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Physicochemical and electrochemical properties of zinc fortified milk

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ABSTRACT

To eradicate Zn deficiency, milk was fortified with 22.5 ppm Zn as Zn acetate based on sensory and physico-chemical evaluation. The pH, acidity, viscosity, rennet coagulation time and alcohol stability of milk were not significantly affected ($p > 0.05$) upon fortification with Zn. The fortification decreased the milk current flow and increased the impedance significantly. FTIR analysis showed that Zn fortification decreased transmittance between 3500 and 3000 cm^{-1} indicating decreased hydrogen bonding. The peaks that disappeared suggested that Zn hindered C-H and C=O stretching. Zn affected the milk system; however these changes were on the micro level and did not significantly ($p > 0.05$) affect the stability of the milk system.

1. Introduction

Minerals cannot be synthesized by the human body, therefore their daily need must come from food (Din, Hassan, Behairy, & Mohamed, 2012). Zn is a mineral that is abundantly distributed throughout all body tissues and fluids and is essential for metabolism including catalytic, structural and regulatory functions and plays an important role in the immune system (Saunders, Craig, & Baines, 2012). It is a component of more than 300 enzymes (Salgueiro, Zubillaga, et al., 2002). It helps in maintaining growth, reproduction, immunological defenses, vision, cognition, collagen synthesis, bone mineralization, neurogenesis and neurotransmission (Maret & Sandstead, 2006) due to the large number of Zn-dependent biological process, Zn deficiency has serious implications for human health (Aquilanti et al., 2012).

Micronutrient malnutrition is a serious threat to the heath and productivity of 2 billion people worldwide; therefore it should be controlled (Sachdeva, Kaushik, & Arora, 2014). Food fortification is one of the relevant approaches (Gupta, Chawla, & Arora, 2014; Kaushik, Sachdeva, & Arora, 2014). The bioavailability of minerals from food obtained from animal sources is higher in comparison to plant source foods (Kaushik, Sachdeva, Arora, Kapila & Wadhwa, 2014). Milk is the best source as its origin is from animals and it is consumed by vegetarians also (Kaushik, Sachdeva, Arora, & Gupta, 2015). Singh et al. (2007) reported that milk has all of the prerequisites required for an ideal carrier as it is nutritionally excellent and considered as a complete food. It also provides a convenient and useful vehicle for the addition of certain nutrients as (1) it is widely available commercially, (2) consumed widely in predictable amounts, (3) the cost is minimal, (4) fortificants are generally stable with high bioavailability and (5) minimum deterioration in color, taste, and appearance generally occurs after addition of fortificants (Nair, Sharma, & Arora, 2003).

Milk is a biological system which when fortified with Zn may effect physico-chemical properties. Therefore, the present study was carried out to develop Zn fortified milk and check for any changes in physico-chemical and the electrochemical properties of milk.

2. Materials and methods

2.1. Materials and reagents

Three L of milk from just milked sahiwal cows were collected (37 ± 1 °C) from the Mohan Dairy from January to July 2016. Zn sulphate and Zn acetate were obtained from Himedia Chemicals Limited (Mumbai, India), meito rennet (600 International Milk Coagulating Units/mL) from *Mucor meihei* (R5876, Sigma Aldrich, St. Louis, Missouri, USA), ethyl alcohol (99.9%) was obtained from Jiangsu Huaxi International (Beijing, China). All the chemicals and reagents used during the present study were analytical/HPLC grade. All glassware used during the present study was acid washed (HCl 0.1 N) before use.

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2.2. Preparation of milk samples

Zn sulphate and acetate were added as a powder to milk as per their respective molecular weights of salt at levels of 15, 17.5, 20 and 22.5 ppm Zn at 45 °C accompanied by uniform mixing for 15 min in a shaking incubator (Scigenics Biotech Pvt. Ltd., Chennai, India) for complete dispersion of salts. Heat stability of the milks was measured using the method of Kaushik, Sachdeva, Arora, and Wadhwa (2014). Each unfortified milk sample was divided into eight lots of 50 mL each and kept in a 20 °C water bath. The first lot was at the natural pH while the seven other lots were adjusted to pH 6.4–7.0 at 0.1 pH intervals with the addition of either 10% sodium dihydrogen phosphate or disodium hydrogen phosphate. After the adjustment of pH, all the lots of milk were kept at refrigerated temperature (4–6 °C) for 30 min and then the temperature returned to 20 °C and the pH reestablished. Milk samples were pasteurized at 63 °C for 30 min in air tight glass bottles using a temperature controlled water bath (PolyScience, New York, New York, USA). The samples were immediately cooled to 4 °C using a chilled ice bath. After 2 h of storage at 4 °C, milk samples were analyzed for their physico-chemical properties. Every experiment was done in triplicate on three consecutive days.

2.3. Analysis of milk using electrochemistry

The fortified milk samples were analyzed for electrochemical changes using the electrochemical impedance spectroscopy based biosensor (FRA2 μAutolab Type III, Metrohm, New Delhi, India). Amperometric based analysis was done to check the effect of addition of Zn acetate in the milk using the cyclic voltammetry (CV) at different voltage conditions. An Au nano-coated strip was used to detect electrochemical changes in the milk using 1 mM methylene blue (MB) in phosphate buffered saline (PBS) pH 7 (Singh, Kaushal, Khare, & Kumar, 2014) as the redox indicator for CV and it represents current flow (Ip) and differential pulse voltammetry (DPV), whereas electrochemical impedance (EI) was measured using 5 mM K3[Fe(CN)6] as an artificial electron donor in PBS (pH 7) using the same spectroscope. The data were analyzed using NOVA software (Utrecht, The Netherlands). The CV and DPV graph are drawn as working electrode (WE) current flow (Ip) as the X axis versus potential applied (V) as the Y axis (Fig. 1). The electrochemical impedance graph is drawn as real impedance (Z') versus virtual impedance (Z″). Impedance (Z) is a measure of the circuit characteristics that impede the flow of electrons through the circuit, measured in Ohm when subjected to periodic electrical perturbations. Virtual impedance is purely frequency dependent and appears in capacitors and inductors as a consequence of the applied AC frequency. Virtual impedance is represented by the imaginary part of the complex impedance and is symbolized by Z″ for capacitive reactance (Zia and Mukhopadhyay, 2016).

2.4. FTIR

The samples were analyzed from 4000 to 650 cm^{-1} wavelength (Jothi, Babu, & Ramamurthi, 2014). The fortified milk samples were coagulated using aqueous solution citric acid (10% w/v). The coagulum represents casein and supernatant represents whey. The casein and whey were freeze dried (Labcanco, Thermo Scientific, Bombay, India). The pasteurized milk, casein and whey were analyzed using a FTIR (CARY 630 Agilent Technologies, Santa Clara, California, USA) to determine the effect of Zn fortification on functional groups in milk. The Microlab Software (Bozeman, Montana, USA) was used to generate data with a resolution of 8 cm^{-1}. The graphs obtained using the FTIR were analyzed using a method described by Pavia, Lampman, Kriz, and Vyvyan (2010).

2.5. Quality characteristics of fortified milk

pH, titratable acidity (% lactic acid), alcohol stability (equal quantity of milk and 68% alcohol were mixed and if flakes were observed then the sample failed) and clotting with boiling (milk was boiled and if milk coagulates then the sample fails) of control and fortified milk samples were determined using the method described in the BIS manual (IS1479-2, 1981). The curd tension of milk was determined using the method of Chandrasekhara, Swaminathan, Bhatia, and Subramanyam (1957). An h shaped curd tension knife (attached with a pulley to a pan) was placed in a beaker and 50 mL milk added and 0.5 mL of 0.015% (w/v) meito rennet, was added with immediate stirring and incubated at 30 ± 1 °C. After 1/2 h the pan was loaded with weights until the curd tension knife was able to cut its way through the curd. The weight (g) was taken as a measure of curd tension. Rennet clotting time (RCT) of control and Zn fortified milk were determined according to the method of Berridge (1952): 10.0 mL milk in a 20 mL open test tube and 1.0 mL of 0.2% meito rennet solution was added. The contents were mixed by inverting the test tube, tilted at an angle of 45°, and observed at regular intervals for the appearance of clot formation. The time taken from

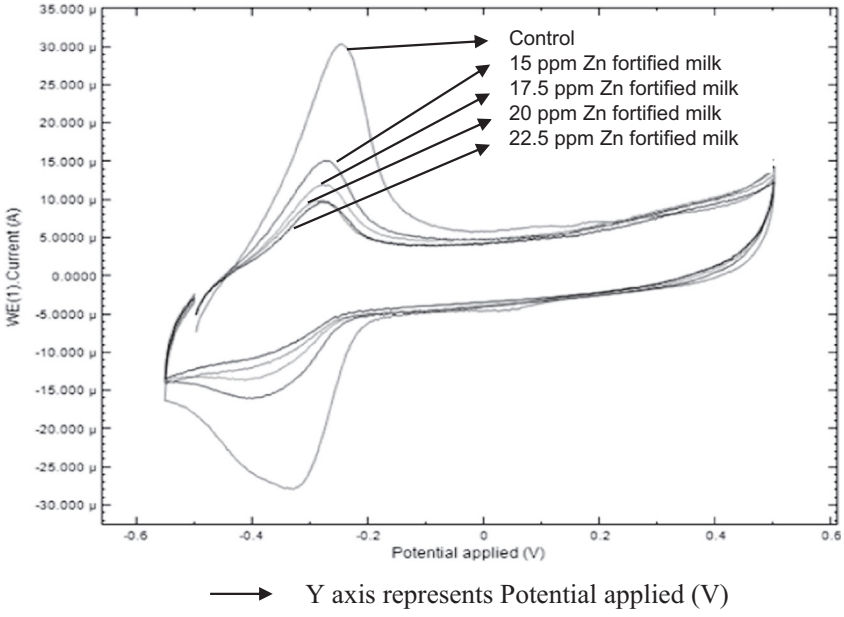

Fig. 1. Cyclic voltammetric curve of Zn acetate fortified milk.