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Fortification of yogurt with nano and micro sized calcium, iron and zinc, effect on the physicochemical and rheological properties

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A B S T R A C T

Yogurt is a highly consumed dairy product, regarded as healthy. The objective of this study was to fortify a set-type yogurt with two levels of iron oxide, zinc oxide, and calcium phosphate nanoparticles. Minerals were also used to make a comparison between nano and micro-sized minerals, to determine their effect on the physicochemical and rheological properties during 28 days of storage. The pH decreased while acidity increased in all samples during storage. Density and moisture did not show differences between samples, or during storage. Color parameters showed variations in iron-fortified samples, whereas an increase in net color change through storage was recorded for all samples. Syneresis increased significantly in micro-mineral samples, being lower in nano-fortified ones; during storage the separation significantly increased in all samples. The Herschel-Bulkley flow model fitted well the non-Newtonian behavior of the yogurt. The yogurts fortified with calcium and zinc nanoparticles increased their consistency and firmness concerning to the other samples, both parameters decreased during storage in all samples; yield stress and flow index did not significantly change during storage. In vitro digestion analysis of the yogurt with nanoparticles showed more solubility than micro-minerals, for the three minerals. In general, nanoparticles showed advantages over conventional fortification.

1. Introduction

Low intake or absorption of minerals like calcium, iron and zinc might generate deficiencies which in turn are related to many human health problems including stunted growth in children, weak bones, and immune system disorders. Food fortification could play a key role to overcome this problem. Yogurt has gained wide acceptance among consumers as it is perceived as a healthy product rich in nutrients such as calcium and high-quality proteins (Mckinley, 2005). However, as it is common with all dairy products, the content of iron and zinc is naturally very low (Mehar-Afroz, Swaminathan, Karthikeyan, Pervez, & Umesh, 2012). Due to its nature and widespread consumption, yogurt might be a suitable vehicle for these minerals.

Several studies about the fortification of yogurt with minerals have been published in recent years (Gahruie, Eskandari, Mesbahi, & Hanifpour, 2015; Gupta, Chawla, Arora, Tomar, & Singh, 2015; Karam, Gaiani, Hosri, Burgain, & Scher, 2013; Ocak & Köse, 2010). It is well known that fortification of yogurt with minerals as iron and zinc ions can chemically interact with various food ingredients. Induced chemical reactions can cause changes in physicochemical properties important for the quality of yogurt, such as syneresis as well as on rheological features; also off-flavors have been associated with fortification of dairy products. The quality of fortified dairy products depends on the selected mineral source, concentration and potential effects on physicochemical and functional properties on the food chosen as a carrier (Fayed, 2015). Thus, it is paramount to find alternatives to reduce the potentially undesirable effects of mineral fortification in dairy products while maximizing absorption and quality (Mehar-Afroz et al., 2012; Sharifi, Golestan, & Sharifzadeh, 2013). The use of nanomaterials in food fortification has experienced significant growth in recent years and is very promising (Santillán-Urquiza, Ruiz-Espinosa, Angulo-Molina, Velez-Ruiz, & Méndez-Rojas, 2017). This trend has been

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driven by the ability of these structures to improve bioavailability and solubility of active ingredients due to their large surface-to-volume ratio. That could be achieved without compromising other food properties (Sanguansri & Augustin, 2006).

The core-shell nanostructures used for the fortification of the yogurt have been previously designed, prepared and evaluated by our research group. Santillán-Urquiza et al. (2015) reported inulin-coated nanoparticles with an inorganic iron and/or zinc oxide core. Inulin made these minerals more soluble and bioavailable while reducing their reactivity, avoiding thus possible detrimental effects (Dickinson, 2012), but these nanoparticles have been not tested yet in an appropriate carrier. Therefore, the goal of this study was to determine the effect of adding inulin coated calcium phosphate, iron oxide and zinc oxide nanoparticles in a set-type yogurt, evaluating potential changes in physicochemical and rheological properties right after manufacturing and during refrigerated storage, comparing the results with those of added with micro-sized minerals and a control yogurt.

2. Materials and methods

2.1. Materials

Nanoparticles of $CaHPO_4$, $\alpha-Fe_2O_3$ and ZnO coated with inulin (Fructagave SP750 Monterrey, México) and micro-minerals commercially available of $CaHPO_4$, α-Fe_2O_3 and ZnO (Sigma-Aldrich, México) were used for fortification. Pasteurized whole milk (Alpura®, México) and skim milk powder (Svelty®, México) were used for preparation of the yogurt, as well as lyophilized microorganism Choozit® (Danisco, Mexico) containing: *Lactobacillus delbrueckii* spp. *bulgaricus* y *Streptococcus salivarius* spp. *thermophilus*. For the analysis of digestion, the enzyme pepsin (Golden Bell, México) and pancreatin Sigma-Aldrich, México were also utilized.

2.2. Methods

2.2.1. Synthesis of inorganic nanoparticles

Inorganic nanoparticles of zinc oxide (ZnO), hematite with zinc oxide ($\alpha-Fe_2O_3$@ZnO) and $CaHPO_4$, all coated with the polysaccharide inulin, were prepared accordingly to previously reported methods by Santillán-Urquiza et al. (2015).

2.2.2. Characterization of nanoparticles

The nanoparticles were characterized by powder X-Ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), transmission electron microscopy (TEM) and thermogravimetric analysis (TGA) (Yue-Jian, 2010; Santillán-Urquiza et al., 2015).

2.2.3. Determination of solubility of nano and micro minerals by in vitro digestion

The *in vitro* digestion protocol was applied to samples of Ca_{30N}, Ca_{30M}, FZ_{50N}, FZ_{50M}, Zn_{50N} and Zn_{50M} as described previously by Cilla, Perales, Lagarda, Reyes-Barbera, & Farre. (2008), with minor modifications and comprising two sequential steps: gastric and intestinal. To evaluate the gastric digestibility of nanoparticles and micro-minerals, the dissolution process of 8 g of the samples of fortified yogurt in a solution of HCl (6 mol/L) adjusted to pH 2 was followed. A solution of the enzyme pepsin (20 mg per gram of sample) was then added and the mixture incubated at 37 °C with stirring (120 agitations per min) for 2 h. At the end of this time, the mixture was kept on ice for 15 min to stop the enzyme digestion. For the intestinal digestion phase, the pH was raised to 6.5 with a solution of sodium bicarbonate (1 mol/L), and then, 5 mg per gram of sample of pancreatin were added; incubation continued for 2 h after the pH was adjusted to 7.2 with a solution of NaOH (0.5 mol/L). The samples were centrifuged at 1252 g for 20 min and filtered. The concentration of Zn (II) and Fe (III) ions was determined by atomic absorption spectrophotometry (Varian SpectrAA 220Fs, Midland, ON, Canada). The concentrations of the specified ions were measured in an air/acetylene flame for Zn and Fe and NO_2/acetylene flame for Ca. The amount of metal ions released was calculated from a calibration curve previously obtained (Argyri, Birba, Miller, Komaitis, & Kapsokefalou, 2009).

2.2.4. Preparation of yogurt samples

For the yogurt preparation, milk was standardized adding 6 g of milk powder per 100 mL of pasteurized milk. Then, a heat treatment was applied by raising the temperature of the milk at 90 °C for 20 min and then cooling down to 40–45 °C. After cooling at 42 °C, the milk was added with the lyophilized culture directly and stirred for 10 min, and poured into 100 mL plastic containers, being the same procedure for all samples; then the minerals (both, nanoparticles and micro-minerals) were added at a concentration as described in Table 1 and stirred for 20 min (120 agitations/min) until complete dissolution. Subsequently, the milk with minerals was incubated at 45 °C for 5 h until a pH of 4.6 was reached, as well as the control (Lee & Lucey, 2010), all yogurt samples were stored for 28 days at 4 ± 1 °C.

2.2.5. Physicochemical analysis

The pH was measured by a digital potentiometer (Beckman, Denver, CO, USA), previously calibrated, at room temperature. Moisture content was determined through water evaporation (method 16.032, A.O.A.C, 2000). Acidity was quantified by titration of 9 mL of sample using phenolphthalein and NaOH (0.1 mol equi/L) (method 16.023, A.O.A.C, 2000). Density was determined by a gravimetric method using Grease pycnometers (Fisherbrand, ON, Canada). The color of yogurt was measured in a Color Gard System/

Table 1
Yogurts formulations, control and fortified 100 mL per portion.

Samples	Minerals	Size	Amount (mg)	RDI (%)	Inulin (mg)	Moisture (g/100 g)	Total solids (g/100 g)
Control	0	0	0	0	0	84.02	15.98
Ca_{30N}	Ca	nano	240	30	15	82.08	17.98
Ca_{30M}	Ca	micro	240	30	15	82.24	17.76
Ca_{15N}	Ca	nano	120	15	15	82.67	17.33
FZ_{50N}	Fe/Zn	nano	7.5/12	50/80	15	83.50	16.50
FZ_{50M}	Fe/Zn	micro	7.5/12	50/80	15	83.76	16.24
FZ_{25N}	Fe/Zn	nano	3.7/6	25/40	15	82.70	17.30
Zn_{50N}	Zn	nano	7.50	50	15	82.59	17.41
Zn_{50M}	Zn	micro	7.50	50	15	82.96	17.04
Zn_{25N}	Zn	nano	3.75	25	15	82.11	17.89

Ca = calcium, Fe = iron, Zn = zinc, N = nanoparticles, M = micro-minerals *RDI (recommended daily intake).