% moisture and 95% solubility was purchased from Tamil Nadu Co-operative Milk Producers' Federation (Aavin) and used to standardize the milk solids not fat content of ice cream. Commercially available good quality cane sugar was used in the preparation of yoghurt. Freeze dried DVS culture containing yoghurt bacteria *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus salivarius ssp. Thermophilus* (YC-XII) was obtained from Chr. Hansen, Denmark was used. Good quality carrot (*Daucus carota*) purchased from local market was used to obtain juice as a source of carotene in the preparation of yoghurt. Carrot roots were washed thoroughly and the juice was obtained by blending in blender with sieves.

The crystalline vitamin D_3 (40,000,000 IU/g) was procured from Sigma Aldrich (Saint Louis, MO, USA). Plate Count Agar (Himedia-M091A, Mumbai) was used for the enumeration of microorganisms in yoghurt by pour plate technique. Violet Red Bile Agar (Himedia-M049S, Mumbai) was used for the enumeration of coliforms in yoghurt by pour plate technique. Potato Dextrose Agar (Himedia-M096, Mumbai) was used for the enumeration of yeast and mould in yoghurt by pour plate technique. The guidelines prepared by IS: 12898(1989) [4] and the flow chart indicated by De (1980) [5] were followed in the preparation of yoghurt.

Flow chart for the preparation of Vitamin D & Carotene enriched yoghurt



The enriched yoghurt samples were evaluated by a semi trained panel of seven judges for the attributes of flavour, body and texture, colour and package, acidity and Overall acceptability scores on a 9- point hedonic scale (Singh *et al.*, 2014) [6]. The texture profile was assessed according to (Akalin *et al.*, 2008) [7]. The analysis was performed at 15 ºC using a TA.XT plus Texture Analyzer (Stable Micro System, United Kingdom). The yoghurt samples enriched with vitamin D and carotene was analysed by reversed phase High Performance Liquid Chromatography (HPLC) as described by (Lyan *et al.*, 2001) [8] at 0 day, 7 days and 14 days of storage.

Results and Discussion

The developed vitamin D_3 enriched yoghurt was assessed by sensory evaluation using the 9-point hedonic scale by a semi-trained panel of seven members, and the scores were presented in the following table.

Table 1: Optimizing the enrichment levels of crystalline vitamin D_3 in yoghurt by sensory evaluation using 9-point hedonic scale

Attributes	Control	T_1	T_2	T_3
Flavour	8.62±0.08 ^{b}	8.50±0.08^{b}	8.55±0.08^{b}	7.41±0.10^{a}
Body & texture	8.52±0.09^{c}	8.36±0.10^{bc}	8.10±0.06^{b}	7.29±0.12^{a}
Colour & Package	8.07±0.11	8.50±0.10	8.07±0.11	8.00±0.10
Acidity	8.38±0.10	8.50±0.11	8.36±0.11	8.21±0.12
Overall acceptability	8.60±0.09^{c}	8.38±0.09^{c}	8.14±0.09^{b}	7.45±0.09^{a}

Mean ± SE with different superscripts in a row differ significantly (p<0.05).
C - Control (unenriched)
T_1 - Treatment with 1000 IU/L Crystalline vitamin D_3
T_2 - Treatment with 1500 IU/L Crystalline vitamin D_3
T_3 - Treatment with 2000 IU/L Crystalline vitamin D_3
N = 42 for each treatment
Sensory scores based on 9-point hedonic scale, where 1: dislike extremely and 9: like extremely

ANOVA for optimizing the enrichment levels of crystalline vitamin D_3 in yoghurt by sensory evaluation

Attributes	Source of variation				
	Treatment			Error	
	D.F.	MSS	F-Value	D.F.	MSS
Flavour	3	11.022	33.341**	164	0.331
Body & texture	3	12.349	30.642**	164	0.403
Colour & Package	3	0.054	0.125	164	0.429
Acidity	3	0.329	0.724	164	0.447
Overall acceptability	3	11.149	35.275**	164	0.316

** Highly significant (p<0.01)

Statistical analysis revealed that there was significant difference (p<0.05) in flavour, body and texture and overall acceptability scores between control and treatments. There was no significant difference observed in acidity and overall acceptability. The enriched yoghurt samples with crystalline vitamin D_3 up to 1500 IU per litre of milk had better acceptability than 2000 IU per litre. Hence the yoghurt enriched with crystalline vitamin D_3 at the concentration of 1500 IU per litre of milk (T2) was selected for further studies.

The developed carotene enriched yoghurt was assessed by sensory evaluation using the 9-point hedonic scale by a semi-trained panel of seven members, and the scores were presented in in the following table.

Table 2: Optimizing the enrichment levels of carotene in yoghurt by sensory evaluation using 9-point hedonic scale

Attributes	Control	T_1	T_2	T_3
Flavour	8.67±0.07^c	8.33±0.09^b	8.14±0.09^b	7.45±0.10^a
Body & texture	8.52±0.08^c	8.29±0.10^{bc}	8.19±0.09^b	7.28±0.11^a
Colour & Package	8.07±0.10	8.07±0.09	8.07±0.11	8.00±0.10
Acidity	8.43±0.10	8.31±0.09	8.31±0.11	8.21±0.12
Overall acceptability	8.69±0.07^c	8.38±0.10^b	8.19±0.09^b	7.48±0.09^a

Mean ± SE with different superscripts in a row differ significantly (p<0.05).
C - Control (unenriched)
T_1 - Treatment with 10% carrot juice/L
T_2 - Treatment with 15% carrot juice/L
T_3 - Treatment with 20% carrot juice/L
N = 42 for each treatment
Sensory scores based on 9-point hedonic scale, where 1: dislike extremely and 9: like extremely

ANOVA for optimizing the enrichment levels of carotene in yoghurt by sensory evaluation

Attributes	Source of variation				
	Treatment			Error	
	D.F.	MSS	F-Value	D.F.	MSS
Flavour	3	11.022	33.341**	164	0.331
Body & texture	3	12.349	30.642**	164	0.403
Colour & Package	3	0.054	0.125	164	0.429
Acidity	3	0.329	0.724	164	0.447
Overall acceptability	3	11.149	35.275**	164	0.316

* Highly significant (p<0.01)

Statistical analysis revealed that there was significant difference (p<0.05) in flavour, body and texture and overall acceptability scores between control and treatments. There was no significant difference observed in acidity and overall acceptability. The enriched yoghurt samples with carotene up to 15% per litre of milk had better acceptability than 20% per litre. Hence the yoghurt enriched with carotene at the concentration of 15% per litre of milk (T_2) was selected for further studies.
Further, the optimized level of enrichment of vitamin D_3 at the concentration of 1500 IU per litre of milk and carotene at the concentration of 15% per litre of milk were incorporated in yoghurt and subjected to sensory evaluation using the 9-point hedonic scale by a semi-trained panel of seven members, and the scores were presented in in the following table.

Table 3: Sensory evaluation of vitamin d and carotene enriched yoghurt using 9-point hedonic scale

Sensory attributes	Control Yoghurt (Mean ± SE)	Enriched yoghurt (Vitamin D 1500 IU & Carrot juice 15%), (Mean ± SE)
Flavour	8.625±0.078	8.50±0.08
Body & texture	8.500±0.087	8.36±0.10
Colour & Package	8.100±0.112	8.50±0.10
Acidity	8.400±0.106	8.51±0.09
Overall acceptability	8.600±0.093	8.58±0.11

Statistical analysis revealed that there was significant difference (p<0.05) in flavour, body and texture and colour and package scores between control and enriched yoghurt while there was no significant difference observed in acidity and overall acceptability.
The optimized level of enrichment of vitamin D_3 at the concentration of 1500 IU per litre of milk and carotene at the concentration of 15% per litre of milk were incorporated in yoghurt and subjected to texture analysis and the results were presented in in the following table.

Table 4: Texture analysis of vitamin d and carotene enriched yoghurt

Texture parameters	Control Yoghurt (Mean ± SE)	Enriched yoghurt (Vitamin D 1500 IU & Carrot juice 15%), (Mean ± SE)
Firmness (g)	30.372±0.412	20.732±0.309
Consistency (g/sec)	499.217±21.124	495.105±22.951
Cohesiveness (g)	-12.116±1.142	-12.287±1.599
Viscosity index (g/sec)	2.198±0.924	2.005±1.568

The Vitamin D and Carotene enriched yoghurt samples were subjected to microbial analysis and there is no significant difference between control and enriched yoghurt up to 14 days during storage pertaining to total viable count. The coliforms and yeast and moulds were absent both in control and enriched yoghurt. Throughout the study period of 14 days as shown in the following table.

Table 5: Microbial count of vitamin d and carotene enriched yoghurt during storage

Storage period (in days)	Control yoghurt (Mean ± SE)			Vitamin D & Carotene enriched yoghurt (Mean ± SE)		
	Total Viable Count ($x10^3$) CFU/g	Coliform count CFU/g	Yeast and mould count CFU/g	Total Viable Count ($x10^3$) CFU/g	Coliform count CFU/g	Yeast and mould count CFU/g
0	3.4±0.17	Nil	Nil	3.7±0.10	Nil	Nil
7	6.3±0.30	Nil	Nil	6.4±0.20	Nil	Nil
15	55.0±1.00	Nil	Nil	56.0±1.00	Nil	Nil

The Vitamin D and Carotene enriched yoghurt samples were subjected to High Performance Liquid Chromatography (HPLC) to assess the retention of Vitamin D and Carotene in the enriched product during storage and found satisfactory.

Table 6: Stability of vitamin d and carotene enriched yoghurt by HPLC during storage

Parameter	Control Yoghurt (Mean ± SE)	Vitamin D & Carotene enriched yoghurt (Mean ± SE)		
		Day 0	Day 7	Day 14
Vitamin D (IU/L)	42.47±0.52	1477.53±2.55	1469.42±2.55	1452.36±4.15
Carotene (microgram/ml)	0.166±0.42	13.51±0.18	12.63±0.13	11.46±0.10

Conclusions

It is concluded that the yoghurt enriched with Vitamin D @1500 IU and natural beta carotene @15% in one litre of milk revealed better sensory acceptability, textural properties, microbial quality and also stability of Vitamin D and carotene during storage up to 14 days. The developed Vitamin D and

carotene enriched yoghurt will address the nutritional deficiency prevalent among the public and thereby improves the nutritional status.

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