Microencapsulation Can Be a Novel Tool in Wheat Flour with Micronutrients Fortification: Current Trends and Future Applications – a Review

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Abstract

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Wheat flour fortification can be a novel and effective food based approach to improve effective micronutrient deficiencies that affect millions of people worldwide especially in the developing countries. Wheat is an important cereal crop grown worldwide and its per capita consumption is high even in the developing countries. Being a most popular dietary food component, fortification of wheat flour with micronutrients like iron, vitamin A, folic acid, zinc, and iodine is expected to be the most effective strategy to overcome the related deficiencies and, if mandated, could be helpful in achieving the international health goals. However, on the other hand food fortification (Direct mixing) with micronutrients might cause unwanted sensory changes and interaction with food components resulting in a lower bioavailability. Microencapsulation may be helpful to prevent unwanted sensory changes and diminish micronutrients interactions with wheat flour components. The current review will focus on the technical issues related to the fortification (Direct mixing) of wheat flour and prospects of microencapsulation technology in fortification.

Keywords: bioavailability; folic acid; iron; sensory changes; vitamin A; zinc

Deficiencies of iron, zinc, iodine, and vitamin A have been a focus of the governments and world organisations for decades and extensive efforts are being made to address this malnutrition (Akhter *et al.* 2011). The reliance of communities on plant based foods rather than meat and its products has resulted in boosting the prevalence of these nutritional deficiencies in the developing countries. According to WHO (2002), more than two billion people are anaemic, 254 million school age kids have vitamin A deficiency, and about two million have iodine deficiency. Wheat flour fortification with micronutrients will be helpful to eradicate the related deficiencies.

Cereals, especially wheat (*Triticum aestivum* L.), are widely used for human consumption in different parts of the world. Wheat is milled into flour which results in a loss of its components. Prabhasankar and Rao (2001) studied the effect of the milling methods on the composition of whole wheat flour in terms of protein and lipid contents. There were substantial losses in starch and lipid contents. The lipid content was low in stone milled flour (1.3 ± 0.01) followed by plate milled flour (1.5 ± 0.01) when compared to hammer (1.7 ± 0.02) and roller milled flours (1.9 ± 0.05). On the other hand, total amino acid content was high in roller milled flour compared to hammer, stone and plate milled flours. Similarly, another research group found vitamin E content to be 18.7 of vitamin E equivalents (α-TE) in stone milled flour and 10.8 α-TE in roller milled flour (Nielson & Hansen

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2008). According to Hussain *et al.* (1985), whole wheat flour contains moisture (12%), lipids (1.6 g), proteins (10 g), carbohydrate (72.6), fiber (1.3 g), ash (1.4 g), Ca (43 mg), P (284 mg), and Fe (45 mg) per 100 gram.

Flour possesses antinutritional factors such as phytic acid, trypsin inhibitors, and polyphenols, it interacts with multi charged ions like Ca, Fe, and Zn and reduces their bioavailability. Wheat grain contains 1–2% phytic acid located in the outer two layers (Nadeem *et al.* 2010). Phytic acid (myoinositol hexa-phosphoric acid, IP6) is the major phosphorus storage compound in most seeds and cereal grains. It can chelate multivalent metal ions, especially zinc, calcium, and iron. The binding results in the formation of insoluble salts which limits the bioavailability of the minerals (Rhou & Erdman 1995). Phytic acid is hydrolysed enzymatically by phytases or chemically during storage, fermentation, germination, food processing, and digestion in the human gut to lower inositol phosphates such as inositol pentaphosphate (IP5), inositol tetraphosphate (IP4), inositol triphosphate (IP3), and inositol di and monophosphates (Burbano *et al.* 1995). Among these, IP6 and IP5 have negative effects on the bioavailability of minerals, while the other hydrolytic products have a reducing capacity to bind minerals (Sandberg *et al.* 1989).

To compensate for such losses, micronutrients are added through fortification to overcome the related deficiency. Wheat flour (WF) is considered as a suitable vehicle for fortification with micronutrients and may be helpful to combat micronutrients deficiencies (Olivares *et al.* 2007). The incorporation of minor nutrients causes changes in the sensory properties of the fortified food like off flavour and change in colour. These changes might be due to the additives or interactions between, the additives and components of the food vehicle. Akhtar and Anjum (2007) reported that direct addition of iron to whole wheat flour produces off flavour and colour changes in fortified chapattis. The interaction of iron and zinc with whole wheat flour components reduces protein and moisture contents (Akhtar *et al.* 2008). Hence, a suitable technology must be used to deliver fortificants into fortified foods.

Microencapsulation is considered as an excellent approach to protect sensitive food ingredients from oxidative degradation, increase the retention of nutrients, avoid interaction with food components, and prevent undesirable changes in fortified food. It can help to maximise the bioactivity of the added compounds during processing and storage of the fortified food (Schrooyen 2000; Korhonen 2002; Ghosh 2006; Mourtzinos 2008). Microencapsulation involves various chemical (coacervation and molecular inclusion) and mechanical techniques (spray drying, spray chilling, liposomes, fluidised bed coating, and extrusion). Wegmuller *et al.* (2006) prepared microcapsules containing iron, iodine, and vitamin A using spray chilling and then fortification in salt. Colour and taste of the fortified salt remained the same as in the unfortified one. In this review, we will discuss the micronutrients deficiencies and their consequences, fortification (direct mixing) of micronutrients in wheat flour and related technical factors that affect food quality. Moreover, we will also emphasise the promising approach to microencapsulation technology in fortified foods.

Micronutrient deficiencies and consequences. Micronutrients deficiency is a major threat for more than half of the world population living in the developing countries (Mayer *et al.* 2008). According to FAO (2004), micronutrients deficiency has been affecting at least two billion people worldwide. The deficiencies of iron, iodine, zinc, and vitamin A are the main manifestations of malnutrition in the developing countries (Muller & Krawinkel 2005). Iron deficiency is the most common and widespread nutritional disorder in the world and it makes a public health problem in both industrialised and non industrialised countries. In severe cases iron deficiency causes anaemia. Anaemia is the outcome of a reduced haemoglobin level in blood causing mental disorders, severe pregnancy problems, and premature delivery (Bui & Small 2012).

According to Bhutta (2008), most of the vitamin A deficiencies occur in the developing countries and are common in South and South East Asia. Women, being the most susceptible group, are influenced by vitamin A deficiency (VAD) during pregnancy and lactation (Zimmermann *et al.* 2006). VAD affects normal functioning of the visual system, cell function, epithelial cellular integrity, immune function, and reproduction (WHO/FAO 2006). VAD has affected over two million people, especially pregnant women and young children, in the developing countries (WHO 2003).

Folic acid deficiency causes megaloblastic anaemia, increases the susceptibility to cancer, leads to neural tube defects and vascular diseases (Garcia-Casal 2005). The consequences of iodine deficiency include goitre, cretinism, paralysis, and deaf-mutism (Diosady *et al.* 2002). Zinc interferes with the cell division, protein synthesis, and growth which indicates its need for infants and pregnant and lactating women (Romana *et al.* 2011). Zinc deficiency affects infants, pregnant and lactating women (Brown *et al.* 2007), and also leads to growth retardation and cognitive impairment (Prasad 2003). Food fortification has contributed effectively to improving the micronutrient status in European adults and children (Baltussen *et al.* 2004). According to WHO (2001), in the Eastern Mediterranean region bread consumption is high in most countries and flour fortification offers an opportunity to deliver adequate levels of iron. In this study, the fortification with micronutrients of wheat flour is strongly recommended to overcome the related deficiencies. Micronutrient fortified foods have certain technical limitations which must be considered on the priority basis to make this program successful.

Micronutrients fortification: unencapsulated vs. microencapsulated forms

Iron. Akhtar *et al.* (2008) fortified whole wheat flour with ferric sodium ethylenediaminetetraacetic acid (NaFeEDTA), elemental iron, zinc sulphate, zinc oxide, and studied the effects of fortificants on the flour quality. The moisture content decreased due to elemental iron (8.44%) and zinc oxide (8.35%), respectively, being almost (5.4%) compared to unfortified flour (8.83%). However, protein content of flour decreased significantly due to elemental iron (11.11%) and zinc oxide (11.16%) as compared to the control (11.88%). However, fat and fibre contents of the flour remained unchanged whereas ash content was increased (26%) due to elemental iron and zinc oxide.

Quality attributes of fortified chapatti (used in pieces as scoop to pick up curry and they must be soft and pliable) like flexibility (8%), texture (15%), and chewiness (11%) were lost as compared to the control. Fortification had deteriorative effects on the chemical stability of flour as well as on the texture attributes of the product.

In this work, whole wheat flour (WWF) was fortified with a premix containing ferrous sulphate, ethylenediaminetetraacetic acid (EDTA), and folic acid (20:20; 1.5 ppm). The effect of storage stability was also evaluated. Whole wheat flour, and Naan (a product of wheat flour, especially used in Asia), were individually fortified with ferrous sulphate and iron content was analysed. No effect was observed of storage time on the quantity of iron but the ferrous form was converted to ferric, which affected bioavailability. Moreover, phytic acid content was also estimated in both WWF and Naan, average phytic acid content in WWF was in the range of 0.810–0.886%. This was due to high temperature and humidity which stimulated phytase enzyme to degrade phytic acid (Hinnai *et al.* 2000). Phytic acid content (0.54–0.611%) decreased drastically in Naan due to the enzyme activity as well as fermentation and baking processes involved in its preparation. Naan fortified with (40 ppm) iron was acceptable over (60 ppm), whereas the sensory characteristics also showed decreasing trends in flexibility, chewing ability, and overall acceptability. The softness of Naan was also affected by the electrolytic effect of iron on the dough proteins (Rehman *et al.* 2006). In another study by Salgueiro *et al.* (2005), ferrous sulphate and ferrous gluconate (65 mg/kg) were used to fortify wheat flour to confirm the stability and bioavailability of the stable ferrous gluconate in flour compared to control (ferrous sulphate). Both of them showed the same bioavailability in wheat flour and water. On the other hand, phytic acid, a component of wheat flour, was found responsible for lowering the bioavailability of iron. Biscuits prepared from flour fortified with ferrous sulphate showed unpleasant sensory attributes, grey colour and taste were different from the unfortified ones. In this study, 30 and 40 mg/kg of ferrous sulphate was used to fortify wheat flour. The effects of fortificants on the flour stability were evaluated on storing at room temperature and the elevated to 40°C, respectively. The elevated temperature and high humidity (70–100%) caused oxidation and ultimately affected the fat content (Ayelign *et al.* 2012).

Tripathy and Platel (2011) used finger millet flour as a vehicle for ferrous fumarate and ferric pyrophosphate fortification, the bioaccessibility of both fortificants having been analysed. Minor differences were found in the bioaccessible iron content of fortified flour (ferrous fumarate 0.29, ferric pyrophosphate: 0.27 mg/100 g) compared to the unfortified flour (0.23 mg/100 g). This was due to the presence of large quantities of phytate,

Table 1. Summary of different micronutrients fortified foods, fortification methods, fortificants used and their characteristics

Fortification method (Direct addition)	Reference	Fortificants	Product/food	Level	Results
Mixer blender	Salgueiro *et al.* (2004)	ferrous sulphate, stable iron gluconate (SIG)	biscuits	65 mg/kg	Sensory characteristics (colour and taste) remained unchanged in SIG fortified biscuits but altered in case of ferrous sulphate fortified one
Mixingwith volumetric screwtype feeder	Akthar *et al.* (2008)	sodium iron EDTA, elemental iron, zinc oxide and sulphate	whole wheat flour	60–90 mg/kg	Moisture content reduced due to elemental iron and zinc oxide. Like moisture content both fortificants reduced the protein content nearly 6%. However, ash content increased to 23%
Mixing with chakkies (small scale local grinder	Alam *et al.* (2007)	ferrous sulphate, EDTA(ethylene diamine tetraacetic acid), folic acid	whole wheat flour	20, 20, 1.5 ppm	Naan were prepared and significant deterioration occurred in appearance, odour, and texture and folding ability, as storage period increased to 3 months. Moreover, starch and fats degraded due to heat and oxidation
Mixer blender	Ayelign *et al.* (2012)	ferrous sulphate	wheat flour	40, 30 mg/kg	No change in iron level observed at both room and elevated temperature for a period of 45 days storage. However, oxidation of iron from Fe^{2+} to Fe^{3+} occured due to humidity which ultimately reduced bioavailability
Wet method of fortification	Ayelign *et al.* (2012)	pottasium iodate	salt	66 mg/kg	Potassium iodate reduced to elemental iodine due moisture intake by packaging material at both elevated and room temperature. Elemental iodine rapidly sublimes and lost due to diffusion
Mixingwith volumetric screwtype feeder	Akhtar and Anjum (2007)	Sodium iron EDTA, elemental iron, zinc oxide and sulphate	whole wheat flour	60–90 ppm	Chapattis prepared from fortified flour with elemental iron rejected due to off flavour production. However, blackish colour appeared on chapattis due to elemental iron
Mixer blender	Rehman *et al.* (2006)	ferrous sulphate, EDTA and folic acid	whole wheat flour	20, 20, 1.5 ppm	Ferrous iron significantly reduced in flour and naan due to ferric iron oxidation during storage
Screw ribbon blender	Wegmuller *et al.* (2006)	iodised salt, ferric pyro phosphate	salt	3 mg Fe/g salt	Organoleptic properties of control and double fortified salt dishes were same but, bioavailability of ferric pyrophophste found to be low than ferrous sulphate
Direct mixing	Popovici *et al.* (2006)	crystalline iodine (I2)	sunflower oil	100, 10, 1 µg/ml	Increased in oxidation products (aldehydes 2,4 dienale, 2- alchenale) were observed with the severity of thermal treatment
Direct mixing	El-Din *et al.* (2012)	ferrous chloride, sulphate, zinc sulphate and acetate	buffalo milk	40, 60, 120 mg/kg	No variation in the ash and moisture content while pH was significantlyaffected. TBA number increased in first month of storage but in latter two months decreased due to degradation of fortificants
Direct mixing	Tripathi and Platel (2011)	ferricfumarate, pyrophosphate	finger millet flour	6 mg/g	Iron content decreased in fortified flour due to antinutritional factors
Wet agglomeration	Rutkowski and Diosady (2006)	retinylacetate, palmitate, ferrous fumarate, sulphate, NaFeEDTA, potssium iodate, iodide	salt	250 Vit. A IU/g salt 1000 ppm iron 50 ppm iodine	Loss of nutrients occured and colour of TFS^{c} changed
Direct mixing	Abd-Rabou *et al.* (2010)	zincacetate, chloride and sulphate	cheese	150 mg/kg	Considerable increase observed in the native nutrients content, acidity, TBA, PV and fat acidity value in fortified cheese compared to control. Finally cheese on the basis of texture was unacceptable

TBA – thiobarbituric acid; PV – peroxide value; TFS – triple fortified salt tannin, and calcium which inhibit the accessibility of iron. Akhtar *et al.* (2010) studied the effects of storage conditions on the stability of the added iron content in WWF. A significant decrease was observed in the iron content of fortified flour. However, the levels of fortificants had no effect but their type significantly influenced the iron content. Out of all iron fortificants, NaFeEDTA was more susceptible to degradation during storage. The effects of fortificants on the quality were also studied on curry powder fortified with iron (ferrous slphate, ferrous fumarate, and NaFeEDTA) (Kam *et al.* 2011). Dark colour developed in the curry powder fortified with elemental iron while no adverse effect was observed on the physical, chemical or sensory qualities.

Kwak and Yang (2002) reported that thiobarbituric acid (TBA) absorption was higher in the milk fortified with unencapsulated iron than with the encapsulated iron. Recently Jayalalitha *et al.* (2012) also prepared microencapsulated iron whey protein complex to fortify yogurt, which had good sensory quality and suppressed the oxidised flavour of iron. High TBA absorption was observed in yogurt fortified with unencapsulated iron. This was due to the interaction of iron with casein of milk, the presence of oxygen acted as pro-oxidant and triggered lipid oxidation. When oxidation occurs, free fatty acids accumulate and ultimately TBA absorption increases. The same phenomenon was also observed when encapsulated ferric ammonium sulphate was used to fortify drinking yogurt; TBA absorption was low as compared to the control (Kim *et al.* 2003). Choi *et al.* (2009) prepared W/O/W emulsion to encapsulate iron in the inner aqueous layer to overcome oxidation. Emulsion of water in corn oil with Tween 60 as an emulsifier was prepared. Encapsulation efficiency of iron was 99.75% and TBA reactive substances (TBARS) production was negligible. They also studied the impact of iron on the stability of fish oil. Fish oil emulsion was prepared and mixed with first emulsion W/O droplets, iron interacted with fish oil and triggered oxidation and ultimately increased TBARS. In another study, a stable form of iron (iron pyrophosphate) was encapsulated by spray drying, palm oil with 1% lecithin was heated at 85°C, and ferric pyrophosphate suspension was prepared, passed through the spraying tower, atomised, and cooled with liquid nitrogen. Iron microcapsules of variable sizes were prepared and evaluated for their bioavailability assessment. The highest bioavailability was observed with the lowest particle size (Wegmuller *et al.* 2004). Martin and Jong (2012) used cold set gelation method to encapsulate iron in whey protein. Iron bioaccessibility was high at low pH and the authors also concluded that iron amount can be increased by thermal treatment of whey protein.

Yogurt (4, 7, and 10 mg iron/100 g yogurt) was fortified with ferrous sulphate, iron whey protein complex, and microencapsulated iron whey protein complex. Lipid oxidation and sensory properties of the fortified yogurts were monitored over seven days of storage at 4°C. There was no significant difference observed in TBA and PV (peroxide value) between unfortified yogurt and those fortified with iron whey protein complex and microencapsulated iron whey protein complex. However, yogurt fortified with ferrous sulphate was highly oxidised and metallic taste developed. The flavour and overall quality of yogurt fortified with microencapsulated iron-whey protein complex were similar to those of unfortified yogurt and were well accepted by the sensory panellists (Azzam 2009). Abbasi and Azzari (2011) prepared milk fortified with microencapsulated ferrous sulphate and ferric ammonium sulphate by liposome and fatty acid ester (FAE) methods. The stability of microcapsules in milk was studied. TBA absorption in milk fortified with microencapsulated iron did not change even with the addition of further iron to milk. However, TBA absorption increased significantly (0.0174–0.0213) in milk fortified with unencapsulated iron. It increased to about (0.0174–0.0900) when stored at 4°C for three days. Both techniques reduced the rate of lipid oxidation by 60%. Sensory characteristics like astringency or bitterness of milk fortified with microencapsulated iron were similar to those of control. On the other hand, significant differences in terms of metallic taste was found in milk fortified (7 and 14 mg/l) with unencapsulated iron. Microencapsulation techniques considerably mask the metallic taste of iron. In another study, ferric ammonium sulphate microcapsules were also prepared using airless paint sprayer method. In this method, the emulsion of polyglycerol monostearate and iron salt was nebulised into tween 60 chilled solution, the resulted mixture was centrifuged and microcapsules were obtained. The encapsulation efficiency of microcapsules was 75% and fortified in milk. Chemical lipid oxidation rate was high in milk fortified with unencapsulated iron. TBA

Table 2. Summary of different microencapsulated micronutrients fortified foods, fortification methods, fortificants used and their characteristics

Encapsulation technique	Reference	Fortified foods	Load	Encapsulation efficiency (%)	Mean particle size (μm)	Micronutrient	Results
Fatty acid ester	Abbasi and Azzari (2011)	milk	1 g	85	–	iron	Lipid oxidation reduced in microencapsulated iron fortified milk. Both FAE and liposome reduced rate of lipid oxidation by 60%. Unencapsulated iron fortified milk sensory characteristics like astringency; bitterness and color were significantly different compared to microencapsulate one
Liposome	Abbasi and Azzari (2011)	milk	15 g	81.3	–	iron	Microcapsules prepared were stable and protected iron from degradation
Airless paint sprayer	Kwak *et al.* (2003)	milk	1 g	75	2–5	iron	TBA absorption value (0.35) remained same before & after storage in encapsulated iron fortified milk. Microcapsules stability was excellent and retained 88 & 84% iron respectively after storage
Airlesspaint sprayer	Kim *et al.* (2003)	yogurt	1 g	75	2–5	iron	pH, acidity and TBA values remained unaffected in encapsulated iron fortified yoghurt. However, sensory characteristics like astringency, bitterness showed significant variation compared to yogurt fortified with unencapsulated iron
Spray chilling	Zimmerman *et al.* (2004)	salt	2 mg, 30 μg, 60 μg	–	100	iron iodine vitamin A	Microcapsule contained iron, iodine & vitamin A was stable and only 12–15% iodine and vitamin A content lost, while colour of salt remained unchanged
Spray chilling	Wegmuller *et al.* (2006)	Moroccan meals	100 mg, 2 mg, 5 mg	–	100	iron iodine vitamin A	Iron, iodine and vitamin A microcapsules fortified in salt showed no change in color and overall acceptability of TFS was good
Solvent evaporation	Lee *et al.* (2003)	–	0.5 g	100	38.6	vitamin A	–
Spray drying	Porrarud and Pranee (2010)	–	10	13.12 mg/kg	16.13	zinc	Modified starch spray dried zinc powder was stable and capable of being used as food additive
Spray drying	Cheung *et al.* (2008)	Asian noodles		97	–	folic acid	Encapsulated folic acid remained same during and after cooking
Cooling extrusion	Li *et al.* (2010)	salt	50–100 μm	87–94	300–700	iodine	50–60% iodine content lost in unencapsulated iron extruded particles fortified salt whereas, only 15% iodine content reduced even with high quantity of iron when microencapsulated extruded iron fortified in salt
Spray drying	Romita (2011)	salt	10% (w/w)	85	< 20	iron and iodine	DFS (microencapsulated iron & iodine) retained 70-85% and 80–90% iodine when stored in environmental chamber & room temperature conditions. However salt fortified with unencapsulated ferrous fumarate retained only 60% iodine
Polymer complex	Jayalalitha *et al.* (2012)	yogurt	–	–	–	iron	Oxidised flavour of iron was suppressed and TBA absorption was low in yogurt fortified with microencapsulated iron whey protein complex

Table 2 to be continued

Encapsulation technique	Reference	Fortified foods	Load	Encapsulation efficiency (%)	Mean particle size (μm)	Micronutrient	Results
Polymer complex	Azzam (2009)	yogurt	–	–	–	iron	TBA and PV remained unchanged when yogurt was fortified with iron whey protein complex and microencapsulated iron whey protein complex. The yogurt fortified with unencapsulated ferrous sulphate had metallic taste
Polymer complex	Madziva *et al.* (2005)	–	–	–	–	folic acid	Alginate-pectin microcapsules increased folic acid retention and was found to be 80–100%
Spray drying	Tomiuk *et al.* (2012)	bread	–0.0130 g/l	87.1	–	folic acid	Microencapsulated folic acid (L-5-methyltetrahydrofolic acid) in skimmed milk with and without sodium ascorbate gave better recovery of 95.1 and 96.4%, respectively

FAE – fatty acid ester; TBA – thiobarbituric acid; TFS – tripple fortified salt; DFS – double fortified salt; PV – peroxide value

absorption increased (0.38–0.64) in milk fortified with unencapsulated iron after 12 days of storage, whereas TBA absorption (0.35) remained unchanged in milk fortified with encapsulated iron even after storage. Microcapsules lost iron (12 and 16%) after three days of storage, the loss rising to 18 and 21% after 12-day storage at 4 and 20°C, respectively. Astringency, bitterness, metallic taste, and colour changes were significant milk fortified with in unencapsulated iron compared to control after three days of storage (Kwak *et al.* 2003). Similarly, iron microcapsules were also prepared by using Airless paint sprayer method (Kim *et al.* 2003). This group fortified yogurt with microencapsulated iron, and the stability and effects on the yogurt composition were studied. Unencapsulated iron caused a decrease in pH and subsequently acidity increased during storage. The fortification with encapsulated and unencapsulated iron did not affect the microbial count of yogurt. Encapsulated iron masked off the taste and flavour of iron while significant difference in astringency and bitterness was observed in yogurt fortified with unencapsulated iron. Microcapsules containing ferric pyrophosphate, potassium iodide, and retinyl palmitate were prepared by using spray chilling technique. In this, ferric pyrophosphate (40% w/w) and lecithin (1% w/w) in hot molten palm fat were filled in spray tower, iron salt, and retinyl particles were also added into the mixture. This mixture was transferred immediately to pre-cooled spray tower to avoid oxidation and the atomised particles were solidified. The size of microcapsule was 138 and 132 μm, respectively. The microcapsules were added in salt to prepare Triple fortified salt (TFS). There was no difference in colour or taste, and the overall acceptability was good (Wegmullar *et al.* 2006). Similarly, another research group prepared microcapsules containing potassium iodide, ferric pyrophosphate, and retinyl palmate using the spray chilling method. The size of capsule was 100 μm. The were after fortification of Moroccan salt tested on school age children suffering from goitre, anaemia, and high prevalence of vitamin A deficiency. The loss of iodine and vitamin A during six month period of storage was 12–15% while the colour of salt remained stable (Zimmermann *et al.* 2004). The salts fortification with microencapsulated iron showed better results than direct mixing, avoided sensory changes, and ensured the stability of fortificants in food.

Vitamin A. Wheat flour fortified with vitamin A at 30, 40, and 50% of the recommended daily allowance (RDA) was used to prepare cookies in order to evaluate the effect of retinyl acetate on the chemical components of cookies. It was found that the moisture content increased from 2.70% to 2.76% in cookies fortified with 50% RDA, whereas ash, protein, fibre, and fat contents remained unchanged. On the other hand, significant increase in the moisture content (2.53–2.88%) was observed after 90 days of storage. Significant changes in the sensory characteristics of the fortified cookies in terms of colour and flavour were also observed. However, vitamin A content decreased (9.96–9.13 μg) when stored at ambient temperature for a period of 90 days (Mahmood *et al.* 2008). Butt *et al.* (2007) reported that at elevated temperature vitamin A was oxidised, lost its ability to bind with lipids and its absorption was ultimately affected. Ilic and Ashoor (2010) fortified plain and raspberry low fat yogurt with vitamin A and C. The changes in pH, titratable acidity, and vitamin A and C contents were evaluated to interpret the effects of fortificants on yogurt quality. Yogurt with 10 000 IU of vitamin A and 300 mg of vitamin C provided at least 100% RDA of both fortificants while other formulations showed changes in pH, titratable acidity, and sensory attributes. This group also studied the effect of cooking on vitamin A stability in porridges, in soy fortified bulgur (SFB), and in dumplings with fortified vegetable oil. SFB was fortified with folic acid, thiamin, riboflavin, niacin, vitamin A, calcium, and iron in a dry blended premix, whereas vegetable oil with retinyl palmitate only. 33 and 6% (w/w) losses in the vitamin A content of SFB porridges and dumplings fried in vegetable oil were observed (Rowe *et al.* 2009). Raileanu (2002) and Rutkowski and Diosady (2006) used the pan agglomeration method to prepare a granular premix of vitamin A, iodine, and iron. Three premixes were prepared, i.e. vitamin A as the only ingredient, vitamin A and iodine, and vitamin A, iron, and iodine in single premixes. In these experiments, the granulated premix was passed through a mesh sieve with the size range of 300 and 700 μm. Vitamin A retention in triple fortified salt (TFS) after three months of storage was 39% at 40°C and 60% RH (relative humidity), and 25% at 40°C and 100% RH, respectively. The effects of iron and iodine on the stability of vitamin A were also evaluated. This was found to be more stable in the presence of NaFeEDTA where more than 50% retention was achieved. Ferrous sulphate had deteriorative effects on vitamin A content in triple fortified salt and was found to be unacceptable organoleptically as the products exhibited yellow/green coloration with strong metallic taste.

Diosady *et al.* (2002) found that vitamin A, iron and iodine interact with one another and also with impurities in salt which depleted the added micronutrients. They prepared double fortified salt (DFS) with microencapsulated iron and iodine, and their reactivity was reduced. Similarly, another group prepared triple fortified salt (TFS) with iron, iodine, and vitamin A, encapsulated granulated particles of selected sizes (300 and 700 μm) in pan agglomerator, and hydrophilic coating agents, i.e. shellac, methylcellulose, and hexaethylcellulose were used. However, soy stearine as hydrophobic agent was used to coat the particles. The retention of vitamin A was found to be 35 ± 8%, when 40% soy stearine with NaFeEDTA was stored at 40°C and 60% RH for three months (Rutkowski & Diosady 2007).

Folic acid. Boeneke and Aryana (2008) studied the effect of fortification with folic acid on the characteristics of lemon yogurt. Lemon yogurt was fortified with 25, 50, 75, and 100% RDA of 400 μg folic acid, added before and after pasteurisation. Folic acid did not alter the composition of the yogurt. Protein, fat, moisture, and ash contents remained unchanged after fortification. Pasteurisation had no effect on the folate content but influenced the viscosity of yogurt. Yogurts prepared after pasteurisation had lower average mean viscosity values compared to those before pasteurisation. The mean value for syneresis ranged from 92.67–137.33 ml and was significantly expressed in lemon yogurt fortified with folic acid after pasteurisation. This was due to the casein micelle network alterations. Folic acid levels and storage time showed significant effects on yogurt yellowness, the mean values decreased to 28.6 g/100 g after three weeks but then increased to 42 g/100 g after five weeks. Similarly, the flavour scores of lemon-flavoured yogurt were significantly affected by the levels of folic acid and storage time. Yogurt fortified with folic acid prior to pasteurisation showed less free whey (syneresis) but its colour and appearance was not acceptable. Significant change in pH value of strawberry yogurt was also observed when fortified with folic acid (Boeneke & Aryana 2007).

In another study, the effects of fortification with vitamins – thiamine (B_1), riboflavin (B_2), niacin (B_3), folic acid (B_9), and magnesium on the physiochemical, microbiological, and sensory attributes of yogurt were evaluated. Yogurt mixes contained 30, 60, and 90% RDA of vitamins, minerals, and fibre (Ceolus fiber DF-17) 176 g/5.570 kg. Viscosity of the yogurt containing 60% RDA of vitamins and minerals had a high viscosity whereas syneresis was low in 30% RDA mix compared to 60% and 90%. The highest *b** value (yellowness) was detected when the yogurt mix was fortified with 90% RDA (Boeneke & Aryana 2008). Achanta *et al.* (2007) analysed the effect of folic acid (400 μg RDA) addition on the physicochemical attributes of the reduced fat milk and on consumers' acceptability. Significant differences were found in pH, colour, and fat content of milk. However, sensory panellists did not find any difference in the colour, texture, and appearance of the fortified milk compared to control.

Extruded ultra rice fortified with folic acid (3.68 % to 11.04%) was prepared using the extrusion method. Different iron salts were added as fortificants along with folic acid. The effect of iron fortificants on folic acid stability was evaluated. The retention of folic acid content in rice after three and nine months of storage was > 80 and 60%, respectively. The ferric pyrophosphate did not affect folic acid retention in any formulation with low iron content. However, the formulation with higher iron content was detrimental. Ferric pyrophosphate affected the extrusion; the grains produced were rough and of irregular size. Finally, all the rice formulations prepared after extrusion resembled native rice in shape and size but had darker colour (Li *et al.* 2011a). The effect of baking on the folic acid content was also studied with wheat and rye bread, and significant losses of folic acid in wheat (237 ± 7–184 ± 5) and rye breads (223 ± 9–158 ± 6) after baking were observed. They found that only the added folic acid content decreased but the endogenous one remained unchanged during baking (Gujska & Majewska 2005).

According to Li *et al.* (2011b), folic acid was added to iodised salt and sugar fortified with vitamin A by direct blending as powder, and was sprayed on the target in aqueous or suspended states, and blended with microencapsulated premix. The retention of folic acid when blended in microencapsulated form was > 80% in iodised salt and 70% in sugar, respectively. It is evident that the method of fortification strongly influenced the stability of fortificants. Alginate-pectin microcapsules were prepared to incorporate folic acid in food, as it is sensitive to light, temperature, and processing conditions. Capsules were prepared by mixing both polymers in different proportions (A70:P30, 60:40, 80:20, 100:0), the retention of folic acid in freeze dried capsules after 11 weeks of storage at 4°C was 100% (A70:P30, 60:40), 80% (80:20), and 30% (A100: P0), respectively. The blending of polymers increased the stability of folic acid in capsules, prevented its leakage and ultimately enhanced its bioavailability (Madziva *et al.* 2005).

Another research group prepared biscuits with encapsulated and unencapsulated 5-methyltetrahydrofolic acid (5-CH_3THF) and studied its stability under different baking conditions. 5-CH_3THF was encapsulated using spray drying with variable ratios of pectin and sodium alginate (60:40, 70:30, 80:20). The microcapsules prepared with pectin to alginate in 60:40 ratio revealed the highest retention (68.8%) of folic acid and were preferred as fortificants in biscuits. Microcapsules (426 μg/g) and unencapsulated 5-CH_3THF (563 μg/g) were mixed with flour using a planetary mixer in dark room; other ingredients used in the biscuit preparation were subsequently added. Biscuits containing unencapsulated 5-CH_3THF and encapsulated 5-CH_3THF were baked at three different temperatures, 180, 200, and 220°C. The retention of folic acid content in the biscuits fortified with the encapsulated 5-CH3THF was high (19.1 ± 3.0, 8.5 ± 1.1, 4.9 ± 0.5 at 180, 200, and 220°C, respectively) compared to that in the biscuits fortified with the unencapsulated 5-CH3THF (5.4 ± 0.4, 6.7 ± 1.0, 4.4 ± 0.6 at 180, 200, and 220°C, respectively) (Shrestha *et al.* 2012).

Tomiuk *et al.* (2012) prepared L-5-methyltetrahydrofolic acid (L-5-MTHF) microcapsules using skim milk as the encapsulating agent for the fortification of bread to increase its bioavailability. Skim milk powder (99.9870 g/l), L-5-MTHF (0.030 g/l) and skim milk powder, L-5-MTHF with sodium ascorbate (ASC) 1 g/l were mixed in the dispersed form and finally spray dried to obtain microcapsules. The recovery of L-5-MTHF with ASC was 95.1% while it was 96.4% without ASC. In order to evaluate the bioavailability of L-5-MTHF, the loaded microcapsules were passed through the simulated gastric fluid (SGF) for 30 minutes. It was found that both microencapsulated L-5-MTHF and L-5-MTHF without ASC were released during 10 min in GIF, its quantity being 0.26 μg/ml when released completely. After this, microencapsulated L-5-MTHF, L-5-MTHF with ASC, and free (unencapsulated) L-5-MTHF, L-5-MTHF with ASC were used for bread fortification. The addition of L-5-MTHF to breads using microencapsulated ingredients resulted in a significantly increased content of microencapsulated L-5-MTHF, L-5-MTHF with ASC (138.2 ± 2.2 and 149.6 ± 1.2) compared to breads with free L-5-MTHF, L-5-MTHF with ASC (98.4 ± 2.4 and 113.9 ± 3.2).

Iodine. Tulyathan *et al.* (2007) studied the retention of iodine in parboiled rice and its effect on the pasting characteristics. The pasting temperature (94.97–95.08°C) and peak time (6.93–7.00 min) increased significantly in iodine fortified parboiled rice compared to parboiled milled rice. However, the peak viscosity (1273–1223 cp) and breakdown viscosity (126–122 cp) were decreased. Fortified Parboiled milled rice flour retained 86–90% of iodine while milled rice 97–100% after dialysis. Parboiled milled rice flour fortified with potassium iodide and iodate retained 78.44 and 81.84 μg iodine/100 g rice, respectively. This group used different formulations of iodine and iron, to interpret the iodine stability in salt. The double fortified salt (DFS) with ferrous sulphate and potassium iodide lost the whole iodine content when stored for one month at 40 and 60% RH while with potassium iodate the loss was 93% iodine. DFS with potassium iodate and ferrous fumarate retained 80.9% of iodine at room temperature and 79.7% after seven months storage at 40°C and 60% relative humidity (RH). Potassium iodide which is more stable at room temperature retained 79% iodine content and showed random decrease after six month storage (27% at 40°C and 60% RH, 23% at 40°C and 100% RH) when the temperature and humidity increased (Diosady *et al.* 2002).

DFS having iodine microcapsules mixed with either ferrous fumarate or sulphate were prepared and the effects of storage temperature and humidity on the fortificants stability were evaluated. Potassium iodide (KI) and iodate (KIO_3) microcapsules were prepared using spray drying and fluidised bed coating. The salt was fortified with 1000 mg/kg iron and 50 mg/kg microencapsulated KI or KIO_3, mixed in a blender, and the powders were added into the salt. KI and KIO_3 were encapsulated by using gelatin, sodium hexametaphosphate, dextrin (modified starch), and pure sodium chloride as wall materials in fluidised bed coating and spray drying techniques. In DFS, dextrin coated microcapsules obtained after spray drying were found to be suitable in terms of stability when wall-core ratio was 1:200. The effects of the storage time and relative humidity on the stability of iodine were evaluated in different formulations (ferrous sulphate and KI spray dried in dextrin, ferrous fumarate and KI spray dried in dextrin, ferrous sulphate and KIO_3 spray dried in dextrin, ferrous fumarate and KIO_3 spray dried in dextrin, ferrous lactate and KIO_3 spray dried in dextrin). Iodine retention (98.4–101.9%) was excellent in ferrous fumarate and KI spray dried microcapsules at 40°C and 60% RH when stored for 12 months. Encapsulation provided a barrier between the iodine compound and the components of the product, the salt, its impurities, and the added iron (Diosady *et al.* 2002). Li *et al.* (2010) studied the stability of iodine in iodised salt fortified with microencapsulated iron (ferrous fumarate) made by extrusion-based encapsulation method. In this ferrous fumarate, durum flour as the binder, and TiO_2 as the colour masking agent were used, the premix was prepared, microencapsulated, and blended with iodised salt in ratios 1:160 to 1:200. The samples were stored in an environmental chamber at 40°C and 60% RH, iron and iodine contents were analysed. The formulation prepared with extruded ferrous fumarate, 30% (w/w) binder, 25% (w/w) TiO_2, 10% (w/w) Methocel hydrophilic polymer, was found to be the best. Only 15% iodine content was lost while the added iron was 10%. A significant loss in the iodine content (50–65%) of the uncoated extruded iron particles was observed, whereas the retention of iodine by extruded particles coated with 15%, 10% polymer (Methocel) retained 75% and 80% iodine, respectively, in DFS. The stability of iodine can be further increased by the preparation of compacted iron premix using extrusion and polymer coating; it may prevent the interaction of iron with iodine and ultimately prevent iodine loss as it occurred in control (directly added ferrous fumarate in iodised salt).

Iron (ferrous fumarate) was microencapsulated using spray drying, microcapsules formed in the size range of < 20 μm. Ferrous fumarate was encapsulated in the suspended (10% w/v) as well as in the dissolved (1.2% w/v) form using different polymers (carboxymethyl cellulose, hydroxypropyl methylcellulose, maltodextrin). Ferrous fumarate oxidised 55 and 4%, respectively, in the dissolved and suspended states. The encapsulation of the suspended ferrous fumarate was advantageous. Iron capsules blended (1000 μg/g) with iodised salt (100 ppm iodine) and

DFS were prepared. Iodine stability was analysed in DFS when stored at room temperature, 20% RH, and in an environmental chamber (40°C and 60% RH) for a period of six months. DFS retained 70–85% iodine in the environmental chamber and 80–90% at room temperature while control (salt fortified with unencapsulated ferrous fumarate) retained only 60%. Iodine loss of 3–15% was also observed in iodised salt (without iron) even at room temperature and low humidity which was due to the impurities in the salt (Romita 2011). In another study, double fortified salt with unencapsulated iodine and microencapsulated iron was prepared and the stability of both fortificants during storage and under the environmental conditions in the distribution networks of the costal (Mombasa) and highland (Nairobi) zones of Kenya was evaluated. Iron premixes were prepared by agglomeration followed by microencapsulation. The iron compound was first granulated and then hot soy stearine along with titanium oxide (TiO_2), which masks colour, was sprayed to coat the particles in pan agglomerator. Ferrous sulphate heptahydrate, ferrous fumarate, sodium ferric EDTA, and elemental iron were used as iron compounds while potassium iodide, potassium iodate, and iodised salt were used as iodine sources to prepare the DFS. DFS with microencapsulated ferrous fumarate and potassium iodide premix retained 92% iron and 90% iodine in the coastal zone (Mombasa), while in the highland zone (Nairobi), the average values were 87 and 86%, respectively. On the other hand, the premix containing microencapsulated ferrous fumarate and iodised salt retained 67% iodine and 90% iron in the coastal zone and 78 and 84% in the highland zone, respectively. DFS, prepared with ferrous fumarate premix, were found resistent to colour change. It is evident that microencapsulated iron in DFS avoids interactions between fortificants and helps to increase the bioavailability (Diosady *et al.* 2006).

Zinc. Cheese was fortified with different zinc salts (zinc sulphate, acetate, and chloride) and the effects of these fortificants on cheese quality were analysed. Significant increases were observed in the native contents of cheese after fortification. Zinc acetate fortified cheese showed the highest contents of Ca (0.89) and P (0.69%) while Mg (1.86%) in cheese having zinc chloride as fortificant. Titratable acidity values increased (1.28–3.90%) after twelve weeks of storage compared to control. Similarly, TBA, nitrogen content, and peroxide values were also increased making 0.085 O.D, 18.88%, and 0.051 ml equivalent/kg, respectively (Abd-Rabou *et al.* 2010). Significant variations in the contents of chemical constituents like fats, proteins, and ash in cheddar cheese were observed when zinc (228 mg/kg) was used as fortificant. The moisture content remained unaffected (Kahraman & Ustunol 2012). El- Din *et al.* (2012) studied the effect of zinc salt addition on the quality of buffalo milk. In this, zinc sulphate and zinc acetate (40, 60, and 120 mg/kg) were used for fortification. TBA absorption increased significantly in the first two months of storage and then decreased due to the degradation of the fortificants. Encapsulation of the zinc compound is possible but is not considered yet. This would be a convenient way to mask the unpleasant taste of zinc compounds (WHO/FAO 2006; Cakmak 2008).

Conclusion

Approximately 95% population in the developing countries consume wheat flour as the dietary staple, which is considered to be a suitable vehicle of micronutrients fortification to combat their deficiency. In order to attain the increased bioavailability, micronutrients can be encapsulated by using various techniques. A vast volume of literature have been studied and it is concluded that the most preferred microencapsulation techniques for micronutrients are spray drying and spray chilling/cooling. The encapsulation of micronutrients for fortification may be advantageous in preventing the unwanted sensory changes and the interactions of nutrients with the components of wheat flour. Keeping in view the importance of microencapsulation and wheat flour, it is suggested that the application of microencapsulated micronutrients to wheat flour fortification should be explored.

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