Physicochemical and sensory characteristics of symbiotic fruit flavoured yoghurt fortified with calcium and vitamin D

Ayyavoo Preamnath Manoharan and C Ashokkumar

DOI: https://doi.org/10.22271/chemi.2020.v8.i3n.9339

Abstract
The present study aimed to evaluate the effect of Calcium and vitamin D on its physical and sensory characteristics of symbiotic drinkable fruit flavoured yoghurt. In present claim for foods with specific functional uniqueness and nutritional balance is increasing as well consumer demand. Work was carried out to analyze the effect of calcium and vitamin D inclusions with different levels of fruits yoghurt thus optimizing the composition of fortified symbiotic drinkable yoghurt. The moisture, protein, fat and ash contents were in increasing array as compared to control sample viz., 76.53 to 87.82, 3.21 to 3.97, 3.35 to 3.39 and 0.81 to 0.93 respectively. Sensory evaluation was conducted to assess the optimum inclusion levels of the above ingredients in the fortified symbiotic drinkable fruit flavoured yoghurt. The statistical analysis was done and significance of difference among the fortified symbiotic drinkable yoghurt were analysed.

Keywords: Yoghurt, symbiotic, calcium, vitamin D, Lactobacillus brevis etc.

1. Introduction
The global development is driven by a shift in consumption habits. People with busy lifestyles increasingly prefer to consume smaller meals on the go thus making dairy products in convenient packaging as an appealing option. Consumers also want more products that support a healthy lifestyle. A convenient, mobile breakfast or lunch should still be nutritionally balanced and beneficial. Therefore, every opportunity can be seized to produce fruit flavoured drinking yoghurts (mango and banana) including fructo-oligosaccharides and honey as prebiotic fortified with calcium and vitamin D that meet these needs.

Vitamin-D deficiency is an unrecognized epidemic among all age groups throughout the world and is now recognized as pandemic. It has been estimated that worldwide about 1 billion people have Vitamin D deficiency. Vitamin D is photosynthesized in the skin on exposure to UVB rays. Sun exposure alone ought to suffice for vitamin D sufficiency. However, vitamin D deficiency is widely prevalent despite plentiful sunshine even in tropical countries like India. In India, despite of ample sunlight (required for the synthesis of vitamin D endogenously), Vitamin D Deficiency prevalence has been documented to be in range of 50-90% among all the age groups. Studies from India also report a widespread prevalence of varying degrees of Vitamin D deficiency (Zahid Naeem, 2010) [17]. The Institute of Medicine (2012) recommended that vitamin D requirements are 400 IU/day in infants (0-1 year), 400 IU/day in children and adults (1-70 years), and 800 IU/day in elderly (800 IU/day). Calcium and vitamin D play important roles in bone homeostasis. Calcium is a major constituent of bones and a second messenger in cell signaling pathways. Obtaining sufficient calcium is important to decrease the risk of osteoporosis, hypertension, colorectal cancer, gastric cancer, nephrolithiasis, neurogenerative disease, and degenerative joint disease Fujita T (2000) [10], Keum et al., (2014) [15], Beto et al., (2015) [5]. The recommended daily allowance of calcium varies for different age groups and ranges from 700 to 1300 mg/day.

Akalin et al., (2007) [1] described that the processing of milk into yoghurt, the addition of FOS alone and storage for 28 days did not affect CLA (conjugated linoleic acid) isomer formation in yoghurts. Guarner et al., (2008) [12] reported that prebiotic compounds include fructo-oligosaccharides (FOSs) such as inulin, galacto-oligosaccharides (GOSs), lactitol and...
lactulose, among others. Fructo-oligosaccharides (FOSs) are among the most important natural prebiotics. It fructose components may reduce blood sugar and cholesterol levels and improve calcium absorption for healthy bone. FOSs has been added to different foods since 1990, resulting in improved mouth feel, creaminess, reduced fat, improved texture and increased viscosity especially in yogurt.

Gardiner (2002) [15] reported some Ca salts that had been widely used in food industry were calcium carbonate, calcium phosphate, calcium lactate, calcium gluconate, and calcium lactate gluconate. Calcium lactate gluconate (CLG) contains 12.74% calcium, has neutral taste, great bioavailability, and the best solubility characteristic compared to other calcium salts. Inadequate dietary calcium has long been associated with osteoporosis. Biancuzzo et al., (2010) [16] studied that Vitamin D2 and D3 found to be stable in fortified orange juice. Vitamin D is also essential for bone health. The Institute of Medicine (2012) recommended that vitamin D requirements are 400 IU/day in infants (0-1 year), 400 IU/day in children and adults (1-70 years), and 800 IU/day in elderly (800 IU/day).

2. Materials and Methods
Calcium (calcium citrate and calcium gluconate) was obtained from M/s, Hi Media Laboratories Pvt. Ltd., Mumbai, India. Vitamin D (Cholecalciferol) was purchased from Hi Media, Mumbai, India. Drinkable yoghurt mix was prepared to contain a final composition of 3.5% fat, 8.5% SNF and 5% sugar in the drinkable yoghurt, as per BIS (IS: 12898, 1989) specification (Sukumar De, 2015). In each treatment, mix was preheated to 60 °C and ingredients were homogenized as described in Arbuckle (1986) and then heated to 85 ºC for 30mins as suggested by Lee and Lucy (2010) [16]. Mixes were cooled to 42 ºC and inoculated with yoghurt culture and Lactobacillus brevis at the rate of 1 per cent each and incubated for 3 to 5 hours in the incubator or till the titrable acidity reaches 0.75 per cent and cooled to 20 to 25 ºC. The minerals and vitamins like calcium citrate, calcium gluconate and vitamin D were added at the rate of 1per cent, 1 per cent and 80 IU respectively with continuous stirring before cooling to a final temperature of 5 ºC. The different treatments carried out are shown in the following table. After cooling to 5 ºC the fortified symbiotic drinkable yoghurt was packed in sterile food grade in polypropylene (91.4x61 cm) bags and sealed with hand sealing machine and stored at 5 ºC for 7 days in refrigerator.

| TC | Control drinkable yoghurt (yoghurt culture + Lactobacillus brevis) |
|----|---------------------------------------------------------------------|
| T1 | Drinkable yoghurt + Calcium citrate (1%)                          |
| T2 | Drinkable yoghurt + Calcium citrate (2%)                          |
| T3 | Drinkable yoghurt + Calcium citrate (3%)                          |
| T4 | Drinkable yoghurt + Calcium gluconate (1%)                        |
| T5 | Drinkable yoghurt + Calcium gluconate (2%)                        |
| T6 | Drinkable yoghurt + Calcium gluconate (3%)                        |
| T7 | Drinkable yoghurt + Vitamin D (80 IU)                             |
| T8 | Drinkable yoghurt + Vitamin D (90 IU)                             |
| T9 | Drinkable yoghurt + vitamin D (100 IU)                            |

2.1 Estimation of calcium (Pirkul et al. (1997)) [18]
Calcium was measured by atomic absorption spectroscopy in a Perkin Elmer Analyst 200 spectrophotometer (Shelton, USA) using a hollow cathode lamp for calcium (422.7 nm) and an acetylene-air flame. Calcium standard solution (1000 mg L-1) (Sigma) was diluted with 2% (v/v) HNO3 (Sigma) to obtain working standards varying from 0.5 to 5 mg L-1. Instrumental parameters and sample preparation were made according to Boen et al., (2008) [19] with some modifications. The samples (~1.0 g) were mixed with 4 ml of 69% HNO3 and heated in a digestion block at 110 ºC for two hours. After cooling, 2ml of 69% HNO3 and 2 ml of 30% H2O2 were added to tubes containing the dehydrated fortified drinkable yoghurt samples, while 1ml of 30% H2O2 was added to tubes containing yoghurt. These samples were heated at 130 ºC for two hours. These conditions led to a complete mineralization of the samples. Mineralized samples were quantitatively transferred to volumetric flasks of 10 ml (yoghurt) and 25 ml (for dehydrated fortified drinkable yoghurt) and the volumes completed with ultra-pure deionized water (Ariumcomfort I ultra-pure water system, Sartorius, Gottingen, Germany). When necessary, samples were diluted with ultra-pure water to obtain absorbance readings between 0.1 and 0.2.

2.2 Estimation of vitamin D (Kazmi, 2007)
Cholecalciferol was evaluated by a method described by kazmi et al., (2007). Saponification was performed by mixing 1 g diluted drinkable yoghurt sample (1:3) with 0.5 ml 60% KOH before extraction by high-performance liquid chromatography (Shimadzu LC-10A, Shimadzu, Kyoto, Japan) using a C18 column (Water Spheri orb ODS2 10 µm 4.6 × 250 mm, Ireland). Methanol:acetonitrile:water (45:45:10) was used as a mobile phase. The sample injection volume was 50 µl. A flow rate of 1.0 ml/min was maintained throughout the test period (5 min, T = 26 ºC). The elution of vitamin D3 was detected at 254 and 228 nm on an ultraviolet detector at 2.7 min.

2.3 Sensory evaluation
The sensory assessments were conducted in the Department of Food Science and Technology laboratory with sensory panelist at College of Food and Dairy Technology, Koduvalli, Chennai. A sensory score card to evaluate colour and appearance, flavor, body and texture, sweetness, sourness and overall acceptability using 9 point hedonic scale was prepared and given to the sensory panelist.

3. Result and Discussion
3.1 Proximate characteristics of different types of symbiotic drinkable yoghurt
The proximate compositions of various types of fortified symbiotic drinkable yoghurt were shown in Table 1. The mean moisture, protein, fat and ash contents were in increasing array as compared to control sample viz., 76.53±0.003 to 87.82±0.004, 3.21±0.006 to 3.97±0.009, 3.35±0.002 to 3.99±0.005 and 0.81±0.001 to 0.93±0.006 respectively. The mean nitrogen free extract content drastically decreased compared to control from 16.10±0.017 to 4.17±0.008. This was in agreement with the findings of Pushkaraj Sawant et al., (2015) [19] who reported that the fresh yoghurt contained 3.21 per cent protein and 3.35 per cent fat. As the mango pulp contains lower fat than milk, the decrease is very apparent and understandable. These results were in accordance with findings of Sengupta et al., (2014).

The addition of fruit has caused the increase in protein content of yoghurt as proved by Amna Mahmood et al. (2008) [21]. As per the Codex standards (2003) for fermented milk, there should be a minimum of 2.7 per cent of milk protein and less than 10 per cent of milk fat. The fortified symbiotic yoghurt in the present study had met the Codex requirement.
3.2 pH value of different types of synbiotic drinkable yoghurt during storage

The pH value of various types of fortified synbiotic drinkable yoghurt was analyzed on during storage is shown in Table 2. The pH values of samples analyzed during 7 days of storage which is ranged viz., 4.65 to 4.52, 4.48 to 4.44, 4.49 to 4.43, 4.52 to 4.45, 4.38 to 4.36 of control, FFM, FHM, FFB and FHB respectively. The pH values decreased as the days of storage increased. This was in agreement to the requirements of IDF (1981) for fermented milk products. This range of pH observed in the present study was also in agreement with the findings of Hauly et al., (2005) who reported a final pH value of 4.63 in soy yoghurt manufactured with inulin. They also reported a decline in pH with increase in storage time. The results of the present study also showed a significant decrease in pH during storage and this was in agreement with the findings of Akalin et al., (2007) [1] who reported slightly increased titratable acidity and decreased pH values due to the inclusion of honey.

The range of pH values for FFB and FHB were observed as 4.52±0.409 to 4.45±0.005 and 4.38±0.006 to 4.36±0.005 for 0 to 7 days, respectively. This was in accordance with the values of 3.73 to 4.19 obtained by Stan and Popa (2013) for banana and apple smoothies, respectively.

The pH in the present study during storage at refrigeration temperature from 0 to 7 days varied from 4.48 ±0.405 to 4.44±0.405 for FFM, 4.49±0.405 to 4.43±0.405 for FHM, 4.52±0.005 to 4.45±0.405 for FFB and 4.38±0.005 to 4.36±0.405 for FHB. This was in accordance to the findings of Pirkul et al., (1997) [18] who also reported the pH range of 4.41 to 4.28 in varying inclusion of calcium salts to yoghurt.

3.3 Titrable acidity value of different types of synbiotic drinkable yoghurt during storage

The titratable acidity value of various types of fortified synbiotic drinkable yoghurt was analyzed on during storage is shown in Table 3. The titratable acidity values of samples analyzed during 7 days of storage which is ranged viz., 0.72 to 0.83, 0.92 to 1.014, 0.92 to 1.02, 0.83 to 0.87, 0.87 to 1.01, of control, FFB, FHM, FFB and FHB respectively. In general the mean titratable acidity was lower at the earlier days of storage and increased slowly. It is evident that titratable acidity was highest in different types of drinkable yoghurt over the increasing period of storage (from 0 to 7 days) at refrigeration temperature. Blending of yoghurt drink with increased level of pulp increased titratable acidity, similar to the total solids. The reason behind the increase of acidity was the acidic nature of mango pulp. Similar results have also been reported by Chougrani et al., (2009).

The mean acidity values of the samples during storage at refrigeration temperature varied from 0.72±0.043 to 0.83±0.021 per cent, 0.92 ±0.043 to 1.01±0.043 per cent, 0.92±0.043 to 1.02 ±0.005 per cent, 0.83±0.005 to 1.00±0.043 and 0.87±0.43 to 1.01±0.005 for control, FFM, FHM, FFB and FHB, respectively. This was in agreement with Pirkul et al., (1997) [18], also reported a similar increasing trend in acidity of control and calcium lactate fortified plain yoghurt during storage. The range of acidity values throughout storage in the present study for control and different types of fortified synbiotic drinkable yoghurt complied with the consumer acceptance and preference. Acidity values of control, FFM, FHM, FFB and FHB samples obtained in the present study were more than 0.31 to 0.29 per cent for a mixture of sapota, pomegranate and grape drinkable yoghurt as reported by Balaswamy et al., (2013). However, the values in the given table were in accordance to the range of 0.528 and 0.984 per cent/100g for banana and apple based drinkable yoghurt as observed by Stan and Popa (2013).

3.4 Viscosity (cP) value of different types of synbiotic drinkable yoghurt during storage

The Viscosity value of various types of fortified synbiotic drinkable yoghurt was analyzed on during storage is shown in Table 4. The Viscosity values of samples analyzed during 7 days of storage which is ranged viz., 125.14 to 122.40, 195.13 to 192.42, 232.08 to 230.09, 299.38 to 296.13 and 388.60 to 337.10, of control, FFM, FHM, FFB and FHB respectively. In general the mean viscosity values of FHB sample were higher than the mean viscosity values of the other treatment samples. The viscosity values decrease as the days of storage increased. It is evident that viscosity was highest during zeroth day and decreased during storage at refrigeration temperature for all the treatments. According to European patent standards, the viscosity of drinkable yoghurt at 10°C should be in the range of 50 to 400 centipoise (cP) and the viscosity estimates of developed yoghurts fall within this range. The decreased viscosity of fortified synbiotic drinkable yoghurt in the present study was in accordance with the findings of Serafettin and Ihsan (2003) where in addition of mulberry pekmez (concentrated juice) led to an increase in the fermentation time and a decrease in the viscosity of yoghurt.

**Table 1: Proximate compositions of different types of synbiotic drinkable yoghurt® (Mean± SE)**

| Treatments | Moisture | Protein | Fat | Ash | Nitrogen Free Extract |
|------------|----------|---------|-----|-----|-----------------------|
| Control    | 76.53±0.003 | 3.21±0.006 | 3.35±0.002 | 0.81±0.001 | 16.10±0.017 |
| FFM        | 87.61±0.004 | 3.91±0.002 | 3.37±0.002 | 0.89±0.07 | 4.22±0.008 |
| FHM        | 87.82±0.004 | 3.71±0.009 | 3.39±0.005 | 0.91±0.006 | 4.17±0.008 |
| FFB        | 87.41±0.005 | 3.97±0.009 | 3.35±0.008 | 0.93±0.006 | 4.34±0.007 |
| FHB        | 87.57±0.009 | 3.82±0.015 | 3.36±0.009 | 0.95±0.006 | 4.30±0.009 |

*Average of eight trials

**Table 2: pH value of different types of synbiotic drinkable yoghurt on storage ® (Mean± SE)**

| Treatments | Storage period in days | 0th day | 3rd day | 6th day | 9th day | Overall average |
|------------|------------------------|--------|--------|--------|--------|----------------|
| Control    | 4.65±0.405            | 4.61±0.405 | 4.55±0.405 | 4.52±0.405 | 4.59±0.194 |
| FFM        | 4.48±0.405            | 4.78±0.405 | 4.45±0.405 | 4.44±0.405 | 4.46±0.194 |
| FHM        | 4.49±0.405            | 4.47±0.405 | 4.46±0.405 | 4.43±0.405 | 4.46±0.194 |
| FFB        | 4.52±0.405            | 4.49±0.405 | 4.47±0.405 | 4.45±0.405 | 4.48±0.184 |
| FHB        | 4.38±0.405            | 4.37±0.405 | 4.37±0.405 | 4.36±0.405 | 4.37±0.194 |

*Average of eight trials
3.5 Sensory values of various levels calcium citrate (CaC) fortified drinkable yoghurt
The mean sensory values of Calcium citrate added synbiotic drinkable yoghurt sample viz., one, two and three per cent were shown in Table 5. The overall average score for the control one, two and three per cent inclusion of calcium citrate were 6.91, 7.36, 6.66 and 6.49 respectively. From the table it may be noticed that the inclusion level of one per cent had the maximum overall score and was statistically similar to that of control samples. Hence the level of one per cent of calcium citrate was considered as optimum. Selgas et al., (2009) [20] revealed that the calcium salts at any form can be included at the lowest level which gives better sensory attributes and in the present study also one per cent inclusion level of calcium citrate in the preparation of fortified synbiotic drinkable yoghurt was found to be optimum and scored higher on sensory attribute than 2 and 3 per cent inclusions (Table 5).

3.6 Sensory values of various levels calcium gluconate (CaG) fortified drinkable yoghurt
The mean sensory values for fortified synbiotic drinkable yoghurt prepared by inclusion of calcium gluconate viz., one, two and three per cent were shown in Table 6. The overall average scores for the control, one, two and three per cent inclusion of calcium gluconate were 6.91, 7.18, 6.42 and 6.12 respectively. From the table it may be noticed that the inclusion level of one per cent had the maximum overall score and was statistically similar to that of control sample. Hence the level of one per cent of calcium gluconate was considered as optimum. In the present study one per cent inclusion level of calcium gluconate in the preparation of fortified synbiotic drinkable yoghurt was found to be optimum and scored maximum on sensory attribute. Similar to the present findings, Yonis et al., (2013) [21] revealed that 1 to 2 per cent fortification of calcium gluconate in banana stirred yoghurt scored maximum without any negative influence on the sensory properties. As stated by Black et al., (1996) [7], 82% of patients with osteoporosis were taking less than the recommended daily intake (1000mg) of calcium. This highlights the need for calcium supplementation in all patient populations. In fact, patients with osteoporosis are more likely to have a history of inadequate dietary calcium intake (NIH 1994).

3.7 Sensory values of various levels vitamin D fortified drinkable yoghurt
The mean sensory values of vitamin D added synbiotic drinkable yoghurt sample viz., one, two and three per cent were shown in Table 7. The overall average scores by three levels of inclusion namely 80IU, 90IU and 100IU were 7.01, 6.86 and 6.72, respectively. The control sample had overall average score of 6.91 and all sensory characters were not statistically different from the sample prepared with 80IU vitamin D (Table 7). The results of the study showed that the vitamin D can be added with the synbiotic drinkable yoghurt mix at rate of 80IU.
It appears that vitamin D supplementation with calcium may prove more beneficial in reducing falls and increasing bone density in elderly women who have suffered a hip fracture (Harwood et al., 2004) [11]. This vitamin is generally added at levels of 40-400 IU per serving of the fortified food and it has been estimated that people cannot earn <2µg (80 IU)/day of vitamin D dietary intake (Calvo et al., 2004) [8]. In the present study as seen in Table 7, 80 IU inclusion level of vitamin D into yoghurt for the preparation of fortified synbiotic drinkable yoghurt was found to be optimum.

3.8 Calcium content of different types of synbiotic drinkable yoghurt
The mean sensory values of Calcium added synbiotic drinkable yoghurt sample viz., one, two and three per cent were shown in Table 8. The value for control was 0.133, for FFM the value was 1.212 for FHM the values was 1.227 for FFB the value was 1.016 for FHB the value was 1.015 respectively. The results of the present study were in agreement to the results of Singh and Muthukumarappan (2008) [22] who reported the calcium level in yoghurt as131.66mg/100g.

Table 3: Titrable acidity value of different types of synbiotic drinkable yoghurt on storageθ (Mean± SE)

| Treatments | Storage period in days | 0th day | 3rd day | 5th day | 7th day | Overall Average |
|------------|------------------------|---------|---------|---------|---------|-----------------|
| Control    |                        | 0.72±0.043 | 0.74±0.043 | 0.78±0.043 | 0.83±0.043 | 0.77±0.021 A |
| FFM        |                        | 0.92±0.043 | 0.95±0.043 | 0.97±0.043 | 1.01±0.043 | 0.96±0.021 C |
| FHM        |                        | 0.92±0.043 | 0.95±0.043 | 0.98±0.043 | 1.02±0.043 | 0.96±0.021 C |
| FFB        |                        | 0.83±0.043 | 0.84±0.043 | 0.86±0.043 | 1.00±0.043 | 0.96±0.021 C |
| FHB        |                        | 0.87±0.043 | 0.87±0.043 | 0.89±0.043 | 1.01±0.043 | 0.91±0.021 NC |
| Overall Average |                  | 0.85±0.19 a | 0.87±0.19 b | 0.90±0.19 A b | 0.95±0.19 b |
θ Average of eight trials

Table 4: Viscosity (cP) value of different types of synbiotic drinkable yoghurt on storageθ (Mean± SE)

| Treatments | Storage period in days | 0th day | 3rd day | 5th day | 7th day | Overall Average |
|------------|------------------------|---------|---------|---------|---------|-----------------|
| Control    |                        | 125.14±17.07 | 124.07±17.07 | 123.91±17.07 | 122.40±17.07 | 123.88±8.19 C |
| FFM        |                        | 195.13±17.07 | 194.76±17.07 | 192.52±17.07 | 192.42±17.07 | 193.71±8.19 B |
| FHM        |                        | 232.08±17.07 | 232.60±17.07 | 231.10±17.07 | 230.09±17.07 | 231.84±8.19 C |
| FFB        |                        | 299.38±17.07 | 298.52±17.07 | 297.65±17.07 | 296.13±17.07 | 297.95±8.19 B |
| FHB        |                        | 388.60±17.07 | 340.70±17.07 | 339.20±17.07 | 337.10±17.07 | 351.40±8.19 B |
| Overall Average |                  | 237.34 ±7.33 a | 237.54 ±7.33 a | 222.28 ±7.33 a | 236.10 ±7.33 a |
θ Average of eight trials
1. A study was conducted for the development of fortified synbiotic drinkable yoghurt prepared by incorporating fruit pulp with 10 percentages is the highly acceptable product. All the other combinations remained acceptable till the 7th day of storage and hence commercialization of all these products will help in utilizing various perishable seasonal fruits into nutrient rich value added products which in turn would balance the economic aspects. Hereby the drinkable yoghurt might act as a good source of calcium, Vitamin D and other nutrients. The incorporation of fruits also enhances the flavour of yoghurt establishing the need to optimize the level of inclusion of these fruits in yoghurt. This study was carried out to analyze the effect of inclusion of different levels of fruits and prebiotic into yoghurt thus optimizing the composition of fortified synbiotic drinkable yoghurt.

5. References
1. Akalin AS, Tokoşoglu O, Gońc S, Aycan S. Occurrence of conjugated linoleic acid in probiotic yoghurts supplemented with fructooligosaccharide. International Dairy Journal. 2007; 17(9):1089-1095.
2. Amna Mahmood, Abbas N, Gilani AH. Quality of stirred buffalo milk yoghurt blended with apple and banana fruits. Pak. J Agric. Sci. 2008; 45(2):275-279.
3. Arbuckle WS. Ice cream, 2nd Edn. The AVI Publishing Co., West port, Conn, 1977.
4. Balasawmy K, Prabhakara Rao PG, Nagender A, Rao GN, Sathiya Mala K, Jyothirmayi T et al. Development of smoothies from selected fruit pulps/juices. International Food Research Journal. 2013; 20(3):1181-118.
5. Beto JA. The role of calcium in human aging. Clin Nutr. 2015; 41:1-8.
6. Biancuzzo RM, Young A, Bibuld D, Cai MH, Winter MR, Klein EK et al. Fortification of orange juice with vitamin D2 or vitamin D3 is as effective as an oral supplement in maintaining vitamin D status in adults. The American journal of clinical nutrition. 2010; 91(6):1621-1626.
7. Black DM, Cummings SR, Karpf DB. Fracture Intervention Trial Randomized trial of effect of alendronate on risk of fracture in women with existing vertebral fractures: the Fracture Intervention Trial. FIT Research Group. Lancet. 1996; 348:1535-41.
8. Calvo MS, Whiting SJ, Barton CN. Vitamin D fortification in the United States and Canada: current status and data needs. The American journal of clinical nutrition. 2004; 80(6):1710S-1716S.
9. Chougurani F, Cheriguene A, Bensoltane A. Sensorialand Physico-Chemical Characteristics of Yoghurt Manufactured with Ewe’s and SkimMilk”. World Journal of Dairy and Food Sciences. 2009; 4(2):136-140.
10. Fujita T. Calcium paradox: Consequences of calcium deficiency manifested by a wide variety of diseases. J Bone Miner Metab. 2000; 18:234-6.
11. Gardiner GE, Ross RP, Kelly PM, Stanton C, Collins JK, Fitzgerald G. Microbiology of therapeutic milks. Dairy microbiology handbook. The microbiology of milk and milk products, 2002, 431-478.
12. Guarnier F, Khan AG, Garisch J, Eliakim R, Gangl A, Thomson A et al. World Gastroenterology Organisation
Practice Guideline: Probiotics and Prebiotics-May (2008): guideline. South African Gastroenterology Review. 2008; 6(2):14-25.

13. Harwood RH, Sahota O, Gaynor K. The Nottingham Neck of Femur (NONOF) Study. A randomized, controlled comparison of different calcium and vitamin D supplementation regimens in elderly women after hip fracture: The NONOF study. Age Ageing. 2004; 33:45-51.

14. Hauly MC, Fuchs RH, Prudencio-Ferreira SH. Review of Nutrition. 2005; 18(5):613-622.

15. Keum N, Aune D, Greenwood DC, Ju W, Giovannucci EL. Calcium intake and colorectal cancer risk: Dose-response meta-analysis of prospective observational studies. Int J Cancer. 2014; 135:1940-8.

16. Lee WJ, Lucey JA. Formation and Physical Properties of Yoghurt. Asian-Australian Journal of Animal Science 2010; 23(9):1127-1130.

17. Naeem Z. Vitamin D deficiency—an ignored epidemic. International journal of health sciences. 2010; 4(1):5-6.

18. Pirkul T, Temiz A, Erdem YK. Fortification of yogurt with calcium salts and its effect on starter microorganisms and yogurt quality. Int. Dairy J. 1997; 7:547-552.

19. Sawant P, Kumar D, Patil V, Ingale Y, Sawant D. Physico-chemical, Sensory and Microbial Quality of Yoghurt Drink Fortified with Pineapple Pulp. International Journal of Food and Fermentation Technology. 2015; 5(1), 59.

20. Selgas MD, Salazar P, García ML. Usefulness of calcium lactate, citrate and gluconate for calcium enrichment of dry fermented sausages. Meat science. 2009; 82(4):478-480.

21. Boeni S, Pourahmad R. Use of inulin and probiotic lactobacilli in synbiotic yogurt production. Scholars Research Library. 2012; 3(7):3486-3491

22. Singh G, Muthukumarappan K. Influence of calcium fortification on sensory, physical and rheological characteristics of fruit yogurt. LWT-Food Science and Technology. 2008; 41(7):1145-1152.

23. Stan A, Popa ME. Research on the correlation between physico-chemical, sensory analysis of smoothie type products and consumer preferences. Scientific Bulletin. Series F. Biotechnologies. 2013; 17(1):193-197.

24. Sukumar De. Outlines of dairy technology. Published by Oxford University Press, New Delhi. 2008, 183 - 219.

25. Yonis AAM, elzamzamy FM, Shimmaelmorsi A. Fortification of banana stirred yogurt with calcium. J Food and Dairy. Mansoura univ. 2013; 4(5):183-192.