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Iron Fortification in Yoghurt: Methods and Processes, as well as their Influence on Physico-chemical and Sensory Qualities

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ABSTRACT

It has been stated that anaemia affects 30% of the world's population, according to the World Health Organization. Iron fortification in food is the most efficient approach of relieving iron deficiency condition, according to research. Because iron fortification manifests itself in food in the form of colour, flavour, and general acceptability, researchers are attempting to increase the nutritional value of food without compromising the sensory aspects of iron fortified food products. A large number of researches have shown that yoghurt is the most effective vehicle for iron fortification in this approach. This study discusses the fortification techniques and diverse sources of iron utilised in the development of iron fortified yoghurt, as well as the influence of these methods and sources of iron on the physico-chemical and sensory aspects of the yoghurt. It is anticipated that the general concept of iron enrichment in yoghurt will lead to improvements in iron fortification in the near future.

HIGHLIGHTS

- Anaemia is a worldwide issue that must be addressed by dietary fortification. Yoghurt is a fermented dairy product that has been fortified with micronutrients in recent studies.
- This review investigated the issue of iron fortification in yoghurt in terms of physico-chemical qualities, organoleptic features, and shelf-life.
- To achieve the intended distribution of fortified iron in yoghurt, numerous efforts have been made utilising various methods and materials. This paper examines the most recent methods and processes for iron fortification in yoghurt that do not jeopardise chemical or microbiological quality.
- It is determined that it is imperative that safety standards are met prior to commercialization of fortified yoghurt.

Keywords: Yoghurt, iron, fortification, encapsulation, nanocapsulation

Milk is a full and balanced diet due to its high nutritional content. An issue arises when cow milk is given to a newborn since it has no or little iron, resulting in anaemia. Iron deficiency is a worldwide problem that affects the most susceptible groups, which include babies, school-age children, and women who are in the reproductive period of their lives. Approximately 20 percent of all maternal deaths are caused by iron deficiency anaemia, while approximately 30 percent of the world's population (2 billion people) is anaemic, according to the World Health Organization (WHO, 2001). In addition to a lack of iron-rich foods in one's diet, anaemia can be caused by other reasons. Because of their restricted capacity to ingest iron-rich meals, children and pregnant women are less likely than adults to

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consume the recommended daily intake of 10-12 mg/day of iron. Lactic acidification of milk can help to reduce iron deficient disorders (Silva *et al.* 2008).

Oral iron treatments normally work effectively for most patients, but long-term usage is restricted by mild side effects that concern patients (Gereklioglu *et al.* 2016). Hence, Iron fortification in food is the most effective method of alleviating this condition. Due to the fact that iron reacts with other dietary elements, iron fortification is more difficult than fortification of any other nutrient.

The source of iron is a critically important aspect in determining its bioavailability, and the oranoleptic quality of the diet should not be altered. Iron compounds, such as ferrous sulphate, have a high bioavailability, and the oxidative processes that it catalyses induce colour and taste changes in the food. In contrast, chelated iron sources were less reactive and had higher bioavailability (Drago and Valencia 2002). The ideal fortification is one that provides high bioavailability iron while without altering the sensory characteristics of a particular food. Food scientists came up with a variety of molecules to improve the iron content, including chelated versions, microencapsulated molecules, and nano molecules, among others. Fermented foods, particularly dairy products, are the most effective carriers for iron fortification, and the bioavailability of iron can be enhanced by the addition of ascorbic acid (Gillooly *et al*. 1984; Stekel *et al*. 1986). Darwish *et al*. (2021) reported in his recent study, nanoencapsulated iron fortified yoghurt improves the anemic condition in albino rats. Such different modification of iron to fortify in yoghurts and its effect on physico chemical and rheological characters of yoghurt is reviewed in this study.

Modification of iron molecules for fortification

As new technologies emerge, they are being used to optimise the effects of iron molecules in protected iron compounds, which include the following: Encapsulation in a liposome (Ding *et al*. 2009; Ding *et al*. 2011; Yuan *et al*. 2013; Xu *et al*. 2014), microencapsulation of iron (Kim *et al*. 2003; Jayalalitha *et al*. 2012; Gupta *et al*. 2015; Nkhata *et al*. 2015). These techniques not only help to avoid sensory properties of iron-fortified foods, but they also prevent iron oxidation caused by external agents, enhancing their bioavailability and intestinal absorption (Zimmermann 2004).

Zhang and Mahoney, (1989) used 3 different forms of iron that are ferric chloride, ferric citrate, Fe-casein complex to fortify in cheddar cheese.

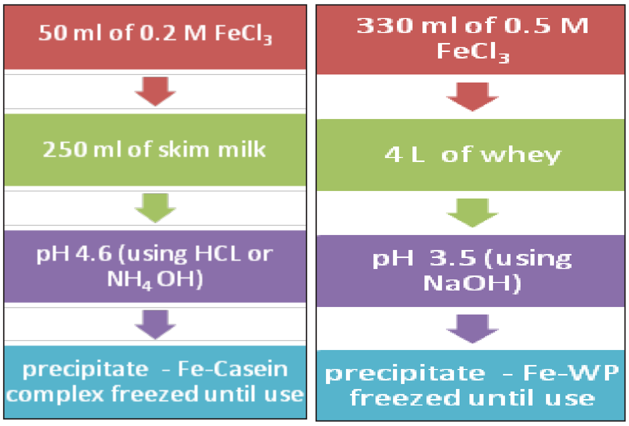

Fig. 1

Iron microcapsules prepared with polyglycerol monostearate (PGMS) coating material and ferric ammonium in the ratio of 5:1 and 50 mL distilled water was additionally added. The spray solution was heated at 55°C for 20 min, and stirred at 1,200 rpm for 1 min during spraying into a cylinder containing a 0.05% polyethylene sorbitan monostearate (Tween 60) solution at 5°C. The diameter of the nozzle orifice was 0.33 mm. The chilled fluid was centrifuged at 2,490×g for 10 min to separate unwashed microcapsule suspension. Microcapsules were formed as lipid solidified in the chilled fluid. The microencapsulation of iron and ascorbic acid were done in triplicate.

Kim *et al.* (2003) prepared Iron microcapsules with poly glycerol monostearate (PGMS), ferric ammonium sulfate and L-ascorbic acid. Coating material to core material ratio was 5:1, and 50 mL distilled water was additionally added and the spray solution was heated at 55°C for 20 min, and stirred at 1,200 rpm for 1 min during spraying. An airless paint sprayer nebulized a coating material-iron emulsion at 45°C into a cylinder containing a 0.05% polyethylene sorbitan monostearate (Tween 60) solution at 5°C. Microcapsules were serperated by centrifuging the chilled fluid at 2,490xg for 10 min. Sadiq and Doosh, 2019 encapsulated the iron molecules at the concentration of 5mg to 15 mg/100ml with sodium alginate and calcium

Table 1: Physico-chemical Properties of Iron Fortified Yoghurt in different Storage Period

Method of iron in to yohurt	Storage days	pH	Acidity	Syneresis (g/100 ml)	Moisture (M) / Total solids(TS)	Density (Kg/m³)	TBA values	References
Nano Inorganic particles	0	4.77 ± 0.01	0.87± 0.01	43.00± 0.01	83.75±0.02 (M)	1049.18± 0.99	—	Santillán-Urquiza *et al.* 2017
	28	4.30 ± 0.01	0.90± 0.01	53.45± 0.01	84.00± 0.04 (M)	1044.01± 0.08	—	
FeWP complex	0	4.33	—	11.00	14.30 (TS)	—	0.047	El-Kholy *et al.* 2011
	10	4.00	—	9.60	15.18(TS)	—	0.057	
Casein and whey protein chelated iron	0	4.30 ± 0.1	—	—	—	—	0.318	Hekmat and McMohan, 1997
	30	4.00 ± 0.07	—	—	—	—	—	
Iron microcapsules using PGMS	0	4.15	1.02	—	—	—	0.25	Kim *et al.* 2003
	20	4.03	1.10	—	—	—	0.40	
Whey protein chelated iron microcapsules	0	—	—	—	—	—	0.0132±0.02	Subash *et al.* 2017
	21	—	—	—	—	—	0.0345±0.04	
Bovine serum albumin nanoparticle of iron	0	4.58 ± 0.01	0.86 ± 0.04	64.80 ± 0.14	—	—	—	Darwish *et al.* 2021
	21	4.43 ± 0.03	0.98 ± 0.03	47.34 ± 0.28	—	—	—	
Ammonium ferrous sulphate	0	—	0.27±0	—	87.4 ±0.5 (M) 12.6±0.5(TS)	—	—	De and Nayak, 2017
	5	—	0.29±0.005	—	89.14 ±0.91 (M) 10.86 ±0.91(TS)	—	—	
Microencapsulated Fe-WP	0	—	—	—	—	—	0.002 ±0.00	Jayalalitha *et al.* 2012
	7	—	—	—	—	—	0.015± 0.00	
Aminoacid chelated Iron	0	—	0.9754	—	—	—	—	Ziena and Nasser, 2019
	7	—	1.2660	—	—	—	—	
Ferrous sulphate	0	—	1.0036	—	11.3 (TS)	—	—	
	7	—	1.4300	—	11.4(TS)	—	—	
Ferrous fumarate	0	—	1.04	—	10.9(TS)	—	—	
	7	—	1.49	—	10.8(TS)	—	—	
Ferric hydroxide polymaltose	0	—	0.8628	—	11.5(TS)	—	—	
	7	—	1.350	—	11.6(TS)	—	—	
Ferrous lactate hydrate solution	0	4.61 ± 0.06	—	—	—	—	—	Simova *et al.* 2008
	5	4.02± 0.06	—	—	—	—	—	

chloride by cold spraying method for fortification in to yoghurt.

Niosomes

Nanovesicles formed by the self-assembly of non ionic surfactants in aqueous media resulting in closed bilayer structures are called niosomes (Uchegbu & Vyas 1998). Gutiérrez *et al.* (2016) prepared the iron entrapped niosomes using the modified ethanol injection method as follows: aqueous phase of solution prepared with 300 mol/L of ferrous, 100 mol/L of ascorbic acid and 200 mol/L of citric acid with the iron concentration of 0.4 g/L. The organic phase was prepared by dissolving appropriate amounts of surfactants (P, PO, S80) and membrane stabilizer (D) at the same concentration into absolute ethanol with concentration of 2.4 g/L. Organic phase was injected, with a syringe pump at a flow of 130 mL/h, into the aqueous phase at 40 °C, stirring at 5000 rpm with a homogenizer. Vigorous agitation produced narrower size niosomes. Ethanol was removed using rotary evaporator and the total concentration of niosome membrane components was 4.8 g/L.

Bio iron nanoparticles

Organic form of iron improved the bioavailability in fortified foods. Hence, researchers made effort to prepare the biological form of iron, zinc and other microelements for food fortification. Kanamarlapudi

Table 2: Rheology of Iron Fortified Yoghurt

Method of iron in to yohurt	Firmness	Adhesiveness	Cohesiveness	Springiness	Chewiness	Reference
Noisome entrapped	0.263±0.002	-0.033±0.002	—	—	—	Gutiérrez *et al.* 2017
	0.256±0.003	-0.039±0.001	—	—	—	
Inorganic Nano particles	0.60 ± 0.01	—	-0.43 ± 0.01	—	—	Santillán-Urquiza *et al.* 2017
Iron aminoacid chelate	0.3	0.138	0.462	0.45567	0.063	Ziena and Nasser, 2019
Ferrous sulphate	0.5	0.211	0.423	0.422	0.089	
Ferrous fumarate	0.7	0.320	0.457	0.511	0.163	
Ferric hydroxide polymaltose	0.5	0.282	0.564	0.515	0.145	
$Fe(NO_3)3.9\ H_2O]$	0.35	—	0.25	—	—	Ocak and Kose, 2010

and Muddada, 2019 prepared the metal binding biomass using *Bacillus* subtilis and 100 mg/L of iron and used for fortification in chocolates. El-Saddony *et al.* (2021) prepared the biological ferrous nanoparticles (Bio-Fe NP) with the help of *Bacillus subtilis* and fortified in to yoghurt @ 200 - 400 µg/ml. That Bio-Fe NP was acted as natural preservative of yoghurt as well as antioxidant. They proved that iron fortified yoghurt improved the blood parameters values such as haemoglobin, ferritin and iron content in their study.

Physico-chemical changes

Incubation time

Since the yoghurt culture doesn't require iron for their growth, iron fortification did not affect the incubation time for setting of yoghurt as reported by El Kholy and Osman (2011) and Hekmat and Mc Mohan (1997).

Moisture

Moisture is not affected by the addition of minerals supported by Karam *et al.* (2013) and Santillán-Urquiza *et al*. (2017).

pH and acidity of iron fortified yoghurt were not differed with control yoghurt significantly and in storage period pH decreased from 4.65 to 4.3 and acidity increased from 0.86 to 0.9 g/ml due to production of lactic acid. (Santillán-Urquiza *et al*. 2017; Drago & Valencia 2002), they reported values of acidity for a yogurt enriched with iron was raised upto 1.8 g/ml. Iron fortifications did not affect fermentation time required for the yogurt mixes to reach pH 4.10-4.20.

Hekmat and McMahon (1997) reported that, Fe-WP fortified yoghurt showed no or minor effect on yoghurt quality and yoghurt fortified with Fe-CN gave accepted yoghurt quality. Azzam (2009) reported that the source of iron used for fortification had no significant effect or smoothness of yoghurt samples when fresh and during storage period.

Syneresis

Achanta *et al*. (2007) revealed that water holding capacity of yoghurt fortified with iron was improved. El Kholy and Osman (2011) revealed that no significant difference between control and iron fortified yoghurt, whereas it decreased during storage irrespective of control and fortified yoghurt. Jasim *et al.* (2020) observed that, upto 5 mM concentration of ferrous sulphate, ferrous chloride in yoghurt, there was reduced Syneresis, increased Water holding capacity, and improved viscosity and hardness. Whereas in increased concentration, there was a negative effect on rheological properties of yoghurt.

Sensory qualities of iron fortified yoghurt

Many previous studies revealed that, the colour, body and texture, overall acceptable quality of iron fortified yoghurt had not significantly differed with normal yoghurt (El-Kholy *et al*. 2011; Augustin 2000 and Hekmat and McMahon 1997).When the yoghurt fortified with iron salts as such like ammonium ferrous sulphate, it will have metallic flavour and it was suggested that Fe-WP or Fe-CN or Ferrous lactate could be fortified at the level of 20 mg/kg of milk (El-Kholy *et al.* 2011). Apart from metallic flavour, major off flavour observed in iron fortification was oxidised flavour since iron catalyse the lipid oxidation. When encapsulated iron coated with PGMS used in fortification of yoghurt, no deviation in the sensory properties of normal yoghurt (Kim *et al.* 2003). Azzam (2009) reported that iron source had not influenced the smoothness of yoghurt. Fresh iron fortified yoghurts were reported for better sensory scores than stored yoghurt after 3 days or 7 days when using different source of iron viz., iron amino acid chelate, ferrous sulphate, ferrous fumarate and ferric hydroxide poly maltose (Ziena and Nasser 2019). Simova *et al*. (2008) studied for the oxidised, metallic and bitter flavour of the yoghurt fortified with iron as ferrous lactate hydrate and noted that no significant difference was observed in flavour compared with control (unfortified) yoghurt. Iron can be fortified in to yoghurt upto 20 mg /litre as unencapsulated form and upto 80 mg/litre as encapsulated form without affecting the organoleptic quality (Subash *et al*. 2015).

Viability of probiotics in iron fortified yoghurt

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; Subash *et al.* 2015; Hekmat and Mcmahon 1997; Osman and Ismail 2004).

Bioavailability of iron in yoghurt

Many previous studies supported that yoghurt was a good vehicle to carry microbnutrients, probiotics, phytosterols etc. But in bioavailability of iron in calcium rich dairy products is little complicated due to chemical interaction of calcium and iron moleculels. However studies supported that bioavailability of iron was good in yoghurt (Drago and Valencia 2002; Dabour *et al.* 2019). Fortification of iron, reduced the bioavailability of calcium with respect to the nature of iron salts. According to an in-vitro study on bioavailability of iron in fortified yoghurt, ferric sulphate had better bioavailability than ferric chloride (Dabour *et al.* 2019).

CONCLUSION

Yoghurt is a fermented dairy product that is popular across the world due to its health benefits. It will serve as a vehicle for the fortification of a variety of nutrients. Because milk is lacking in iron, numerous researchers throughout the world have experimented with various methods and iron sources to fortify yoghurt. Because of its oxidised response, iron fortification in food alters the appearance, colour, and flavour. To address these issues, techniques to shield the fortified component against such reactions are being developed. The physicochemical changes and sensory qualities of yoghurt fortified with various techniques and different iron sources are discussed in this paper. Novel techniques of iron fortification in yoghurt may be accompanied with appropriate safeguards to counteract the detrimental effects of existing fortification methods. There are still certain research gaps to complete in order to commercialise the iron-fortified yoghurt's safety and requirements.

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