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RESEARCH ARTICLE

# Probiotic evaluation studies and elemental composition of iron-fortified sweet corn milk-based probiotic yoghurt

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**Abstract**: Yoghurt is one of the important fermented milk products with numerous nutrition and health benefits but has low iron content. To further improve the nutritional value sweet corn milk was incorporated at 30% along with cow milk and ferrous lactate for the development of iron-fortified probiotic sweet corn blend milk yoghurt. The premix was fermented with 2% probiotic cultures: *Streptococcus thermophilus MTCC 1938 and Lactobacillus acidophilus MTCC 447* at 42°C for 4h. It was stored for 16 days under refrigerated conditions. Food grade ferrous lactate hydrate salt was used at different concentrations like 10mg and 20 mg per 100 ml of premix for fortification. It proved the addition of ferrous lactate had not affected yoghurt characteristics. The elemental composition analysis was done using an X-ray fluorescence spectrophotometer. 20 mg ferrous lactate fortified probiotic sweet corn blend milk yoghurt remained acceptable even at 16 days of storage. This iron-fortified probiotic yoghurt serves as a potential fermented milk product for commercial use.

**Keywords**: Cow milk (4.5%); Ferrous lactate, Fortification; *Lactobacillus acidophilus;* Sweet corn; *Streptococcus thermophilus,*

## Introduction

Yoghurt as a health food has attracted the attention of the middle class in India because of increased disposable income and better health-benefit awareness. Yoghurt prepared with probiotic cultures is being commercialized in the market with health promoting and gut health improving aspects. This has been proven to promote gut microbiota with numerous therapeutic benefits like cholesterol-lowering effect, increase in the bioavailability of minerals and treatment of gut-related aliments (Hadjimbei et al. 2022).

Daily iron requirement of human may vary based on age and gender. It could be summarized as 8 to 18 mg of iron may be required for humans. For example, females of age 18 and above may require 18 gms of iron whereas males of same age group may require 8 gms of iron. Since iron fortified yoghurt is having 20 gms of iron per 100 ml, it is recommended that females could take 100 ml of iron fortified yoghurt per day whereas males could consume 50 ml per day (NIH, 2023). As cow milk has lower iron content food grade ferrous hydrate can serve as a potential iron fortifier in yoghurt. Food fortification with iron has been recommended as one of the preferred approaches for preventing and eradicating iron deficiency. However, fortification with bioavailable iron sources often presents multiple challenges in product acceptance, product shelf life, and effectiveness in improving iron status. In developing an effective iron fortification technology, it is critical that the chemical property of iron that contributes to the development of undesirable organoleptic properties is taken into consideration (Banjare et al. 2019). It is well known that two major off-flavors may be associated with fortified dairy products: oxidized flavor resulting from catalysis of lipid oxidation by iron and metallic flavor contributed by iron salts. No oxidative rancidity had been detected in freshly prepared and stored samples of yoghurt whereas iron fortified yoghurt showed slight rancidity due to different sources of iron.

Further, sweet corn milk is being used as a supplement or replacer for many milk-based products. This would serve as alternative vegetable milk. Sweet Corn *(Zea mays L.* ssp. *saccharata)* is one of the largest vegetable crops grown. Primary interest has been directed to carbohydrates, since in the milky stage when the grain is harvested for food use, carbohydrates determine flavor and texture (Kokkinidou et al. 2019). Several workers have investigated animal milk or soy-milk yoghurts, but little work has been done on corn–milk yoghurt. Production of yoghurt from

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corn milk was aimed to combine the good sensory characteristics of the corn milk with the well-known yoghurt flavor.

It was reported that the sensory quality of iron-fortified dairy foods has been shown to be affected by the type of iron used, the amount of iron added and the properties of dairy products being fortified (Amira et al. 2011 and Hurrell, 2021). Hence this study was taken up to formulate iron-fortified sweet corn milk incorporated yoghurt with *Streptococcus thermophilus MTCC 1938 and Lactobacillus acidophilus MTCC 447* and analyzed for viability and acid tolerance during storage.

## Materials and Methods

### Materials

Milk (4.5% fat), sweet corn and skim milk powder were obtained from the local market. *Streptococcus thermophilus MTCC 1938 and Lactobacillus acidophilus MTCC 447* were purchased from Centre for Food Technology, Anna University, Chennai. *Lactobacillus* MRS agar for *Lactobacillus acidophilus* and M17 media for *Streptococcus thermophilus* were obtained from Hi-Media and were used for microbial analysis. Food grade ferrous lactate hydrate was obtained from Sigma Aldrich and was used for fortification.

### Preparation of Corn milk

To prepare the corn milk, the corn cobs were firstly husked, the silks removed and washed with water. The seeds were then separated from the cleaned cobs using knives. The corn seeds were ground using a grinder. 50 ml of water was added to 100 g of corn seeds during grinding. The slurry was then filtered using a filter to produce a milky liquid. The corn milk was heated to 80°C for 10 min and stored at -18°C until use. Two types of corn were used in this study. Both corn milk and sweet corn milk were prepared by this method.

### Stock culture preparation

The Slant cultures of *Streptococcus thermophilus MTCC 1938 and Lactobacillus acidophilus MTCC 447* were grown by inoculating into M17 broth and MRS medium respectively for 18 h at 37 °C. One loop of each culture was transferred into 10 ml of litmus milk prepared by mixing 16% (w/v) skim milk powder (SMP) and 0.3% (w/v) yeast extract. The inoculated culture was incubated for 18 h at 37 °C and stored at 5 °C until use.

### Mother culture

An individual mother culture was freshly prepared before conducting the experiment by inoculating one loop of stock culture into 100 ml of sterilized milk medium containing 16% (w/v) SMP and 0.1% (w/v) yeast extract. The inoculated culture was incubated at 37°C for 18 h and kept at 5°C until use.

### Preparation of Iron-fortified Sweet corn milk supplemented yoghurt

In preparation of control yoghurt 100% cow milk and unfortified sweet corn blend milk yoghurt of 70% cow milk and 30% sweet corn milk were used using the below-mentioned method (Fig 1) as described in Geetha et al (2018). Ferrous lactate hydrate of food grade at 10, 20 and 30 mg was incorporated as an iron fortifier. The yoghurt containing 30 mg of ferrous lactate resulted in high whey syneresis, hence it was not considered for optimization. Control = 100 % cow milk, Sample 1 = cow milk: sweet corn milk (70:30) ml, Sample 2 = cow milk : sweet corn milk (70:30) ml + 10 mg Ferrous lactate, Sample 3 = cow milk: sweet corn milk (70:30) ml + 20 mg Ferrous lactate was taken for studies.

### Probiotic evaluation studies

Viable count and acid tolerance tests were done using methods stated in B.I.S.2002 and ICMR-DBT 2011. Analysis was carried out on 0,4,8,12 and 16$^{th}$ days of storage.

### Elemental composition analysis

The mineral composition of yoghurt samples was determined by using the X-ray fluorescence spectrophotometer (Model: Minipal 4 Benchtop XRF, Elemental range : Al…Y, Pd…U, Size : 300x550x450 mm$^3$, Fine focus X-ray tube with MO Target, Multilayer monochromator 17.5 Kev) . All the yoghurt samples were individually freeze-dried in a freeze-dryer (CRYO Technologies, Chennai) to obtain a fine powder. The powdered samples were placed in separate compartments in the XRF analyzer. The elemental analysis was done and results were obtained in graphical representation.

## Results and Discussion

### Probiotic evaluation analysis of yoghurt

#### *Viable count of probiotic organisms in yoghurt during 16 days of storage studies*

The viability of both the strains decreased as the days of storage increased in all the samples along with the control sample (Fig 2). The % viability of *Lactobacillus acidophilus* of control samples were 94.95 %, 87.90%, 84.01% and 78.13% on 4$^{th}$, 8$^{th}$, 12$^{th}$ and end of 16$^{th}$ day of storage respectively. The 20 mg of iron-fortified corn milk yoghurt showed 92.6%, 88.5%, 83.06% and 79.93% of viability on 4$^{th}$, 8$^{th}$, 12$^{th}$ and end of 16$^{th}$ day of storage respectively. Incorporation of elemental iron did not show any influence on the survival of *Lactobacillus acidophilus* in yoghurt during 16$^{th}$ days of storage. The viable count showed colonies greater than $10^9$ CFU/ml during 16 days of storage studies. However the values reported by Codex stated that lactobacilli in yoghurt should be in the range of $10^7$ ( (Lopez, 2014 and CODEX STAN, 2011).

The % viability of *Streptococcus thermophilus* in control samples was 96.3%, 92.43%, 88.42% and 86.31% on 4th, 8th, 12th and end of 16th day of storage respectively. The 20 mg of iron-fortified corn milk yoghurt showed 93.33%, 88.32%, 86.22% and 81.65% on 4th, 8th, 12th and end of 16th day of storage respectively. Incorporation of elemental iron did not show any influence on the survival of *Streptococcus thermophilus* in yoghurt during 16th days of storage. The viable count showed colonies greater than $10^9$ CFU/ml during 16 days of storage studies. Many studies reported that the iron fortification in yoghurt and dairy foods and their impact on sensory qualities and survivability of yoghurt bacteria (Azzam, 2009). There is a lack of studies about iron fortification in probiotic yoghurt and its effect on the viability of probiotics. Many of the studies reported that the viability of the starter microflora as well as probiotic was not affected by the iron fortification in yoghurt (Dabour et al. 2019).

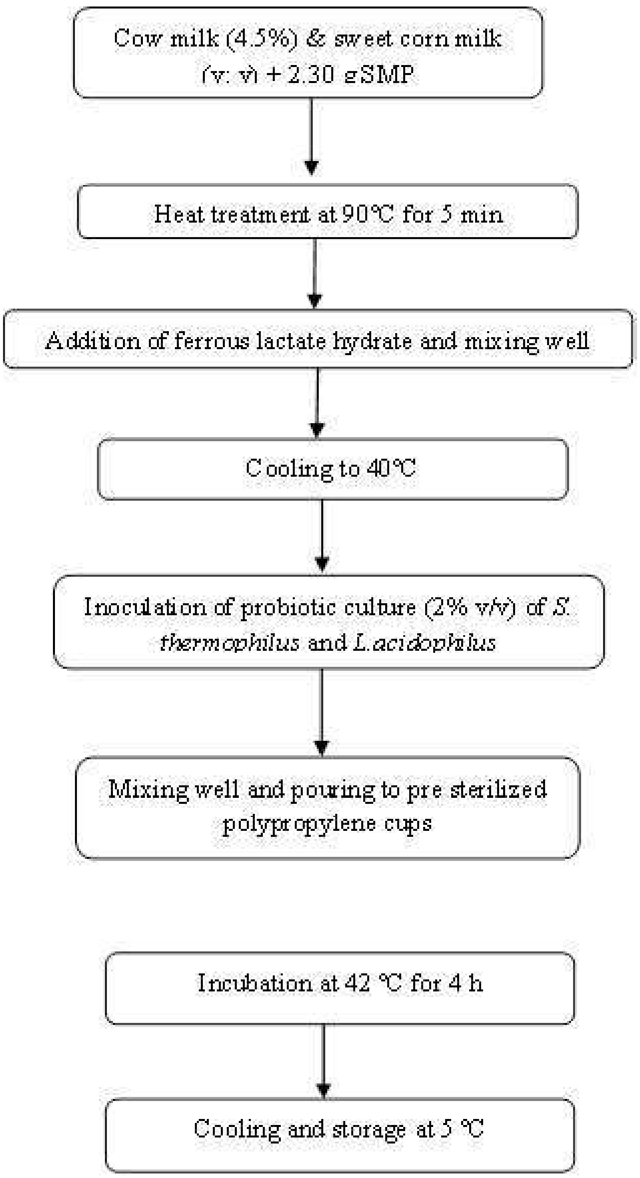


**Fig 1.** Optimized flow diagram for preparation of ferrous lactate fortified sweet corn blend milk yoghurt

**Acid tolerance test for probiotic organisms in yoghurt**

The number of cell counts remained significantly unchanged during all the interval transit at pH 3 and pH 7.2. Good acid tolerance properties exhibited by the bacteria are closely related to their strains specification as they are always strains dependent (Huang and Adams, 2004; Lin et al. 2006). The % viability of *Lactobacillus acidophilus* was 93.25 % and 97.21% at pH 3 and 7.2 after 3 hrs incubation in 20 mg of iron-fortified corn milk yoghurt respectively. Viability counts of the bacteria usually decline tremendously when exposed to simulated gastric juice of pH 1.5 after an incubation period of 3 hours. The threshold point to determine acid resistance was set at a pH value of 3.0 and an incubation period of 3 hours in the *in vitro* studies as it simulates the residence time in the stomach (Prasad et al. 1998; Haddadin et al. 2008). The developed 20 mg of iron fortified corn milk yoghurt has proven to be successful to meet the minimum requirement of $10^6$ viable probiotic cells per ml at pH 3 after exposure for 3 hours (Sahadeva et al. 2011) (Fig 3) FAO/WHO (2002). The % viability of *Streptococcus thermophilus* was 93.29% and 90.21% at pH 3 and 7.2 after 3 hrs incubation in 20 mg of iron-fortified corn milk yoghurt. From the graph, *S. thermophilus and L. acidophilus* strains showed acid tolerance at pH 7.2 and 3.0 after 3 hours of incubation. Many studies supported that bioavailability of iron was good in yoghurt (Drago and Valencia 2002).

**Elemental composition analysis of yoghurt**

The X-ray fluorescence analysis (XRF) of milk and dairy products has not yet become widespread in dairy industry, although the method has a great potential, as the dried samples can be analyzed directly without any chemical treatment and XRF equipment is rather accessible (Galina Pashkova, 2009).

An X-ray photon of sufficient energy strikes an atom; it dislodges an electron from one of its inner shells (K in this case). The atom fills the vacant K shell with an electron from the L shell; as the electron drops to the lower energy state, excess energy is released as a $K_a$ X-ray. The atom fills the vacant K shell with an electron from the M shell; as the electron drops to the lower energy state, excess energy is released as a $K_b$ X-ray. The emission of fluorescence is specific for individual elements. The graphical representation of this electron transition within the orbital is given by concentration cps vs. Kev (energy)

Based on the standard observations made on different elements (Van Grieken, & Markowicz, 2001),

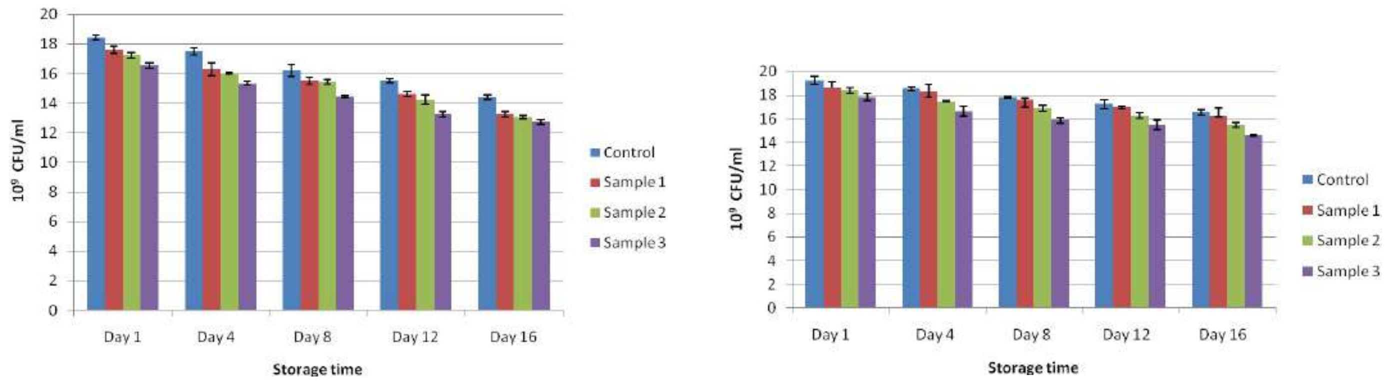


**Fig. 2** Viable count of *Lactobacillus acidophilus* and of *Streptococcus thermophilus* in yoghurt during 16 days of storage studies

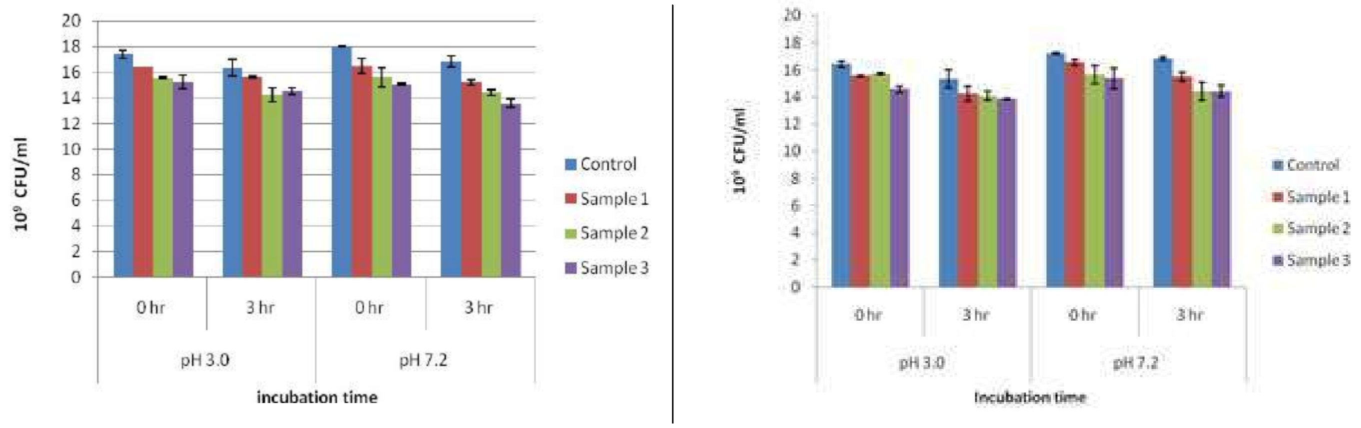


**Fig.3.** Acid tolerance results of *L. acidophilus* and *S. thermophilus* in yoghurt

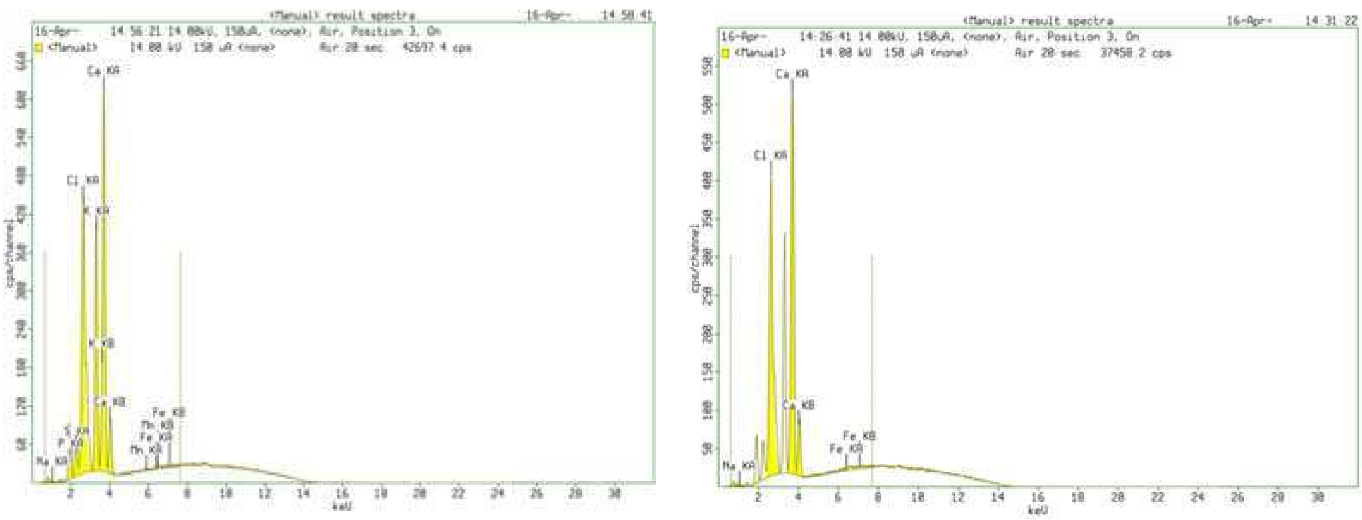


**Fig. 4** XRF analysis graph of control and probiotic sweet corn blend milk yoghurt

- Peak intensity >100 cps corresponds to concentrations >10,000 ppm (% levels)
- Peak intensity of 10-100 cps corresponds to concentrations of ~100-1000 ppm

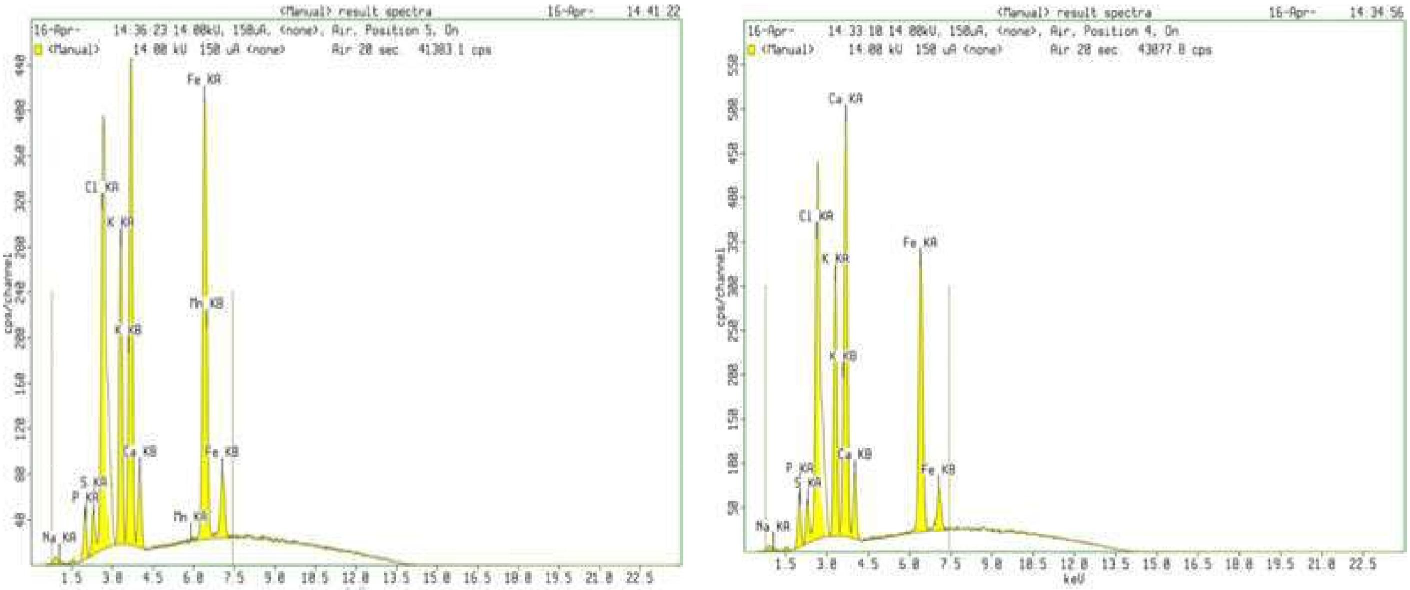


**Fig 5**. XRF analysis graph of probiotic sweet corn blend milk yoghurt fortified with 10 and 20 mg of ferrous lactate hydrate

- Peak intensity of 1-10 cps corresponds to concentrations ~10-100 ppm
- Peak intensity < 1 cps corresponds to concentrations ~1-10 ppm

The Cps (counts per second) versus the energy graphs, depicting the concentration of different elements like sodium (Na), potassium (K), calcium (Ca) and iron (Fe) for the yoghurt made from 100% cow milk are presented in Fig 4. The concentrations for Na, K, Ca, and Fe expressed as cps were 13.986 cps, 5098.625 cps, 8541.48 cps and 13.125 cps respectively. The approximate results show K and Ca are greater than 10000 ppm. Na and Fe are within 100 to 1000 ppm. The concentration for Na, Ca and Fe was 23.986 cps, 7292.234 cps and 16.290 cps for yoghurt without elemental iron fortification. The approximate results show Ca is greater than 10000 ppm. Na and Fe are within 100 to 1000 ppm.

The Cps (counts per second) Vs the energy, the concentration for different elements like sodium (Na), potassium (K), calcium (Ca) and iron (Fe) was found. The concentration for Na, K, Ca and Fe was 21.944 cps, 3963.33cps, 6597.43cps and 5119.99 cps for the probiotic yoghurt incorporated with 10 mg elemental iron. The approximate results show K, Ca and Fe are greater than 10000 ppm. Na is within 100 to 1000 ppm. The concentration for Na, K, Ca and Fe was 26.639 cps, 3620.663 cps, 6090.110 cps and 6574.40 cps for probiotic yoghurt with 20 mg iron fortification (Fig 5). The approximate results show K, Ca and Fe are greater than 10000 ppm. Na is within 100 to 1000 ppm. The peak values corresponding to iron showed a gradual increase from control yoghurt to 10 and 20 mg of iron-fortified corn milk yoghurt. From the results, it was concluded that qualitatively Fe concentration was present.

## Conclusion

Yoghurt is a fermented milk product that is popular around the world for its health benefits. It acts as a medium for accumulating various nutrients. Since milk is deficient in iron, many researchers around the world have experimented with different methods and iron sources to fortify yoghurt. Overall, the study indicates that the iron-fortified corn milk yogurt maintained the viability of Lactobacillus acidophilus and Streptococcus thermophilus during storage and demonstrated good acid tolerance properties, which are important traits for probiotic microorganisms. Elemental analysis showed increased iron concentrations in the 10 mg and 20 mg iron fortified corn milk yogurt samples. Thus, the developed product shows successful results in terms of its properties.

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