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A COMPARTIVE STUDY BETWEEN ADDING COMMERCIAL AND NANO SIZED IRON ON THE PHYSIOCHEMICAL PROPERTIES AND TEXTURE PROFILE OF YOGHURT

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ABSTRACT

This study was carried out to determine the effect of adding Fe_2o_3 nanoparticles (Fe_2O_3NPs) and commercial Fe_2o_3 to yoghurt for improving its functional, physicochemical and sensory properties during storage. The pH values were higher (4.58) after 21 days of cold storage in yoghurt fortified with Fe_2O_3NPs (3mg/100ml) then the control (4.42). Titratable acidity levels were lower in all yoghurt samples supplemented with Fe_2O_3-NPs (0.89%) at the end of the storage. No appreciable effect on total solid content was found. However, the highest ash values at zero time were 0.81.0.82 and 0.87 for Fe_2o_3NPs (3mg/100ml), commercial Fe_2o_3 and Fe_2O_3NPs (1.5mg/100ml) respectively. No cytotoxicity was seen after 1, 2, 3, 24, 48, and 72 hours at a concentration of 3 mg/100 mL for Fe NPs. Moreover, Texture profile analysis (TPA) of yoghurt samples showed that textural properties of yoghurt increased with the addition Fe_2O_3NPs(3mg/100ml). Generally, the addition of Fe_2O_3-NPs(3mg/100ml) improved the sensory properties of yoghurt more than that fortified with commercial Fe_2o_3.

INTRODUCTION

Emerging technologies such as Nano Technol. are using milk, dairy products, and their components to improve nutritional facts and health benefits in a variety of ways, such functional food**.** This is due to the fact that diverse nutritional components of dairy foods, through altering mitochondrial function and gut microbiota composition, can affect body composition, metabolic balance, and inflammatory state **(Trinchese *et al.*, 2015).** These effects can be impacted at the nanoscale due to the nano effect, which happens when distinct improved physical qualities (strength), reactivity, or biological interactions emerge, notably below 100 nm. **(Riviere, 2019).** Companies involved in nanoTechnol. are seeking to add nutrients that are nano-encapsulated to processed dairy and food products. The texture, flavour, functioning, nutritional value, and even the detection of infections can all be changed by adding nanoscale food additives. Nano-developed colours can improve the appearance and taste of food, while nano-modification can reduce fat and sugar content. Food additives containing nano ingredients are being developed in some countries with the sports and health food markets in mind. They primarily contain minerals with nano formulations, and the particle size of these minerals is less than 100 nm. As a result, they can cross the stomach wall and enter body cells faster than minerals with larger particle sizes **(Radha *et al.*, 2014)**. Micronutrient malnutrition is a global problem that affects both developing and

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developed countries, with serious health and economic consequences. Food fortification has been recognized as an important, ongoing, and self-sustaining strategy for improving the health and nutritional status of millions of people. Dairy products are inexpensive and consumed in moderate quantities, making them ideal for mineral fortification. As a result, minerals like Ca, Fe, Zn, and Se are added to milk to fortify it. Consumers profit from dairy product fortification, while the dairy products sector gains new sales prospects. Healthy products that offer alternate sources of micronutrients to suit consumer nutritional demands are advantageous to consumers. The creation of pleasant, appealing fortified products that can be promoted as being high in minerals benefits the dairy industry **(Imran *et al.*, 2010).** Iron is a important microelement that the human body needs for a variety of processes. Iron deficiency anaemia one of the most prevalent nutritional deficiencies in the world, is caused by a lack of this element and affects 20% of the world's population (**Martinez-Navarrete *et al.*, 2002).** Inadequate dietary iron intake, inadequate iron bioavailability from digested foods, or a combination of both the usual causes of iron insufficiency **(Gaucheron, 2000).** According to the National Institutes of Health (NIH), the average daily requirement for iron in adults is 8 mg for men and 18 mg for women, whereas the requirement is 8 mg for kids and 27 mg for pregnant women **(NIH, 2007).** Iron bioavailability can be affected by food components; for example, phytic acid has a detrimental effect on iron bioavailability, but ascorbic acid has a beneficial effect. **Wieringa *et al.* (2016)** noted that adding iron compounds to yoghurt caused the production of off-flavour, oxidising flavour, and mineral flavour linked to lipid peroxidation. Fortification of food with nanomaterials has become particularly promising in recent years due to the bioavailability and solubility of the active chemicals due to the huge surface to volume ratio without compromising the food qualities **(Santillan-Urquiza *et al.*, 2017 a).**

McClements and Xiao (2017) found that reducing the particle size of ferric pyrophosphate from 8 to 4 nm raised iron absorption by 2-4 times in adults. These studies demonstrated that increasing iron bioavailability through particle size reduction is a successful tactic. Particle size reduction procedures increase the iron compound's surface area, which enhances its solubility in gastric acid and results in greater absorption. It is difficult to include iron into diets that are well absorbed by the body. The organoleptic characteristics of conventional fortificants ($FeSO_4$, $FeCl_3$) were altered, and they resulted in gastrointestinal disorders, black stool, and other health-related problems. The usage of water-soluble iron complexes may be resolved by nanosized iron salts. Iron bioavailability is increased by the nanoscale synthesis of iron complexes, while difficulties with undesirable colour, flavour, metallic taste, and rancidity in food carriers are resolved by employing water-soluble iron salts. As a result, the fortification of food and food products for the treatment of Iron Deficiency Anemia (IDA) involves the use of nanosized iron powder **(Kumari and Chauhan, 2022)**. When applied in food formulation, nanoTechnol. has the potential to deliver benefits. It is a potential strategy to avoid iron deficiency. $FePO_4$ NP is a highly bioavailable form of fortification with great sensory performance in foods. The safety of it is, however most untested. Thiobarbituric acid reactive substances were produced as a byproduct of reactive oxygen species (ROS), which are known to cause a number of health problems and were used to test the oxidative stress of iron repletion. The formation of ROS, which is ascribed to the preferential mobilization of NPs to mitochondria and also to its presentation as free iron, was highlighted in previous *in vivo* and *in vitro* studies on NP toxicity. The term "ROS" refers to a class of incompletely reduced oxygen compounds, primarily superoxide anion (O_2), hydrogen peroxide

(H_2O_2), and hydroxyl (**Ghosh and Arcot 2022)**. Yoghurt was fortified with bio-Fe (II) nanoparticles. After three weeks of cold preservation, Bio-Fe (II)NPs prevented lipid oxidation while having no impact on the overall lactic acid bacterium count. The highest colour, taste, and flavour ratings were found in yoghurt that had Bio-Fe (II) NPs added **(El-Saadony *et al.*, 2021).** Yoghurt containing iron oxide nanoparticles coated with inulin increased the iron's solubility and availability while lowering its unfavourable sensory qualities, such as metallic colour and taste. **(Santillan-Urquiza *et al.*, 2017b).**

MATERIALS AND METHODS

Materials

Buffalo's milk was purchased from a private farm in North Sinai Governorate, Egypt, and had 6% fat, 4% protein, and 18.21% T.S. Yoghurt culture (*Streptococcus thermophillus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*) was obtained from DANISCO, Rue de Clemencieres-BP 32, Sassenage, Denmark. Ferric oxid nano and commercial components were purchased from Egypt's Nakaa NanoTechnol. Network (NNN). The analytical grade chemicals utilised in this study were all provided by BDH and Sigma chemical companies.

Methods

Manufacture of Yoghurt Fortified With Nano and Commercial Ferric Oxid

Fresh buffalo milk was heated to 90°C for 3 minutes before being chilled to 42°C and inoculated with 3% yoghurt starter. Following that, the inoculated milk was separated into four equal portions (Table 1).

Iron was added to milk in 3 treatments. The first treatment consisted of adding the particles in their commercial form, the second of adding nano particles at the same concentration as the commercial form, and the third of adding nano particles at half the concentration of the commercial form. Iron was added to the inoculated milk at a concentration that is 100% of the recommended daily intake.

Characterization of Fe2o3 Nanoparticles

These tests were carried out at the Faculty of NanoTechnol., Sheikh Zayed, Cairo University.

X-ray Diffraction (XRD) Analysis

To determined the phase variety and grain size of synthesized Metal oxide nanoparticles was used X-ray diffraction spectroscopy (Philips PAN analytical). The metal oxide nanoparticles were studied using CUK radiation at a voltage of 30 kV, a current of 20 MA, and a scan rate of 0.030/s. X′ pert high score software with search and match capability was used to determine the different phases contained in the synthesized samples. Scherrer's equation was used to calculate the particle size of the produced samples.

$$D \approx \frac{0.9\lambda}{\beta \cos\theta}$$

Where D is the crystal size, λ is the wavelength of X-ray, Θ is the Braggs angle in radians and β is the full width at half maximum of the peak in radians **(Sankaran *et al.*, 2018).**

Chemical Analysis

pH values were measured using JENWAY Digital pH meter Model 3310. Titratable acidity, total solids and ash were determined according to **AOAC (2011)**.

Iron content of the yoghurt samples were determined by inductively coupled plasma optical emission spectrophotometer (ICP-OES) (Perkin Elmer Ltd., Optima 3300 DV, USA) as described by **Prokisch *et al.* (2006).**

Aroma compounands of yoghurt

Yoghurt samples' acetaldehyde and diacetyl contents were determined with some modifications according to **Lee and Jago (1969)**.

Table 1. The Experimental Design

Treatment	Size	amount/100ml
Control	milk sample without any additives	
T1(Fe_2O_3)	Commercial	3mg
T2(Fe_2O_3)	Nano	3mg
T3(Fe_2O_3)	Nano	1.5mg

Texture Profile Analyses (TPA)

This test was conducted at the Agricultural Research Centre, Food Technol. Research Institute, Dairy Research Department. Using a Universal Testing Machine (TMS-Pro) outfitted with a (250 lbf) load cell and connected to a computer running Texture ProTM texture analysis (programme, DEV TPA with hold). Texture profile analysis (TPA) of yoghurt samples was carried out. The yoghurt samples were uniaxially compressed to half their original height using a flat rod probe (49.95 mm in diameter). The trigger force IN, deformation 25%, and holding time 2 seconds between cycles were changed for the texture profile analysis test set condition. At 5 °C, samples of yoghurt were tested for texture. Each sample underwent two additional cycles (bites) **International Dairy Federation (1991)**.

- **Hardness** is the amount of force required to achieve a certain deformation.
- **Cohesiveness**: the amount material may be bent before rupturing.
- **Springines**s: the pace at which a sample returns to its original shape after being deformed.
- **Gumminess:** the amount of force required to breakdown the sample into a state suitable for ingestion.
- **Chewiness:** the amount of effort required to masticate the sample so that it is ready for swallowing.

Toxicity Test

The cytotoxicity of Fe NPs, was determined on rats (pheochromocytoma cell) with **XTT** according to the methods previously described by **Berridge *et al.* (2005).** The sample was tested against pheochromocytoma cell (PC12) at concentration 3 mg/mL, for Fe NPs of 1, 2, 3, 24, 48 and 72 hr.

Microbiological Analyses

Lactic acid bacterial count

Lactic acid bacterial (LAB) was enumerated on M17 agar medium and incubated at 37^0C for 3 days according to **Elliker *et al.* (1956).**

Molud and Yeast counts

Molud and Yeast were enumerated on oxytetracycline glucose yeast extract agar medium as described by **American Public Health Association (2004).** Plates were incubated at 25^0C for 3 days.

Coliform group count

Coliform were determinted according to the **American Public Health Association, (2004).** Approving dilutions of samples were plated on Mac Conk's agar medium and incubated at 37^0 C for 48 hr.

Organoleptic Properties of Yoghurt

A group of Faculty staff and students (90) persons, Faculty of Agriculture, Arish University. Yoghurt was evaluated according to the method of **El-Samragy and Zall (1988)**

Statistical Analyses

The SPSS 19.0 statistical programme was used to analyses all of the study's outcomes, which were all expressed as means and standard deviations of three triplicate analyses. According to the protocol of the study, one-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to identify data with significant (P0.05) differences **(Duncan, 1955).**

RESULTS AND DISCUSSION

Characterization of Nanoparticles

The size of NPs has a significant impact on their biological activity. The effects of NPs of varying sizes and doses on cellular protein levels and enzyme activity of secretory lactate dehydrogenase, intracellular sodium potassium adenosine triphosphatase, glutathione peroxidase, and superoxide dismutase are variable **(Peng *et al.*, 2018)**. XRD in Fig.1 demonstrated that the average particle size are on the nanoparticles.

In addition to illustrate the synthesis of Fe_2O_3 nanoparticles without any impurities from synthesis materials. However, Fe_2O_3 nanoparticles depicts a typical chart of synthesized Fe_2O_3 nanoparticles, with the diffraction peaks (220), (311), (222), (400), (422), (511), and (440) corresponding to the Fe_2O_3 nano crystal (**Anandalakshmi *et al.*, 2016**). These findings revealed that the crystalline nature of the synthesized iron nanoparticles is primarily pure Fe_2O_3 crystal, and the densities of all peaks indexed the face-centered cubic crystal structure of the synthesized Iron with an average crystalline size of 12 nm and space point group of Fd-3 m.

Chemical Properties

pH and titratable acidity

Fig. 2 shows the changes of pH values in yoghurt fortified with commercial and nano sized Fe_2o_3 during storage at 4-6°C up to 21 days. The results showed that the pH values in all treatments tended to decline throughout the course of storage, indicating that the quality of the yoghurt significantly declined after 21 days of storage. As of storage, the pH values in the yoghurts with Fe_2O_3-NPs (3 mg) fortification raised to 4.58 from 4.42 in the control. The lactic acid produced by the microorganisms found in yoghurt during storage may because of the pH drop. Similar trends were reported for pH values by **El-Saadony *et al.* (2021)**.

The titratable acidity levels were lower in all yoghurt samples supplemented with Fe_2O_3-NPs 0.89% at the end of the storage. (Fig. 3). All of the studied samples' titratable acidity values marginally increased after 21 days of storage at 4-6°C. Similar observation was reported by **Santillán-Urquiza *et al.* (2017b)** who Found that there were no significant differences among the fresh formulations with nanoparticles, microminerals and the control. The pH of the yoghurt decreased during storage may be due to the microorganisms present producing lactic acid **(El-Kholy *et al.*, 2011).**

Gross Compostion

Total solid contents

The total solid contents of yoghurt were unaffected by the addition of commercial- and nano-sized Fe_2O_3. This might be as a result of the minor addition of commercial and Nano Fe_2O_3. Due to the storage period, (18.29%) of the control solid was reported in Fig. 4. Additionally, Fe_2O_3NPs(3mg) had the highest T.S value at the end of the storage period (18.86%). **Parivar *et al.* (2016)** found similar trend because of the evaporation of some water from yoghurt surface during cold storage.

Ash contents

Fig. 5 shows that ash (%) of fortified yoghurt with commercial and nano sized Fe_2O_3 increased as the level of adding particles increased in fresh samples. The highest value at the zero time was 0.81, 0.82 and

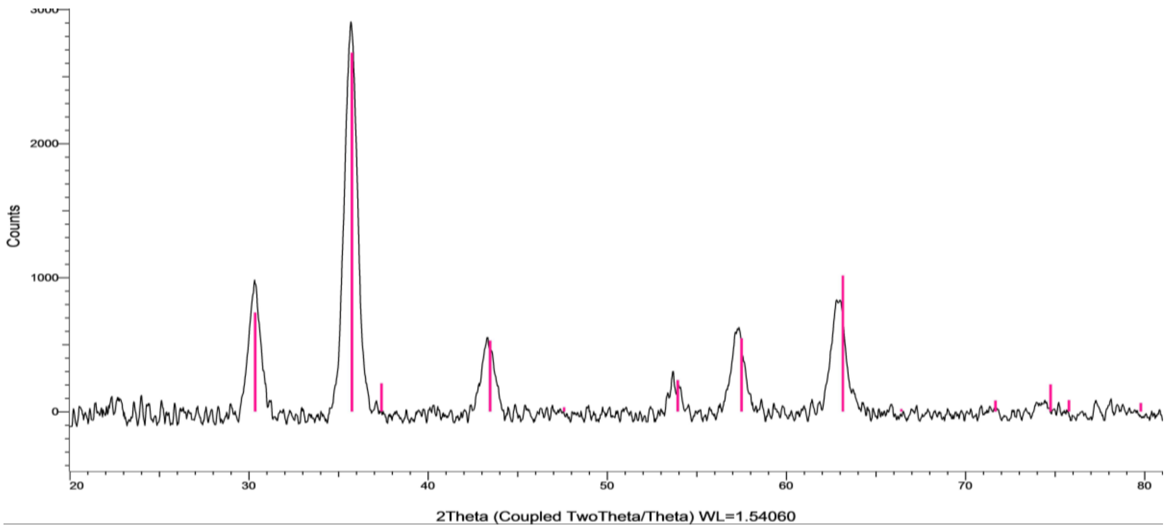

Fig. 1. XRD of synthesis of Fe_2O_3 nanoparticles

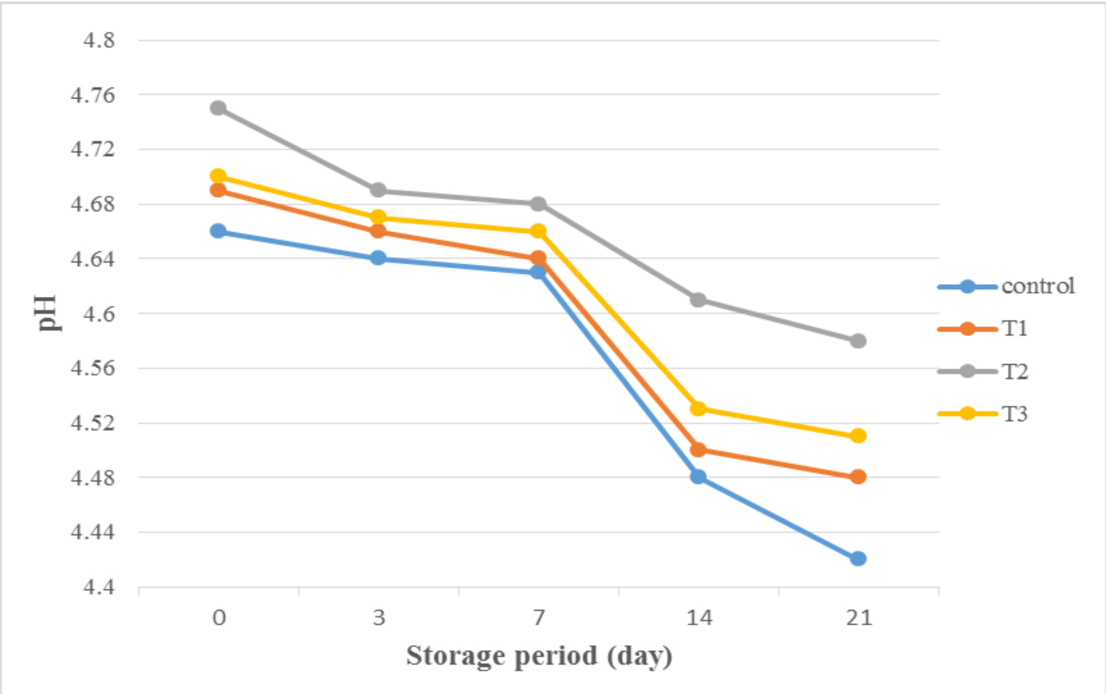

Fig. 2. pH values of fortified yoghurt with commercial and nano sized iron during storage at 4-6°C up to 21 days

Control: yoghurt without any additives, T1: Yoghurt fortified with 3mg Commercial Fe/100ml.

T2: Yoghurt fortified with 3mg FeNPs/100ml, T3: Yoghurt fortified with 1.5mg FeNPs/100ml

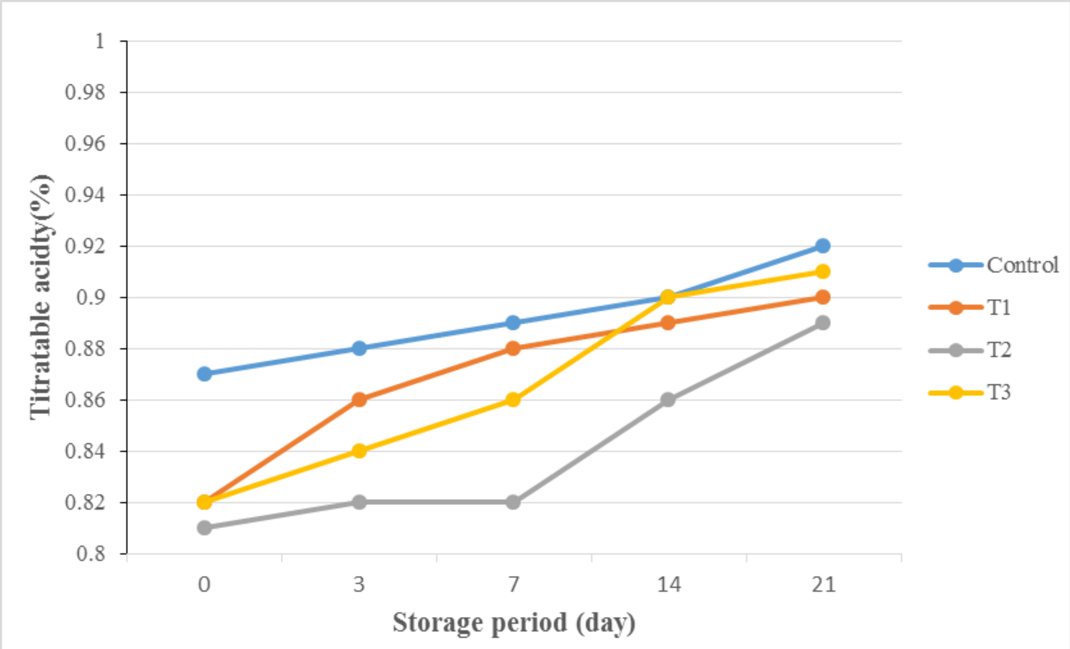

Fig. 3. Titratable acidity percent of fortified yoghurt with commercial and nano sized iron during storage at 4-6°C up to 21 days.

Control: yoghurt without any additives, T1: Yoghurt fortified with 3mg Commercial Fe/100ml.

T2: Yoghurt fortified with 3mg FeNPs/100ml, T3: Yoghurt fortified with 1.5mg FeNPs/100ml.

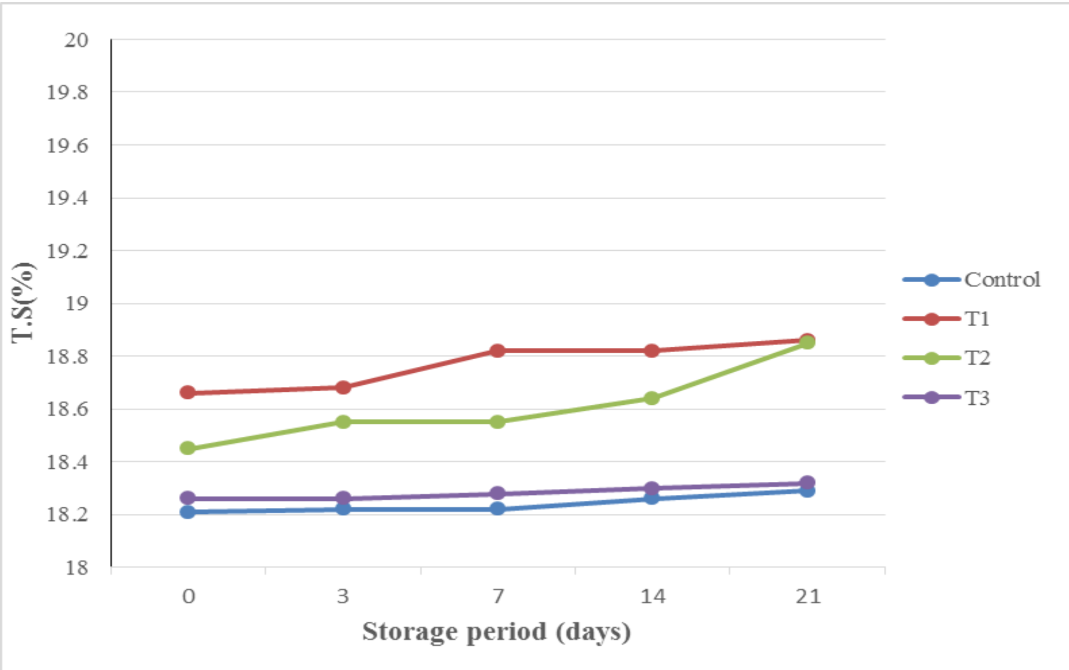

Fig. 4. Total solids (%) of fortified yoghurt with commercial and nano sized iron during storage at 4-6°C up to 21 days

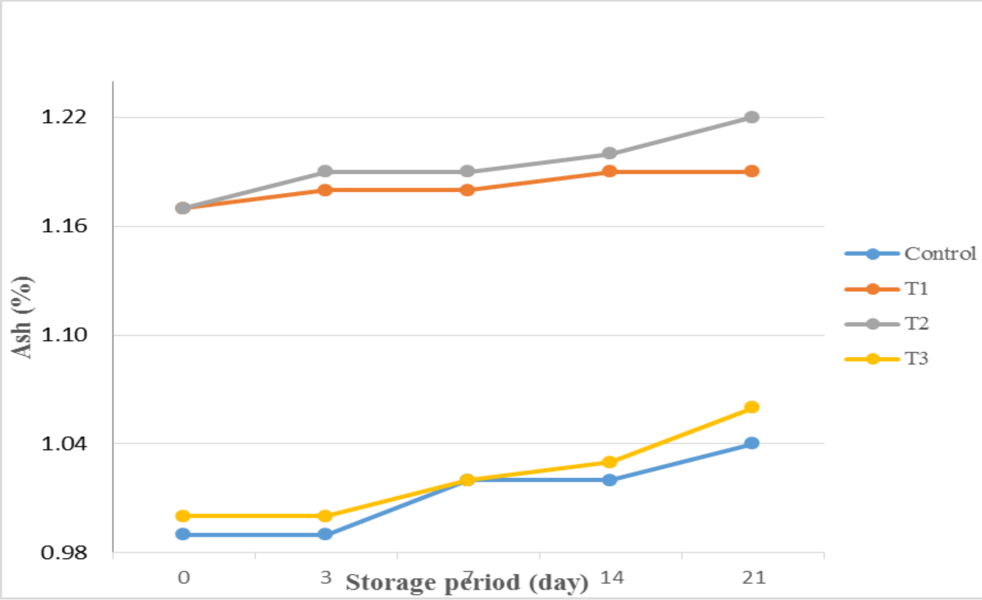

Fig. 5. Ash (%) of fortified yoghurt with commercial and nano sized iron during storage at 4-6°C up to 21 days

0.87 for each of Fe_2o_3NPs(3mg/100ml), commercial Fe_2o_3 and Fe_2o_3NPs (1.5 mg/ 100 ml) and control, respectively. Ash content did not differ significantly among the fresh treatments or during the storage times. Results indicated that adding Fe_2O_3 NPs to fortify yoghurt is more effective than adding commercial Fe_2o_3 that ascribed to the very small size of nanoparticles. This outcome justifies the addition of commercially available nanoparticles, which are regarded as a good source of minerals. The current statistics are consistent with those found by **Gangwar *et al.* (2016)** who reported that control yoghurt had less content of ash compared with those fortified with commercial and nano particles.

Iron content

Fig. 6 shows the overall iron concentrations in samples of iron-free control yoghurt and fortified yoghurt. The results revealed that the average iron concentration in control yoghurt samples was 1.9 and 1.2 mg/100g, respectively, on the first day and at the 21 days of storage at 4°C. The concentration of iron increased in the order T2, T1 and T3. It is clear that the addition of iron in the form of nano increased its concentration more than commercial. This may be due to the very small size of the nano iron, which leads to the ease of its spread in a homogeneous manner in yoghurt. A similar trend was observed in yoghurts and milk by **El-Saadony *et al.* (2021)**.

Aroma Compounds of Yoghurt

During the storage time of the various yoghurt treatments, the use of Fe_2O_3NPs had an impact on the levels of diacetyl and acetaldehyde (mg/L), as shown in Figs. 7 and 8. The maximum value was 29.03 mg/L for Fe_2O_3NPs (3 mg) yoghurt samples at the end of the storage. On the first day, fresh yoghurt had the highest content of acetaldehyde and the lowest amount was recorded for control sample (16.00 mg/L). Due to acetaldehyde's oxidation to acetate, acetaldehyde was reduced by a decreasing pH, according to **Tamime and Robinson (1999).** Compared to Fe_2O_3 commercial treatments, the amount of acetaldehyde rose in the yoghurt that had been enriched with Fe_2O_3NPs.

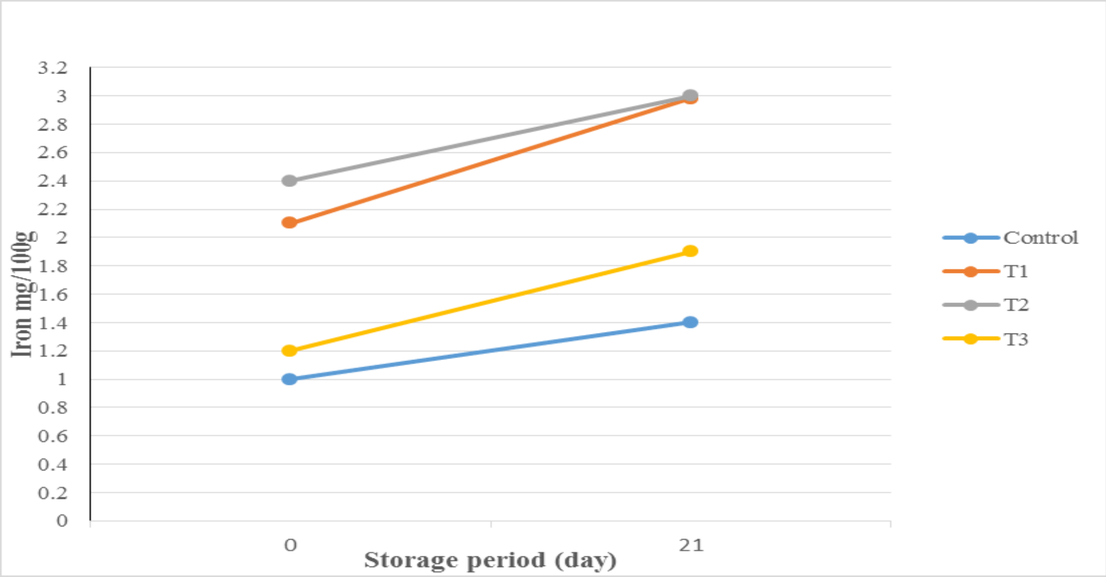

Fig. 6. Iron content (mg/100g) of fortified yoghurt with commercial and nano sized iron, when fresh and after 21 dayes

Control: yoghurt without any additives, T1: Yoghurt fortified with 3mg Commercial Fe/100ml.

T2: Yoghurt fortified with 3mg FeNPs/100ml, T3: Yoghurt fortified with 1.5mg FeNPs/100ml

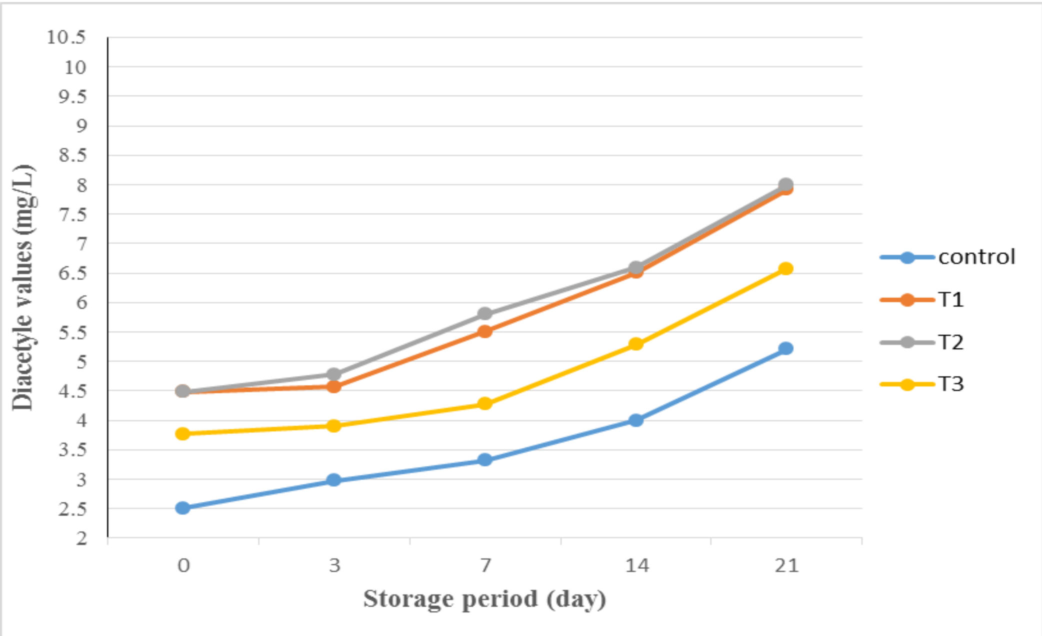

Fig. 7. Diacetyl values (mg/L) of fortified yoghurt with commercial and nano sized iron during storage at 4-6°C. up to 21 days

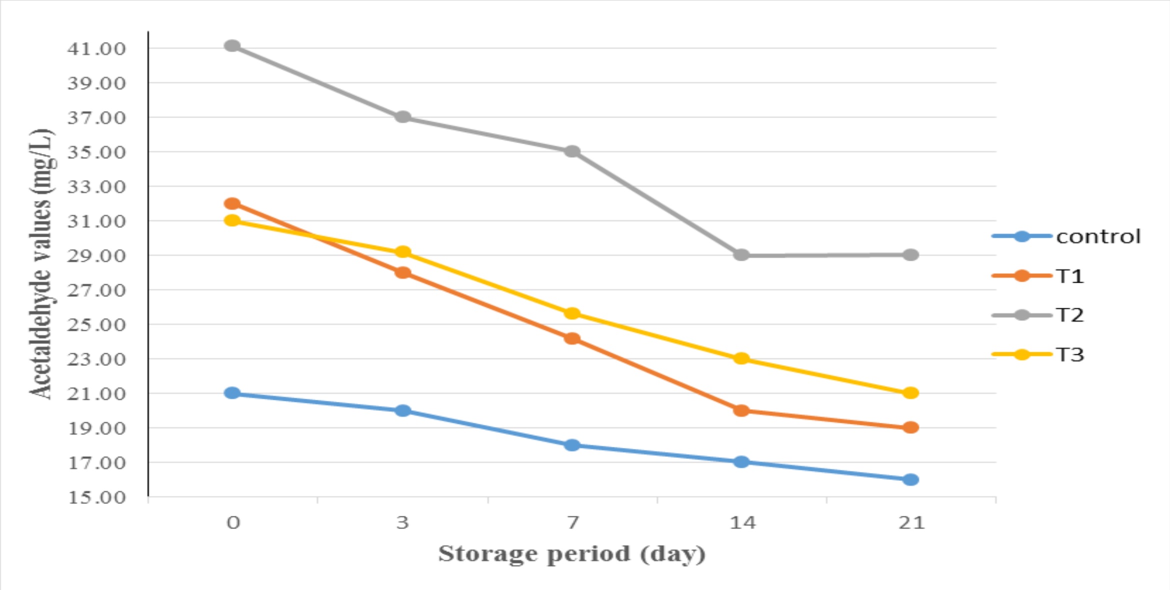

Fig. 8. Acetaldehyde values (mg/L) of fortified yoghurt with commercial and nano sized iron during storage at 4-6°C. up to 21 days

Control: yoghurt without any additives, T1: Yoghurt fortified with 3mg Commercial Fe/100ml.

T2: Yoghurt fortified with 3mg FeNPs/100ml, T3: Yoghurt fortified with 1.5mg FeNPs/100ml

Diacetyl concentration varied significantly amongst all treatments. It was evident that the diacetyl level of each treatment rose and peaked after 21 days of storage. During the yoghurt treatments' storage time, the impact of additional Fe_2O_3NPs on diacetyl was quite evident. The results showed that Fe_2O_3NPs (3 mg) had the highest value while the control treatment had the lowest value. The diacetyl content of the yoghurt sample containing Fe_2O_3NPs (3 mg) was higher than that of the fortified yoghurt at the same concentration. The treatments Fe_2o_3NPs (1.5 mg) showed that there were extremely comparable to those of the commercial therapies. Similar results were reported by **Salama *et al.* (2021).**

Texture Profile Analysis (TPA)

Table 2 shows TPA the structural components of coagulated dairy products influence their textural and rheological properties. **Walia *et al.* (2013)** reported that the textural features of yoghurt products are determined by the network's structural organization, which is controlled by factors such as composition and manufacturing procedures. In general, yoghurt texture is determined by the physical interaction of casein micelles. Overall, yoghurt produced with or without particles had a harder texture during preservation. Gumminess is described as the product of hardness and cohesiveness that is characteristic of semisolid meals with low hardness and high cohesiveness.

Hardness tended to increase during the storage period, with a significant difference) between treatments. The texture profile analysis (TPA) of yoghurt samples revealed that hardness values increased with the addition of Fe_2O_3-NPs to the yoghurt. The hardness of yoghurt increased in samples of higher concentration particles and principally in yoghurts fortified with Fe_2O_3-NPs reflecting

Table 2. Texture Profile Analysis of fortified yoghurt with commercial and nano sized iron during storage up to 21 days at 4-6°C

Parameter	Storage day	Treatment			
		Control	T1	T2	T3
Hardness (N)	Fresh	1.3^{a}±0.1	1.6^{a}±0.1	1.8^{b}±0.1	1.8^{c}±0.1
	3	1.6^{a}±0.1	1.7^{a}±0.1	2.0^{b}±0.1	1.9^{c}±0.1
	7	1.9^{a}±0.1	1.9^{a}±0.1	2.0^{b}±0.1	2.2^{c}±0.1
	14	1.9^{a}±0.1	1.8^{a}±0.1	2.1^{b}±0.1	2.1^{c}±0.1
	21	2.0^{a}±0.1	1.9^{a}±0.1	2.4^{b}±0.1	2.3^{c}±0.1
Cohesiveness (N)	Fresh	0.23^{b}±0.1	0.24^{b}±0.1	0.28^{a}±0.1	0.26^{a}±0.1
	3	0.22^{b}±0.1	0.25^{b}±0.1	0.29^{a}±0.1	0.26^{ac}±0.1
	7	0.23^{b}±0.1	0.25^{b}±0.1	0.29^{a}±0.1	0.28^{ab}±0.1
	14	0.24^{b}±0.1	0.26^{b}±0.1	0.32^{a}±0.1	0.28^{a}±0.1
	21	0.26^{b}±0.1	0.28^{a}±0.1	0.34^{a}±0.1	0.30^{b}±0.1
Springiness (mm)	Fresh	4.00^{b}±0.1	3.80^{b}±0.1	3.82^{b}±0.1	3.83^{b}±0.1
	3	3.95^{b}±0.1	3.71^{a}±0.1	3.82^{a}±0.1	3.82^{ab}±0.1
	7	4.11^{b}±0.1	4.12^{a}±0.1	4.72^{a}±0.1	4.74^{ab}±0.1
	14	5.16^{b}±0.1	5.51^{b}±0.1	5.71^{a}±0.1	5.33^{bc}±0.1
	21	6.12^{b}±0.1	6.40^{b}±0.1	5.62^{a}±0.1	5.36^{c}±0.1
Gumminess (N)	Fresh	0.6^{c}±0.1	0.7^{a}±0.1	0.8^{a}±0.1	0.8^{a}±0.1
	3	0.9^{b}±0.1	0.9^{ab}±0.1	1.2^{a}±0.1	0.9^{ab}±0.1
	7	1.0^{a}±0.1	1.1^{c}±0.1	1.4^{ab}±0.1	1.3^{ab}±0.1
	14	1.2^{ab}±0.1	1.4^{a}±0.1	1.5^{b}±0.1	1.4^{a}±0.1
	21	1.3^{a}±0.1	1.6^{b}±0.1	1.9^{b}±0.1	1.5^{bc}±0.1
Chewiness (m.J)	Fresh	2.09^{b}±0.1	2.46^{a}±0.1	2.92^{bc}±0.1	2.90^{b}±0.1
	3	2.92^{c}±0.1	3.32^{a}±0.1	3.62^{b}±0.1	3.64^{bc}±0.1
	7	3.21^{a}±0.1	3.65^{a}±0.1	3.65^{ab}±0.1	3.68^{a}±0.1
	14	3.36^{a}±0.1	3.67^{a}±0.1	3.85^{b}±0.1	3.74^{c}±0.1
	21	3.80^{a}±0.1	3.91^{a}±0.1	4.10^{b}±0.1	3.95^{ab}±0.1

Control: yoghurt without any additives, T1: Yoghurt fortified with 3mg Commercial Fe/100ml.

T2: Yoghurt fortified with 3mg FeNPs/100ml, T3: Yoghurt fortified with 1.5mg FeNPs/100ml.

a stronger gel structure. These results could be explained by how these minerals interact with the protein matrix of the yoghurt. On the other hand, the hardness of yoghurt sample continued to increase during the course of the storage period of 21 days. The ability of Fe_2O_3-NPs to entrap water within the product's three-dimensional network may be the cause of the raise in viscosity, which may be the cause of the increase in hardness values. This demonstrated that nanoparticles, as opposed to control and commercial particles, more effectively retain the structure of the yoghurt (**Desouky and El-Shaer, 2014)**.

Cohesiveness increased during the storage time, and treatments differed from one another. The cohesiveness values of the control samples was less than in yoghurt fortified with Fe_2O_3-commercial, Fe_2o_3-NPs (1.5 mg/100 ml) and Fe_2O_3-NPs (3 mg/ 100 ml) respectively. Generally, the higher value of cohesiveness led to softer yoghurt texture **(Santillán-Urquiza *et al*., 2017 a).**

Gumminess

There was a difference in gumminess among the treatments, and gumminess values raised for all treatments during the storage period. A considerable increase in gumminess was seen during storage, which may be correlated to its higher hardness values. Additionally, it was noted that yoghurt samples with Fe_2o_3-NPs (3 mg/100 ml) had higher gumminess value than each of fortified yoghurt with Fe_2O_3-NPs and control samples.

Chewiness

The values of chewiness increased for all treatments during the storage period, and there were a significant differences in chewiness among treatments.

Springiness

There were significant differences among treatments, and its values declined for all treatments over the course of storage. This could be a result of the protein matrix's ongoing degradation as well as its strength, which is influenced by things like moisture and fat content **(Lawrence *et al.*, 2002)**. According to these findings, adding Fe_2O_3-NPs to yoghurt had a negligible impact on its textural characteristics.

The solids composition of dairy products influences texture changes **(Lee and Lucey, 2010).** Yoghurt supplemented with Fe_2O_3-NPs has gradually enhanced firmness compared to control. Furthermore, ten days of cold storage for all treatments resulted in an increase in the hardness value **(Osman *et al.*, 2019).** The curd mass's improved ability to hold water after being altered by protein interactions during the coagulation process by starting culture and decreased syneresis may be the cause of this increase **(Achanta *et al.*, 2007).** Additionally, dairy products' hardness significantly increased with storage. This is mostly due to the increase in total solids content, which serves as a plasticizer in the protein matrix **(Desouky and El-Shaer, 2014).** The interaction of Fe_2o_3-NPs with casein micelles and the Fe_2O_3-NPs' capacity to trap water inside the three-dimensional network of the yoghurt may be the causes of these alterations. When combined with protein, Fe_2o_3-NPs can form a gel and keep its structure like yoghurt **(Achanta *et al.*, 2007)**. Overall, the Fe_2o_3-NPs addition has improved the mouth feel qualities, enhancing the product's sensory appeal.

Totoxicity effect

Table 3 shows the toxicity of yoghurt fortified with nano and commercial Fe_2O_3. In the examined rat's pheochromocytoma (PC12) cells, no cytotoxicity was seen at 1, 2, 3, 24, 48, or 72 hours at a concentration of 3 mg/100 mL for Fe NPs. Yoghurt fortified with Fe_2O_3-NP did not enter the cells, according to the results of an XTT test for the examination of cytotoxicity. Instead, it appeared to be sitting on the cell's surface. At any tested of concentration, iron

Table 3. The toxicity of yoghurt fortified with nano and commercial sized of iron at the 7th day of cold strage

Treatment	Rat's pheochromocytoma PC12 cells
Control	22.0±2.3[b]
T_1	21.0±3.2[a]
T_2	21.5±2.1[a]
T_3	20.3±1.9[a]

Control: yoghurt without any additives, T1: Yoghurt fortified with 3mg commercial Fe/100ml.
T2: Yoghurt fortified with 3mg FeNPs/100ml, T3: Yoghurt fortified with 1.5mg FeNPs/100ml.

nanoparticles showed maximal contact and entry in cells without a harmful effect. Therefor, FeNPs are considered safe because they did not induce any noticeable toxicity, even over extended periods of time, and we may conclude that they do not cause any measurable cytotoxicity. Because of milk protein can bind nanoparticles which difficult to release **(El-Sayed *et al.* 2015).** As a result, no harmful activity was noticed whey protein has a high propensity for binding to iron and other hydrophobic compounds. Eight binding sites in whey protein allow minerals to bind to their compact structure, which may account for their potent ability to bind to the additional nanoparticles **(El-Saadony *et al*. 2021).** Some studies showed that FeNPs treatment caused a reduction in reactive oxygen species (ROS), apoptosis, and so protective proteins were also enhanced to resist apoptosis **(Lin *et al.*, 2014)**.

Microbiological evaluation

It was evident that the counts of LAB(Lactic Acid Bacteria) declined as the storage period and the Fe_2O_3-NP fortification process progressed. Fresh yoghurt had the highest rate of LAB in the control sample (Table 4). Due to the antimicrobial activity of Fe_2o_3-NPs, the LAB in yoghurt were considerably reduced as the concentration of nanoparticles Fe_2O_3-NPs (three mg/100 ml) was increased. The lactic acid bacterial count during yoghurt development and throughout cold storage after 21 days was considerably affected by fortification of yoghurt with commercial Fe_2O_3 and Fe_2O_3-NPs. This outcome is consistent with that of **De and BinataNayak (2017).** Lactic acid bacteria concentrations decreased with the advanced of the storage, particularly in the last storage period of shelf life for all samples. Lactic acid bacteria concentration, which should not be less than 10^7 CFU/gram till the product's expiration date, is recorded for each day the product is stored. This shows that the starting culture in the fermented dairy is stable. Coliform and Molds and Yeast were not present in any of the treatments, whether they were stored at 4-6°C for up to 21 days or when they were fresh, proving that set yoghurt processing was done correctly and hygienically.

Sensory Characteristies of Yoghurt

During storage at 4-6°C for up to 21 days, the influence of fortified yoghurt with commercial and nano-sized Fe_2O_3 on the sensory properties of yoghurt is illustrated in Table 5. For all treatments, the scores of flavour steadily declined during the course. However, control received the best flavour ratings for fresh yoghurt. Scores for body and texture for all treatments gradually declined during the course of cold storage up to 21 days. The highest scores, however, were 28 at the end of the storage period for

Table 4. Lactic acid bacteria count (CFU/ml) of fortified yoghurt with commercial and nano sized iron during storage at 4-6°C. up to 21 days

Treatment	Storage period (day)				
	Fresh	3	7	14	21
Control	$8.15\times 10^{10\,a} \pm 0.01$	$1.50\times 10^{10\,a}\pm0.01$	$4.50\times 10^{9\,a}\pm0.01$	$1.70\times 10^{9a}\pm0.01$	$1.75\times10^{9a}\pm0.01$
T_1	$1.09\times 10^{10\,a} \pm 0.01$	$2.15\times 10^{9\,b} \pm 0.01$	$4.51\times 10^{8b}\pm0.01$	$3.25\times 10^{8c}\pm0.01$	$2.56\times 10^{8b}\pm0.01$
T_2	$3.10\times 10^{10\,b} \pm 0.01$	$2.10\times 10^{10\,a}\pm0.01$	$5.40\times10^{8\,b}\pm0.01$	$1.40\times 10^{8c}\pm0.01$	$1.60\times 10^{8b}\pm0.01$
T_3	$8.21\times 10^{10\,a} \pm 0.01$	$3.50\times 10^{10\,a}\pm0.01$	$3.29\times10^{8\,c}\pm0.01$	$2.60\times10^{8\,c}\pm0.01$	$1.50\times 10^{8c}\pm0.01$

Control: yoghurt without any additives, T1: Yoghurt fortified with 3mg commercial Fe/100ml.

T2: Yoghurt fortified with 3mg FeNPs/100ml, T3: Yoghurt fortified with 1.5mg FeNPs/100ml

Table 5. Sensory characteristics of fortified yoghurt with commercial and nano Fe_2O_3 during storage at 4-6°C up to 21 days

Storage day / Treatment	Flavour 50				Body and Texture 35				Appearance 15				Total acceptance 100			
	0	7	14	21	0	7	14	21	0	7	14	21	0	7	14	21
Control	46^{a} ±0.1	43^{a} ±0.1	42^{a} ±0.1	40^{b} ±0.1	31^{b} ±0.1	30^{b} ±0.1	28^{b} ±0.1	27^{b} ±0.1	13^{b} ±0.1	12^{b} ±0.1	12^{b} ±0.1	11^{b} ±0.1	90^{a} ±0.1	85^{b} ±0.1	82^{b} ±0.1	78^{b} ±0.1
T1	43^{ab} ±0.1	42^{ab} ±0.1	40^{ab} ±0.1	38^{a} ±0.1	31^{b} ±0.1	29^{b} ±0.1	27^{b} ±0.1	25^{a} ±0.1	10 ±0.1	9 ±0.1	11 ±0.1	11 ±0.1	84^{a} ±0.1	80^{a} ±0.1	78^{a} ±0.1	73^{b} ±0.1
T2	45^{b} ±0.1	43^{c} ±0.1	40^{b} ±0.1	39^{c} ±0.1	33^{c} ±0.1	30^{c} ±0.1	29^{c} ±0.1	28^{c} ±0.1	12^{c} ±0.1	11^{c} ±0.1	12^{d} ±0.1	11^{c} ±0.1	90^{b} ±0.1	84^{b} ±0.1	82^{b} ±0.1	78^{b} ±0.1
T3	44^{b} ±0.1	42^{b} ±0.1	42^{b} ±0.1	38^{b} ±0.1	31^{b} ±0.1	29^{c} ±0.1	28^{c} ±0.1	26^{c} ±0.1	13^{c} ±0.1	12^{c} ±0.1	11^{c} ±0.1	11^{c} ±0.1	88^{b} ±0.1	83^{b} ±0.1	81^{b} ±0.1	75^{c} ±0.1

Control: yoghurt without any additives, T1: Yoghurt fortified with 3mg commercial Fe/100ml.
T2: Yoghurt fortified with 3mg FeNPs/100ml, T3: Yoghurt fortified with 1.5mg FeNPs/100ml.

Fe_2o_3-NPs(3mg/100ml)**.** Along the storage time, appearance scores for all treatments gradually declined. Commercial Fe_2O_3 (3 mg/100 ml) had the lowest score at the conclusion of the storage period, although the other treatments recorded equal or higher values than T1. Along the storage period, the overall acceptance scores for treatments gradually declined. However, the control and Fe_2O_3-NPs (3 mg/100 ml) received the highest scores. All of the sensory are similar with those that have been reported **(Seo *et al.*, 2009)**. It is noticeable that the addition of Fe_2O_3-NPs (3 mg/100 ml) improved the sensory properties of yoghurt more than which fortified with commercial Fe_2O_3. Because of its small particles size which led to the homogenous body and texture and increased the total acceptability of yoghurt fortified with nano-sized Fe_2O_3 compared to control and commercial particles.

Conclusion

The results referred to fortification of yoghurt with nanoparticles of iron, at a lower concentration has the same effect as fortification with particles in a commercial size at a higher concentration. No cytotoxicity was seen at 1, 2, 3, 24, 48, or 72 hours at a concentration of 3 mg/100 mL for Fe NPs**.** The addition of Fe_2O_3-NPs (3 mg/100 ml) improved the sensory properties of yoghurt. Because of its small particles size which led to the homogenous body and texture and increased the total acceptability of yoghurt fortified with nano-sized Fe2O3 compared to control and commercial particles.

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الملخص العربي

دراسة مقارنة بين إضافة الحديد التجاري والحديد النانوي على الخصائص الفيزيوكيميائية والريولوجية لليوجهورت

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أجريت هذه الدراسة لتحديد تأثير إضافة جزيئات أكسيد الحديديك النانوية والتجاري إلى اليوجهورت بهدف تحسين وظائفه وخصائصه الفيزيائية والكيميائية والحسية أثناء التخزين. زادت قيم الأس الهيدروجيني فى نهاية مدة التخزين المبرد إلى 4.58 في اليوجهورت المدعم بـ3 مجم/100 مل جزيئات أكسيد الحديديك النانوية عن عينة المقارنة (4.42). لم يكن للإضافة تأثيرا ملحوظا على محتوى المواد الصلبة الكلية، ولكن لوحظ ارتفاع قيم الرماد للعينات المعاملة عن عينة المقارنة. لم يلاحظ أي سمية خلوية بعد 1، 2، 3، 24، 48 و72 ساعة بتركيز 3 مجم/100 مل لـ جزيئات أكسيد الحديديك النانوية ،أظهر تحليل المظهر الجانبي للقوام لعينات اليوجهورت أن الخصائص التركيبية لليوجهورت زادت مع إضافة 3 مجم/ 100 مل لـ جزيئات أكسيد الحديديك النانوية بشكل عام، أدت إضافة 3 مجم/100 مل لـ جزيئات أكسيد الحديديك النانوية إلى تحسين الخصائص الحسية لليوجهورت أكثر من تلك المدعمة بنفس التركيز من أكسيد التجاري.

الكلمات الاسترشادية: أكسيد الحديديك، الخصائص الوظيفية، اليوجهورت.

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