

## PART III

Fortificants: physical characteristics, selection and use with specific food vehicles



# Introduction

By providing a critical review of the fortificants that are currently available for fortification purposes, Part III of these guidelines is intended to assist programme managers in their choice of firstly, a suitable food vehicle and secondly, a compatible fortificant. Having established – through the application of appropriate criteria – that the nature of the public health risk posed by a micronutrient deficiency justifies intervention in the form of food fortification, the selection of a suitable combination of food vehicle and fortificant(s), or more specifically, the chemical form of the micronutrient(s) that will be added to the chosen food vehicle, is fundamental to any food fortification programme. Subsequent chapters (Part IV) cover other important aspects of food fortification programme planning, including how to calculate how much fortificant to add to the chosen food vehicle in order to achieve a predetermined public health benefit (Chapter 7), monitoring and impact evaluation (Chapters 8 and 9), marketing (Chapter 10) and regulatory issues (Chapter 11).

In practice, the selection of a food vehicle–fortificant combination is governed by a range of factors, both technological and regulatory. Foods such as cereals, oils, dairy products, beverages and various condiments such as salt, sauces (e.g. soy sauce) and sugar are particularly well suited to mandatory mass fortification. These foods share some or all of the following characteristics:

- They are consumed by a large proportion of the population, including (or especially) the population groups at greatest risk of deficiency.
- They are consumed on a regular basis, in adequate and relatively consistent amounts.
- They can be centrally processed (central processing is preferable for a number of reasons, but primarily because the fewer the number of locations where fortificants are added, the easier it is to implement quality control measures; monitoring and enforcement procedures are also likely to be more effective).
- Allow a nutrient premix to be added relatively easily using low-cost technology, and in such a way so as to ensure an even distribution within batches of the product.

- Are used relatively soon after production and purchase. Foods that are purchased and used within a short period of time of processing tend to have better vitamin retention, and fewer sensorial changes due to the need for only a small overage<sup>1</sup>.

The choice of fortificant compound is often a compromise between reasonable cost, bioavailability from the diet, and the acceptance of any sensory changes. When selecting the most appropriate chemical form of a given micronutrient, the main considerations and concerns are thus:

- *Sensory problems.* Fortificants must not cause unacceptable sensory problems (e.g. colour, flavour, odour or texture) at the level of intended fortification, or segregate out from the food matrix, and they must be stable within given limits. If additional packaging is needed to improve stability of the added fortificant, it is helpful if this does not add significantly to the cost of the product and make it unaffordable to the consumer.
- *Interactions.* The likelihood or potential for interactions between the added micronutrient and the food vehicle, and with other nutrients (either added or naturally present), in particular any interactions that might interfere with the metabolic utilization of the fortificant, needs to be assessed and checked prior to the implementation of a fortification programme.
- *Cost.* The cost of fortification must not affect the affordability of the food nor its competitiveness with the unfortified alternative.
- *Bioavailability.* The fortificant must be sufficiently well absorbed from the food vehicle and be able to improve the micronutrient status of the target population.

Safety is also an important consideration. The level of consumption that is required for fortification to be effective must be compatible with a healthy diet.

The following two chapters consider the above factors in relation to specific micronutrients or micronutrient groups. Chapter 5 deals with iron, vitamin A and iodine; Chapter 6 covers some of the other micronutrients (such as zinc, folate and the other B vitamins, vitamin D and calcium) for which the severity of the public health problem of deficiencies is less well known but is believed to be significant. The discussion is limited to those fortificants and food vehicles that currently are the most widely used, or that have potential for wider application. Details of publications and articles containing more in-depth information about the fortification of foods with specific nutrients are provided in the attached further reading list.

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<sup>1</sup> Overage is the term used to describe the extra amount of micronutrient that is added to a food vehicle to compensate for losses during production, storage, distribution and selling.

## CHAPTER 5

# Iron, vitamin A and iodine

## 5.1 Iron

### 5.1.1 Choice of iron fortificant

Technically, iron is the most challenging micronutrient to add to foods, because the iron compounds that have the best bioavailability tend to be those that interact most strongly with food constituents to produce undesirable organoleptic changes. When selecting a suitable iron compound as a food fortificant, the overall objective is to find the one that has the greatest absorbability, i.e. the highest relative bioavailability<sup>1</sup> (RBV) compared with ferrous sulfate, yet at the same time does not cause unacceptable changes to the sensory properties (i.e. taste, colour, texture) of the food vehicle. Cost is usually another important consideration.

A wide variety of iron compounds are currently used as food fortificants (**Table 5.1**). These can be broadly divided into three categories: (224–226)

- water soluble;
- poorly water soluble but soluble in dilute acid;
- water insoluble and poorly soluble in dilute acid.

#### 5.1.1.1 Water-soluble compounds

Being highly soluble in gastric juices, the water-soluble iron compounds have the highest relative bioavailabilities of all the iron fortificants and for this reason are, more often than not, the preferred choice. However, these compounds are also the most likely to have adverse effects on the organoleptic qualities of foods, in particular, on the colour and flavour. During prolonged storage, the presence of fortificant iron in certain foods can cause rancidity and subsequent off-flavours. Moreover, in the case of multiple fortification, free iron, produced from the degradation of iron compounds present in the food, can oxidize some of the vitamins supplied in the same fortificant mixture.

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<sup>1</sup> Relative bioavailability is a measure which scores the absorbability of a nutrient by comparing its absorbability to that of a reference nutrient that is considered as having the most efficient absorbability.

TABLE 5.1

**Key characteristics of iron compounds commonly used for food fortification purpose: solubility, bioavailability and cost**

Compound	Iron content (%)	Relative bioavailability <sup>a</sup>	Relative cost <sup>b</sup> (per mg iron)
<i>Water soluble</i>			
Ferrous sulfate. 7H <sub>2</sub> O	20	100	1.0
Ferrous sulfate, dried	33	100	1.0
Ferrous gluconate	12	89	6.7
Ferrous lactate	19	67	7.5
Ferrous bisglycinate	20	>100 <sup>c</sup>	17.6
Ferric ammonium citrate	17	51	4.4
Sodium iron EDTA	13	>100 <sup>c</sup>	16.7
<i>Poorly water soluble, soluble in dilute acid</i>			
Ferrous fumarate	33	100	2.2
Ferrous succinate	33	92	9.7
Ferric saccharate	10	74	8.1
<i>Water insoluble, poorly soluble in dilute acid</i>			
Ferric orthophosphate	29	25–32	4.0
Ferric pyrophosphate	25	21–74	4.7
Elemental iron	–	–	–
H-reduced	96	13–148 <sup>d</sup>	0.5
Atomized	96	(24)	0.4
CO-reduced	97	(12–32)	<1.0
Electrolytic	97	75	0.8
Carbonyl	99	5–20	2.2
<i>Encapsulated forms</i>			
Ferrous sulfate	16	100	10.8
Ferrous fumarate	16	100	17.4

EDTA, ethylenediaminetetraacetate; H-reduced, hydrogen reduced; CO-reduced, carbon monoxide reduced.

<sup>a</sup> Relative to hydrated ferrous sulfate (FeSO<sub>4</sub>·7H<sub>2</sub>O), in adult humans. Values in parenthesis are derived from studies in rats.

<sup>b</sup> Relative to dried ferrous sulfate. Per mg of iron, the cost of hydrated and dry ferrous sulfate is similar.

<sup>c</sup> Absorption is two-three times better than that from ferrous sulfate if the phytate content of food vehicle is high.

<sup>d</sup> The high value refers to a very small particle size which has only been used in experimental studies.

Sources: adapted from references (224–226), with additional data supplied by P. Lohmann (cost data) and T. Walczyk (ferrous lactate, H-reduced elemental iron).

The water-soluble forms of iron are especially suited to fortifying cereal flours that have a relatively fast turnover, i.e. one month in warm, humid climates and up to 3 months in dry, cold climates. Water-soluble iron compounds are also useful for dry foods, such as pasta and milk powder, as well as dried milk-based infant formulas. Encapsulated forms, i.e. iron compounds that have been coated

to physically separate the iron from the other food components, can be used for slowing down or preventing sensory changes.

Ferrous sulfate is by far the most frequently used water-soluble iron fortificant, principally because it is the cheapest. It has been widely used to fortify flour (see *section 5.1.5.1*). However, depending on its physical characteristics, the climate and the fat content of the flour to which it is added, ferrous sulfate can cause rancidity, and therefore its suitability as a fortificant needs to be evaluated in trials before use.

#### *5.1.1.2 Iron compounds that are poorly soluble in water but soluble in dilute acid*

Compounds that fall into the second category of iron fortificants (see **Table 5.1**) are also reasonably well absorbed from food, as they are soluble in the gastric acids produced in the stomach of normal healthy adults and adolescents. Some concern has been raised about absorption in infants who may secrete less acid but further research is needed in this area before any firm conclusions can be drawn. In most people, however, with the possible exception of individuals who suffer from a lack of gastric acid due to medical problems, iron absorption from these compounds is likely to be similar to that from water-soluble iron compounds. Poorly water-soluble iron compounds, such as ferrous fumarate, have the advantage of causing fewer sensory problems in foods than the water-soluble compounds, and are generally next in line for consideration, especially if more water-soluble forms cause unacceptable organoleptic changes in the chosen food vehicle.

Ferrous fumarate and ferric saccharate are the most commonly used iron compounds in this group, and in adults are as bioavailable as ferrous sulfate. The former is frequently used to fortify infant cereals and the latter, chocolate drink powders. Ferrous fumarate is used to fortify maize flour in Venezuela and wheat flour in Central America, where it has also been proposed as a potential fortificant for maize masa. Ferrous fumarate can be used in an encapsulated form to limit sensory changes.

#### *5.1.1.3 Iron compounds that are insoluble in water and poorly soluble in dilute acid*

Relative to ferrous sulfate, the absorption of iron from water-insoluble compounds ranges from approximately 20% up to 75%. Despite their reduced absorbability, water-insoluble iron compounds have been widely used by the food industry as fortificants because they have far less effect on the sensory properties of foods (at the levels currently used) and because they are cheaper than the more soluble compounds. However, they are generally regarded as the last resort option, especially in settings where the diet of the target population

is high in iron absorption inhibitors. If it is necessary to use a water-insoluble iron fortificant, it should ideally have an absorption equivalent to at least 50% that of ferrous sulfate (as measured in rat or human assays), and twice as much would need to be added in order to compensate for the reduced absorption rate.

Within this category of iron fortificants, the ferric phosphate compounds – ferric orthophosphate and ferric pyrophosphate – are used to fortify rice, and some infant cereals and chocolate-containing foods. They have a modest iron bioavailability: the relative bioavailability of ferric pyrophosphate is reported to be 21–74%, and that of ferric orthophosphate, 25–32%. However, the relative bioavailability of the ferric phosphates may change during the processing of a food (227,228).

Elemental iron powders are used in a number of countries to fortify cereals, but the bioavailabilities of the different forms of elemental iron that are currently available (**Table 5.1**) are not well established (229). The solubility of elemental iron is very dependent on the size, shape and surface area of the iron particles (characteristics which are governed by the manufacturing process<sup>1</sup>), as well as the composition of the meals in which it is consumed.

According to the conclusions of the Sharing United States Technology to Aid Improvement of Nutrition (SUSTAIN) Task Force, only electrolytic iron powders (diameter <45 microns or 325 mesh) have been proven to be sufficiently bioavailable to humans (229). At the time of the meeting of the Task Force, the only electrolytic iron powders to have been tested were those manufactured by OMG Americas under the trade name “Glidden 131”<sup>2</sup>. More recent data indicate that carbonyl iron and some hydrogen-reduced (H-reduced) iron powders have comparable bioavailability to electrolytic iron. Atomized iron and carbon monoxide-reduced (CO-reduced) iron are not recommended at the present time because of their lower bioavailability. (Atomized iron is a reduced-iron powder that has been processed by striking a stream of molten iron with high-pressure water jets.) Elemental iron with a large particle size (diameter >149 microns or 100 mesh) is probably too insoluble in the intestine and is therefore not generally recommended for use as a food fortificant. Further testing of the bioavailability of various elemental iron powders is ongoing (42).

### 5.1.2 Methods used to increase the amount of iron absorbed from fortificants

The bioavailability of iron from fortificants is dependent not only on the solubility of the fortificant as discussed above, but also on the composition of the

<sup>1</sup> For more details, please refer to the *Handbook of powder metal technologies and applications* (230).

<sup>2</sup> At the time of writing, Glidden 131 was still available.



diet, in particular, on the proportion of inhibitors of iron absorption in the diet, notably iron-binding phytates and certain phenolic compounds. The addition of ascorbic acid (vitamin C) or sodium ethylenediaminetetraacetic acid (sodium EDTA or  $\text{Na}_2\text{EDTA}$ ) and the removal of phytates, all of which reduce the effect of the inhibitors, can be effective ways of increasing the total amount of iron absorbed from iron-fortified foods.

#### 5.1.2.1 Ascorbic acid

The addition of ascorbic acid causes a substantial increase in the amount of iron absorbed from most iron compounds (40,224). Ascorbic acid addition to iron-fortified foods is thus a widely adopted practice throughout the food industry, especially for processed foods. This option is, however, not recommended for staples and condiments because of stability issues (see section 5.1.5.1). For example, Chile fortifies milk powder delivered through its public health programme with both iron and ascorbic acid (as well as some other micronutrients) to control anaemia in infants and young children.

In most studies, the co-addition of ascorbic acid and iron in a 2:1 molar ratio (6:1 weight ratio) increased iron absorption from foods 2- to 3-fold in adults and children (224). This ratio of ascorbic acid to iron is thus recommended for most foods; a higher ascorbic acid:iron molar ratio (4:1) can be used for high-phytate foods. The main problem with using ascorbic acid as a food additive is that substantial amounts can be lost during food storage and preparation. This means that, relative to some of the alternatives, it can be an expensive option.

#### 5.1.2.2 Sodium EDTA

Sodium EDTA is a permitted food additive in many countries, and unlike ascorbic acid, is stable during processing and storage. At low pH (i.e. in the stomach), sodium EDTA acts as a chelating agent, and as such prevents iron from binding to phytic acid or phenolic compounds, which would otherwise inhibit iron absorption (231). Its addition enhances the absorption of both food iron and soluble iron fortificants (232), but not that of the relatively insoluble iron compounds such as ferrous fumarate (233), ferric pyrophosphate (232) or elemental iron (234).

In the case of foods fortified with soluble iron compounds, such as ferrous sulfate, the addition of sodium EDTA in a molar ratio of  $\text{Na}_2\text{EDTA}$ :iron of between 0.5 and 1.0 (between 3.3:1 and 6.6:1 weight ratio) is recommended. Under these circumstances iron absorption is increased by up to 2–3 times (224).

### 5.1.2.3 *Dephytinize cereals and legumes*

The phytic acid content of cereals, pulses and legumes can be substantially reduced by several methods (224), some of which are particularly suitable for ensuring adequate iron absorption from cereal-based complementary foods or soy-based infant formulas. However, the molar ratio of phytic acid:iron needs to be decreased to at least 1:1, or even to less than 0.5:1, in order to achieve a meaningful increase in iron absorption.

Milling removes about 90% of the phytic acid from cereal grains, but the remaining 10% is still strongly inhibitory. The action of phytases (enzymes) is usually necessary in order to achieve complete phytate degradation. Naturally-occurring cereal phytases can be activated by traditional processes, such as soaking, germination and fermentation. At the industrial level, it is possible to completely degrade phytic acid in complementary food mixtures of cereals and legumes by adding exogenous phytases or by adding whole wheat or whole rye as a source of phytases, these being naturally high in phytases (224,235–237). Because of the risk of bacterial contamination, it is better to add the phytases under factory conditions, but as yet, this practice has not been adopted commercially.

### 5.1.3 *Novel iron fortificants*

In recent years, considerable effort has been devoted to the development and testing of alternative iron fortificants, in particular, fortificants that provide better protection against iron absorption inhibitors than those currently available. Among those at an experimental stage are sodium iron EDTA (NaFeEDTA), ferrous bisglycinate and various encapsulated and micronized iron compounds. In recent years, NaFeEDTA has been selected as the iron compound to fortify government-led soy sauce fortification and wheat flour fortification programs in China, and fish sauce fortification in Vietnam.

#### 5.1.3.1 *Sodium iron EDTA*

In high-phytate foods, the absorption of iron from NaFeEDTA is 2–3 times greater than that from either ferrous sulfate or ferrous fumarate. In foods with a low phytate content, however, iron absorption is similar (231,232). In addition to better absorption from high-phytate fortified foods, NaFeEDTA offers a number of other advantages: it does not promote lipid oxidation in stored cereals, or the formation of precipitates in foods that are high in free peptides, such as soy sauce and fish sauce. On the down side, it is expensive, and because it is slowly soluble in water, it may cause colour changes in some foods.

The Joint FAO/WHO Expert Committee on Food Additives has approved the use of NaFeEDTA at 0.2 mg Fe/kg body weight per day (238). Nevertheless, the use of Na<sub>2</sub>EDTA plus ferrous sulfate (or possibly other soluble iron

compounds) rather than NaFeEDTA might yet prove to be the better option for high-phytate foods. In most settings, the choice will depend on the relative costs of, and accessibility to, the EDTA compounds, the acceptability of sensory changes in the food, and current legislation.

#### 5.1.3.2 *Ferrous bisglycinate*

Ferrous bisglycinate is an iron–amino acid chelate in which the iron is protected from the action of absorption inhibitors by being bound to the amino acid, glycine. Absorption from this form of iron has been reported to be 2–3 times better than that from ferrous sulfate in a high-phytate cereal and in whole maize. In contrast, a closely-related compound, ferric trisglycinate, is not well absorbed from maize (239,240).

Ferrous bisglycinate seems to be particularly well suited to the fortification of liquid whole milk and other dairy products where use of ferrous sulfate leads to rancid off-flavours. However, ferrous bisglycinate can also cause rancidity by oxidizing fats in food, which can be a problem in cereal flours and weaning cereals unless an antioxidant is added as well. Furthermore, the bisglycinate is much more expensive than many other iron compounds.

#### 5.1.3.3 *Encapsulated ferrous sulfate and ferrous fumarate*

Several iron compounds are available commercially in encapsulated form, namely ferrous sulfate and ferrous fumarate, and are currently used in dry infant formulas and in infant cereals, predominantly in industrialized countries. In future, use of encapsulated forms of iron compounds may extend to developing countries, although their cost may be a problem. Encapsulation increases costs 3- to 5-fold, which when expressed in terms of iron amounts, is equivalent to a 10-fold increase in cost relative to the use of dried ferrous sulfate (Table 5.1).

As previously indicated, the main purpose of encapsulation is to separate the iron from the other food components, thereby mitigating sensory changes. In double fortified salt (i.e. salt fortified with iodine and iron), encapsulation of iron has been shown to help prevent iodine losses and to slow down colour changes.

When developing encapsulated iron fortificants, it is important to select a coating that provides an adequate balance between stability and bioavailability. Iron compounds are usually encapsulated with hydrogenated vegetable oils, but mono- and diglycerides, maltodextrins and ethyl cellulose, have also been used. Because of the different methods of manufacture, and because different capsule materials and thicknesses are possible, it is imperative to confirm bioavailability, at least in rat assays, before widespread use as a fortificant. Tests have shown that encapsulation of ferrous sulfate and ferrous fumarate does not alter iron

bioavailability to rats. In addition, dual fortification of salt with encapsulated iron has been found to be efficacious in humans (see section 1.3.2.3) (44).

#### 5.1.3.4 *Micronized ferric pyrophosphate*

Just as the bioavailability of elemental iron powders is increased by reducing their particle size, so too can that of insoluble iron salts, such as ferric pyrophosphate. Micronizing insoluble iron salts to an extremely small submicron particle size cannot, however, be achieved by physical grinding, only by a chemical process.

A micronized form of ferric pyrophosphate (diameter, 0.5 microns) has been developed recently for use as a food fortificant. It is available in both liquid and dried forms. In order to make it dispersible in liquids, the particles of ferric pyrophosphate are coated with emulsifiers. Relative to ordinary ferric pyrophosphate (mean particle size of around 8 microns), iron absorption by adult humans is improved by 2–4 four times in milk products (241). Its principal advantage is that, being insoluble in water, it is unlikely to cause many sensory problems, although this remains to be tested adequately. Currently it is added to liquid milk and yoghurt products in Japan, but its more widespread use in the foreseeable future is prohibited by its very high cost.

#### 5.1.4 *Sensory changes*

In the case of iron fortificants, the two most common problems are increased rancidity due to oxidation of unsaturated lipids and unwanted colour changes. The latter typically include a green or bluish colouration in cereals, a greying of chocolate and cocoa, and darkening of salt to yellow or red/brown.

Sensory changes are highly variable and not always predictable. Just because an iron fortificant does not cause adverse sensory changes to a food product in one situation, does not necessarily mean that the same fortificant will not cause a problem with the same food product in another situation. Thus, having selected a potential iron fortificant, it is essential that its effects on the sensory properties of the food to which it is to be added are determined prior to use.

#### 5.1.5 *Experience with iron fortification of specific foods*

Iron fortification is already widely practised in many parts of the world. For example, more than 20 countries in Latin America have implemented mass iron fortification programmes, most of which involve the fortification of wheat or maize flours (237). Elsewhere, other frequently used food vehicles include cereal-based complementary foods, fish sauce, soy sauce and milk. Salt has also been fortified with iron in efficacy trials. Products derived from cereal flours (e.g. bread, cereal snacks and breakfast cereals) are also useful food vehicles, but the amount of iron provided via this route will depend on the quantity of food

TABLE 5.2

**Suggested iron fortificants for specific food vehicles**

<b>Food vehicle</b>	<b>Fortificant</b>
Low extraction (white) wheat flour or degermed corn flour	Dry ferrous sulfate Ferrous fumarate Electrolytic iron (x2 amount) Encapsulated ferrous sulfate Encapsulated ferrous fumarate
High extraction wheat flour, corn flour, corn masa flour	Sodium iron EDTA Ferrous fumarate (x2 amount) Encapsulated ferrous sulfate (x2 amount) Encapsulated ferrous fumarate (x2 amount)
Pasta	Dry ferrous sulfate
Rice <sup>a</sup>	Ferric pyrophosphate (x2 amount)
Dry milk	Ferrous sulfate plus ascorbic acid
Fluid milk	Ferric ammonium citrate Ferrous bisglycinate Micronized ferric pyrophosphate
Cocoa products	Ferrous fumarate plus ascorbic acid Ferric pyrophosphate (x2 amount) plus ascorbic acid
Salt <sup>a</sup>	Encapsulated ferrous sulfate Ferric pyrophosphate (x2 amount)
Sugar <sup>a</sup>	Sodium iron EDTA
Soy sauce, fish sauce	Sodium iron EDTA Ferrous sulfate plus citric acid
Juice, soft drinks	Ferrous bisglycinate, ferrous lactate Micronized ferric pyrophosphate
Bouillon cubes <sup>a</sup>	Micronized ferric pyrophosphate
Cereal-based complementary foods <sup>b</sup>	Ferrous sulfate Encapsulated ferrous sulfate Ferrous fumarate Electrolytic iron (x2 amount) All with ascorbic acid ( $\geq 2:1$ molar ratio of ascorbic acid: Fe)
Breakfast cereals	Electrolytic iron (x2 amount)

EDTA, ethylenediaminetetraacetic acid.

<sup>a</sup> Technical problems, specifically sensory changes and/or segregation, still exist with the iron fortification of these food vehicles.

<sup>b</sup> Recent evidence has indicated that infants may only absorb ferrous fumarate 25% as well as adults, so concentrations of poorly soluble iron compounds in complementary foods may need to be adjusted to allow for this.

eaten and on the level of fortification. Iron compounds suitable for the fortification of specific food vehicles are listed in **Table 5.2**.

#### 5.1.5.1 *Wheat flour*

The nutritional usefulness of iron fortification of wheat flour has recently been confirmed in an efficacy study in Thailand (242). In that study the relative

efficacy of electrolytic iron as compared to ferrous sulfate was about 70% in women consuming fortified wheat flour cookies, compared to 50% for H-reduced iron. Based on this evidence, adding double the amount of electrolytic iron or H-reduced iron as compared to ferrous sulfate, should give an equivalent efficacy to ferrous sulfate.

Ferrous sulfate and elemental iron powders have traditionally been used to fortify wheat and other cereal flours. Electrolytic iron remains the preferred elemental iron fortificant, however H-reduced iron could also be considered. In addition, recent evidence from rat studies suggests that carbonyl iron may be as good as electrolytic iron as a fortificant, however human efficacy studies are still necessary to confirm this.

Although ferrous sulfate has been successfully used for many years in Chile (where fortified flour is consumed within 6–8 weeks of purchase), and ferrous fumarate has been employed in Venezuela and throughout Central America, in other countries the addition of these iron compounds to wheat flours has caused rancidity. This problem could be overcome by using encapsulated forms to improve stability. Ferrous sulfate, and to a lesser extent ferrous fumarate, are also suitable fortificants for pasta, which, because of its low moisture content, is less susceptible than wheat flour to the development of rancidity.

Although potentially useful for some high-phytate flours, NaFeEDTA has not been used widely in any large-scale iron fortification programmes because of reports that it interferes with the bread fermentation process (243). However, China is currently introducing NaFeEDTA to fortify wheat in several provinces, and so far there have been no recorded problems. Although ascorbic acid is often added to iron-fortified foods in order to enhance absorption (see *section 5.1.2.1*), its usefulness in this respect in bread flours is limited by the fact that it is destroyed by the action of heat during baking. Ascorbic acid is nevertheless frequently added to flours, not so much to enhance iron absorption, but rather as a raising agent.

In its guidelines on iron fortification of cereal-based staples, the SUSTAIN Task Force (42) recommended the use of ferrous sulfate in preference, followed by ferrous fumarate, and lastly electrolytic iron (but at twice the iron concentration of the other iron compounds). In order to ensure the successful fortification of wheat flour and wheat flour products, it may be necessary for individual countries to adopt different strategies to take account of differences in climate, wheat flour quality, processing methods and storage conditions, as well as differences in the main uses of flour (i.e. to make bread or other foods).

#### 5.1.5.2 Maize

In general, maize flours are equally, if not more difficult, to fortify with iron than wheat flours. Lime-treated (nixtamalized) corn masa, a staple used to make

tortillas in much of Latin America, goes rancid when soluble iron compounds, such as ferrous sulfate, are added to it. Further colour and texture changes occur during the preparation of tortillas. The difficulties are further compounded by the fact that iron absorption from corn masa is strongly inhibited by its high phytate and high calcium content. For these reasons, iron fortification of maize flours has not been widely adopted, except in a number of Latin American countries where the consumption of maize is high. In Venezuela, for example, ferrous fumarate mixed with elemental iron is used to fortify maize flours.

In view of its highly inhibitory nature (especially if it is not degermed), the Pan American Health Organization (PAHO) recently recommended the use of either NaFeEDTA or ferrous fumarate (at twice the amount) for maize flour fortification (237). These recommendations have yet to be put into practice. Whether or not they are appropriate for maize meal that is used to prepare porridge also needs to be evaluated. For maize flours that are not high in phytic acid (e.g. degermed) and are not lime-treated, the same iron compounds as those recommended for the fortification of white wheat flour can be considered (237).

#### 5.1.5.3 *Cereal-based complementary foods*

Complementary foods (i.e. foods intended for infants during the weaning period) are usually based on dry cereals and consumed as a porridge or gruel with milk or water. Alternatively, they are based on blends of cereals and legumes, which again can be made into a porridge or gruel with water. The addition of ferrous sulfate, ferrous bisglycinate and other soluble iron compounds to these products can cause rancidity, and sometimes colour changes as well, particularly if the porridges are fed with fruits. To overcome such problems, one option would be to use encapsulated forms, such as ferrous sulfate. Although encapsulation helps to prevent fat oxidation during storage, the capsule is removed by hot milk or water, and off-colours may still develop in the presence of some fruits and vegetables.

Another option is to use a less soluble iron fortificant, such as ferrous fumarate or electrolytic iron (but at a higher concentration), both of which are commonly used to fortify complementary foods. Ferric pyrophosphate is another possibility, although it is rarely used in practice. If ferric pyrophosphate were to be used to fortify complementary foods, it too should be added at twice the concentration (relative to ferrous sulfate). Recent evidence has indicated that ferrous fumarate may be less well absorbed in children than in adults (absorption of iron from ferrous fumarate by children may only be 25% of that by adults) and so its use as a fortificant, or at least its level of addition, may need to be re-evaluated (244).

In order to enhance iron absorption, ascorbic acid is usually added together with the iron compound to complementary foods whenever possible (see section

5.1.2.1). Ideally, ascorbic acid and iron should be added in at least a 2:1 molar ratio (ascorbic acid:iron). Dry complementary foods should also be packaged in such a way as to minimize ascorbic acid degradation during storage. As described above (see *section 5.1.2.3*), another useful way of optimizing iron absorption from cereal-based foods is to degrade any phytic acid present with naturally-occurring cereal phytases (i.e. activate those already in the food by soaking, germinating or fermenting) or by adding microbial phytases during manufacture. However, the addition of phytases to processed foods has yet to be attempted on a commercial scale.

#### 5.1.5.4 Dairy products

Dried whole milk powders and dried or ready-to-feed milk-based infant formulas can be successfully fortified with ferrous sulfate (together with ascorbic acid to enhance absorption). In Chile, for example, ascorbic acid (700 mg/kg) and iron (100 mg as ferrous sulfate/kg) are routinely added to dried milk powders consumed by infants. In the case of soy formulas, it has been found necessary to use ferrous sulfate encapsulated with maltodextrin in order to prevent unwanted colour changes (i.e. darkening).

Ferrous sulfate, and many other soluble iron compounds, cannot be used to fortify liquid whole milk and other dairy products because they cause rancidity and off-flavours. Ferric ammonium citrate (245), ferrous bisglycinate and micronized ferric pyrophosphate are generally more suitable for this purpose. Iron fortificants are best added after the milk has been homogenized and the fat internalized in micelles, so as to help protect against oxidation. Ferrous bisglycinate is widely used to fortify whole milk and dairy products in Brazil and Italy; micronized ferric pyrophosphate is added to dairy products in Japan (see also *section 5.1.3.4*).

#### 5.1.5.5 Rice

The fortification of rice grains presents a number of technical challenges. It can be achieved, as is done in the United States, by coating the grain with an appropriate formulation. Alternatively, a rice-based extruded grain that contains a high concentration of iron can be mixed with normal rice grains (usually at a ratio of 1:200). Ferric pyrophosphate, added at a two-fold higher level, and micronized, ferric pyrophosphate (0.5 micron) have recently been recommended for adding to extruded artificial rice grains (246).

Technical difficulties, combined with cultural preferences for specific types of rice, mean that mass fortification of rice, although desirable, remains problematic. The fact that in most of the big rice-producing countries, production takes place in thousands of small mills, also creates problems for mass rice fortification. Not only are smaller mills sensitive to small increases in costs, the sheer



number of them makes it difficult to maintain adequate quality control programmes. Although the extruded grains have found some application in targeted food fortification programmes, such as school feeding programmes, much more research and development is required before mass rice fortification programmes can be implemented on a wider scale.

#### 5.1.5.6 *Cocoa products*

As cocoa is naturally high in phenolic compounds, the addition of ferrous sulfate and other water-soluble iron compounds tends to cause colour changes in cocoa-based products (247). Ferrous fumarate is a useful alternative for some products, but grey or blue/grey colours are still a problem for chocolate drinks, especially if boiling water is used to make up the drink (227). Furthermore, the currently available encapsulated iron compounds are not useful for chocolate drink fortification as the capsules are removed by heat either during product manufacture or during preparation of the drink.

Ferric pyrophosphate, ferric saccharate or ferric orthophosphate are usually used to fortify cocoa products as these tend to produce fewer off-colours. However, relative to ferrous sulfate, larger amounts of these iron compounds would need to be added to allow for their lower absorption. Ascorbic acid addition is also required (in at least a 2:1 molar ratio) in order to offset the inhibitory effects of cocoa phenolics on iron absorption (227,248).

#### 5.1.5.7 *Soy sauce and fish sauce*

Sodium iron EDTA has proved to be a useful fortificant for both fish sauce and soy sauce (see also *section 1.3.1*). Studies have demonstrated that absorption of iron by human subjects fed NaFeEDTA-fortified fish or soy sauce added to rice meals is similar to that from the same meals to which ferrous sulfate-fortified sauces had been added (249). The iron status of iron-deficient Vietnamese women improved significantly following regular intakes of NaFeEDTA-fortified fish sauce over a period of 6 months (28) (see also *section 1.3.1.1*). Similarly, in trials conducted in China, NaFeEDTA soy sauce, providing 20 mg iron per day, significantly improved the iron status of anaemic adolescents (250). Large-scale effectiveness studies of soy sauce fortification with NaFeEDTA are currently underway in both Viet Nam and China.

Until very recently, NaFeEDTA has been the preferred iron fortificant for soy and fish sauces because most of the potential alternatives (i.e. other soluble iron compounds) cause peptide precipitation during storage. However, latterly ferrous sulfate stabilized with citric acid has been successfully used to fortify fish sauce in Thailand, and may offer a less expensive alternative to NaFeEDTA.

### 5.1.5.8 Salt

The success of salt iodization programmes (see *section 5.3.2.1*) has led several countries to consider using salt as a vehicle for iron fortification. In practice, this means the double fortification of salt, i.e. with iron and iodine. Promising approaches that are already being tested include the addition of encapsulated ferrous fumarate, encapsulated ferrous sulfate (see *section 1.3.2.3*) or ferric pyrophosphate (at twice the concentration). Encapsulation is necessary as ferrous sulfate, ferrous fumarate and other soluble iron compounds very quickly cause a yellow or red/brown discoloration in the moist, low quality salt that is currently used in many developing countries. The main disadvantage of the encapsulation options is the increase in the price of the fortified product, which can be by as much as 30%.

### 5.1.6 Safety issues

Concern has been raised about increased iron intakes, particularly in terms of the potential effects on infection rates and on the risk of cardiovascular disease and cancer. Much of this concern, however, relates to the use of pharmaceutical iron supplements and not to fortified foods.

A recent review of intervention studies with iron-fortified milk or cereals, concluded that iron fortification did not increase infectious morbidity in children under 18 months of age (251). Studies in Chile (252), Hungary (253) and South Africa (254) reported that iron added to milk formula had no influence on infectious outcome. Only one study, conducted in a poor community in Chile, reported an increase in episodes of diarrhoea in young infants fed iron-fortified formula (255). On balance, studies have indicated that iron fortification of milk formula is safe (251).

It has been suggested that higher levels of iron intake and elevated body stores are potential risk factors for both coronary heart disease (CHD) and cancer. Results from studies carried out over the last 10 years to test this hypothesis are, however, inconclusive. The association between serum ferritin and risk of CHD has been examined in at least 12 studies, but a meta-analysis of such evidence failed to establish a strong relationship between the two (256). Inflammatory response is an important risk factor for CHD and also increases serum ferritin, which might explain why an association between the risk of CHD and increased serum ferritin is sometimes observed.

Possible links between cancer and iron intake or iron status have been the subject of only a few studies, but are largely unsubstantiated. It has been hypothesized that the presence of unabsorbed fortificant iron in the body, much of which reaches the colon, leads to free radical generation that damages the colon mucosa (257). However, iron is highly insoluble at the pH of the colon, and although unabsorbed ferrous sulfate can increase free radical generation in the

stool (257), there is no evidence to suggest that the free radicals survive long enough to cause tissue damage. The finding that serum transferrin was higher in men who developed colon cancer (258) was not confirmed when the follow-up was extended to 17 years.

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### Summary: iron fortification

- For most food vehicles, the recommended iron fortificants, in order of preference, are: ferrous sulfate, ferrous fumarate, encapsulated ferrous sulfate or fumarate, electrolytic iron (at twice the amount), and ferric pyrophosphate (at twice the amount).
  - The co-addition of ascorbic acid in a 2:1 molar ratio is recommended in order to enhance iron absorption. This applies to infant foods and market-driven foods. In the case of high phytic acid foods, the molar ratio (ascorbic acid:iron) can be increased to 4:1.
  - NaFeEDTA is recommended for the mass fortification of high-phytate cereal flours and for sauces with a high peptide content (e.g. fish sauce, soy sauce).
  - For liquid milk products, ferrous bisglycinate, micronized ferric pyrophosphate and ferric ammonium citrate are the most appropriate fortificants.
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## 5.2 Vitamin A and $\beta$ -carotene

### 5.2.1 Choice of vitamin A fortificant

The choice of a vitamin A fortificant is largely governed by the characteristics of the food vehicle, as well as various technological, regulatory and religious considerations. As preformed vitamin A (retinol) is an unstable compound, in commercial preparations it is esterified, usually with palmitic or acetic acid, to the more stable corresponding esters. Retinyl acetate and retinyl palmitates, along with provitamin A ( $\beta$ -carotene), are thus the main commercial forms of vitamin A that are available for use as food fortificants. The intense orange colour of  $\beta$ -carotene makes it unsuitable for use as a fortificant in many foods, but it is widely used to give an orange-yellow colour to margarines and beverages.

Since vitamin A is fat-soluble, it is easily added to fat-based or oily foods. When the food vehicle is either dry or a water-based liquid, an encapsulated form of the vitamin is needed. Based on this distinction, vitamin A fortificants can be divided into two categories:

- Oily forms that can be incorporated directly into fat-based foods or emulsified into water-based ones (e.g. milk).
- Dry forms that can be dry mixed into foods or dispersed in water, depending on whether they are cold water dispersible or non-cold water dispersible.

TABLE 5.3

**Commercially available forms of vitamin A, their characteristics and their main applications**

Product	Characteristics	Application(s)
Oily vitamin A acetate	Retinol ester of acetic acid which may be stabilized with antioxidants	Fortification of fat-based foods, especially margarine and dairy products
Oily vitamin A palmitate	Retinol ester of palmitic acid which may be stabilized with antioxidants	Fortification of fat-based foods, especially margarine and dairy products
Oily vitamin A palmitate or acetate with vitamin D <sub>3</sub>	Retinol ester and cholecalciferol mix, stabilized with antioxidants	Fortification of fat-based foods, especially margarine and dairy products where the combination of both vitamins is required
Dry vitamin A palmitate or acetate	Vitamin A embedded in a water-soluble matrix (e.g. gelatin, gum acacia, starch) and stabilized with antioxidants	Fortification of dry food products, (i.e. flour and dry milk, beverage powders) and fortification of water-based foods
Dry vitamin A palmitate or acetate with vitamin D <sub>3</sub>	Vitamin A and vitamin D <sub>3</sub> embedded in a water-soluble matrix (e.g. gelatin, gum acacia, starch) and stabilized with antioxidants	Fortification of dry food products, (i.e. flour and dry milk, beverage powders) and fortification of water-based foods

Source: Hector Cori, personal communication, 2004.

Pure vitamin A and  $\beta$ -carotene in solution are unstable when exposed to ultra-violet light, oxygen or air. Thus all forms of vitamin A – oily or dried – are protected with antioxidants to prolong their shelf-life. The use of airtight packaging provides further protection. For example, the loss of vitamin A in sealed cans of oil is minimal, but losses from fortified cereals, fortified sugar or oil can be as high as 40%, depending on ambient conditions and storage times (259–261). Opaque packaging is indispensable for maintaining stability in vitamin A-fortified oils.

The characteristics and applications of the various forms of vitamin A are listed in **Table 5.3**. Each formulation includes stabilizers, and each is compatible with existing food regulations (e.g. contain permitted antioxidants) and/or religious requirements (e.g. Kosher, Halal). The fat-soluble forms of retinol are about one half to one third as expensive as the dry forms. Appropriate vitamin A fortificants for specific foods are given in **Table 5.4**.

### 5.2.2 Experience with vitamin A fortification of specific foods

Of the food vehicles suitable for mass fortification, margarine is the one that is most frequently associated with vitamin A. In both industrialized and

TABLE 5.4

**Vitamin A fortificants and their suitability as fortificants for specific food vehicles**

Food vehicle	Form of vitamin A	Stability
Cereal flours	Retinyl acetate or retinyl palmitate (dry stabilized forms)	Fair
Fats and oils	$\beta$ -carotene and retinyl acetate or retinyl palmitate (oil-soluble)	Good
Sugar	Retinyl palmitate (water dispersible forms)	Fair
Milk powder	Retinyl acetate or palmitate (dry water dispersible forms)	Good
Liquid milk	Retinyl acetate (preferred) or palmitate (oily form, emulsified)	Good/fair depending on packaging
Infant formula	Retinyl palmitate (water dispersible beadlets)	Good
Spreads	Retinyl acetate or palmitate (oily form)	Good

Source: Hector Cori, personal communication, 2004.

developing countries, vegetable oils are also used, and, in recent years, cereal flours have increasingly been fortified with vitamin A in several parts of the world. In parts of Central America, sugar is often the preferred food vehicle for vitamin A. The amount and forms of vitamin A used in a selection of food fortification programmes are detailed in **Table 5.5**. It is estimated that about 90% of fortificant vitamin A will usually be absorbed (262).

#### 5.2.2.1 Oils and margarine

There are two reasons why margarines and oils are the ideal foods for vitamin A fortification. Not only is the oil-soluble form of the vitamin the cheapest available, but the oil protects the vitamin A from oxidation during storage and so facilitates absorption of the vitamin (264). The vitamin A fortification of margarines has a relatively long history, having been introduced in some countries as early as the 1920s, following the realization that the replacement of butter with margarine in the diet was causing widespread xerophthalmia in children (265). Vitamin A fortification of margarine in Newfoundland, Canada, for example, resulted in a marked improvement in vitamin A status (266). Likewise, in India, a hydrogenated oil (vanaspati), which is used as an alternative to ghee, has been fortified with vitamin A since 1953 (267).

Although the technology for adding vitamin A to oils is simple and inexpensive, and oils are widely used, the fortification of oils with this vitamin is relatively rare, at least compared with that of margarines. The fortification of oils is thus a potentially useful means of expanding the present range of vitamin A-fortified foods. Stability may be a problem in some settings; experimental studies have shown that when vitamin A is added to soybean oil in sealed cans, the

TABLE 5.5  
Examples of vitamin A fortification programmes

Food item	Country or programme	Amount of retinol added (mg/kg)	Form of vitamin A added	Amount of food consumed (g/day)	Contribution to recommended daily intake (%)
Margarine	Philippines	25	Retinyl palmitate (oil)	24 (preschool-aged children)	150 <sup>a</sup>
Margarine	Various	1–15	Retinyl palmitate (oil)	15	2–40 <sup>a</sup>
Vegetable oil (PL-480)	US Food Aid	18	Retinyl palmitate (oil)	16	50 <sup>a</sup>
Hydrogenated fat	India, Pakistan	7.5	Retinyl palmitate (oil)	0.3–17	0.4–21 <sup>a</sup>
Maize flour	Venezuela	2.7	Retinyl palmitate (dry)	80	30
Wheat flour	Philippines	4.5	Retinyl palmitate (dry)	40 (bread)	19 <sup>a</sup>
Wheat flour	US Food Aid	6.6–7.9	Retinyl palmitate (dry)	75	80–100 <sup>a</sup>
Sugar	Guatemala	15	Retinyl palmitate (dry)	30–120 (average, 60) (adults) 20–30 (young children)	45–180 (adults) 30 (<3 years)

<sup>a</sup> Assuming no losses during shipping, storage or food preparation. Unless otherwise stated, the contribution to the recommended nutrient intake (RNI) is based on an RNI for an adult male, which is 600 µg/day.

Source: adapted from reference (263).

vitamin was stable for up to 9 months. However, although less than 15% of the vitamin A was lost during boiling or pressure cooking of rice or beans, about 60% was lost when the oil was reused several times for frying (260).

There has been little systematic evaluation of the effectiveness of margarine and oil fortification, although historical data from Europe suggest that it has been effective in controlling vitamin A deficiency. In the Philippines, consumption of “Star margarine”, which is fortified with 25 mg vitamin A/kg plus 3.5 mg  $\beta$ -carotene/kg, significantly reduced the prevalence of low serum retinol. PL-480 vegetable oil, which is distributed in emergency feeding programmes, is intended to provide about 50% of the recommended daily intake of vitamin A for an adult male (assuming a daily intake of 16 g per person) (see **Table 5.5**). The stability of vitamin A in previously unopened pails of PL-480 oil is excellent, although up to 30% losses can occur in opened pails after 30 days of storage. Vitamin A retention in the oil is also good, with only a 10% loss after 30 minutes of heating (268).

#### 5.2.2.2 *Cereals products and flours*

Wholegrain cereals and flours contain negligible, if any, amounts of intrinsic vitamin A. Flours are, nevertheless, potentially good vehicles for vitamin A fortification, because dry forms of vitamin A can easily be mixed in with other additives. Despite this, cereal flours are not fortified with vitamin A in most industrialized countries, because, for historical reasons, margarines are the preferred vehicle and, furthermore, because vitamin A deficiency is no longer a significant problem. The United States Title II Food Aid Program has been fortifying wheat-soy and corn-soy blends with vitamin A for about 30 years; working on the assumption that the recipients are likely to be highly dependent on these fortified foods for their vitamin A needs, it adds sufficient amounts to provide 100% of the recommended daily intake of this particular vitamin (269). However, between 30% and 50% of the vitamin A that is added to the blended cereals is lost in shipping and storage (268,270).

Wheat flour is fortified with 4.5 mg retinol/kg in some mills in the Philippines, a practice which provides an average concentration in bread of 2.2  $\mu$ g retinol/g (**Table 5.5**). This supplies about 33% of the recommended daily intake for vitamin A for school-age children. At this level of fortification, retinol liver stores in deficient children were significantly increased at the end of a 30-week efficacy trial (33) (see also *section 1.3.1.2*).

Pre-cooked maize flour has been fortified with vitamin A in Venezuela since 1993 (**Table 5.5**). A fortification level of 2.7 mg/kg and an intake of 80 g flour/day supplies about 40% of an average family's recommended intake (271). However, the impact of maize fortification on the vitamin A status of the general population is not known.

### 5.2.2.3 Sugar

In the 1970s, vitamin A fortification of sugar was implemented in Costa Rica and Guatemala, because it was the only centrally processed food vehicle that was consumed in adequate amounts by the poorer segments of the population. Such programmes ceased for a time during the 1980s but are again functioning in Guatemala, and also in El Salvador, Honduras and Nicaragua where they receive strong support from the sugar industry (272). An early evaluation of vitamin A fortification of sugar in Guatemala showed that it is an effective strategy for improving vitamin A status and for increasing the amount of the vitamin in breast milk of lactating mothers (273) (see also *section 1.3.2.4*). Fortified sugar in Guatemala provides children with about one third of their recommended intake of vitamin A (274) (**Table 5.5**). Sugar fortification is now being implemented in other parts of the world, such as Zambia.

Large quantities of sugar are used in a wide range of commercial foods, such as confectionery and soft drinks. Retinol in fortified unrefined sugar survives the baking process but is lost during soft-drink production (in fortified unrefined sugar only one third of the initial level remains after 2 weeks of storage). Depending on the level of soft drink production, these losses can have important cost implications and it may be appropriate for the soft drink sector to be exempt from having to use fortified sugar (275).

### 5.2.2.4 Rice

Given that rice is an important staple in many countries where the prevalence of vitamin A deficiency is high, vitamin A fortification of rice has the potential to be an effective public health strategy for the elimination of VAD. However, as is the case with iron, for technical reasons, rice fortification with vitamin A is still at an experimental stage. Again, the predominance of small-scale mills in the rice-producing countries hinders the implementation of fortification programmes using rice as the chosen food vehicle.

### 5.2.2.5 Other foods and beverages

Other foods that have been fortified successfully with preformed or provitamin A include:

- dry milk;
- complementary foods for infants and young children;
- biscuits and beverages, which are sold commercially or used in school feeding programmes such as those implemented in Indonesia, Mexico and other countries in Central America (276), (277), Peru (278) and South Africa (34);



- instant noodles (in Thailand), the vitamin A (and elemental iron<sup>1</sup>) being supplied in the spices that are provided in a separate sachet (279);
- yoghurt (worldwide) (280).

### 5.2.3 Safety issues

Adverse physiological effects have been associated with both acute hypervitaminosis A and chronic high intake. The routine consumption of large amounts of vitamin A over a period of time can result in a variety of toxic symptoms including liver damage, bone abnormalities and joint pain, alopecia, headaches, vomiting and skin desquamation (93).

For long-term daily intakes, the United States Institute of Medicine's Food and Nutrition Board (IOM/FNB) have defined Tolerable Upper Intake Levels (ULs) for vitamin A, as follows (91):

- 600 µg/day for children <3 years,
- 900 µg/day for children 4–8 years,
- 1 700 µg/day for children 9–13 years,
- 2 800 µg/day for adolescents,
- 3 000 µg/day for both women at risk of becoming pregnant and adult men.

The UL for children, i.e. the highest level of daily vitamin A intake that is likely to pose no risk of adverse health effects, is a factor of 10 lower than the level of intake at which any toxic effect has been observed in this age group.

The ULs as defined by the United States Food and Nutrition Board are based on data obtained from healthy populations in developed countries. They may not apply, nor are intended to do so, to communities of malnourished individuals that receive vitamin A prophylactically, either periodically or through fortification, as a means of preventing vitamin A deficiency. A recent review has indicated that the risk of excessive vitamin A consumption from fortified foods in women and young children is likely to be negligible (281), but that it is nevertheless a matter that deserves attention as many foods are increasingly being fortified with vitamin A.

β-Carotene and other provitamin A carotenoids are less of a concern in terms of potential toxicity, not being active forms of the vitamin and because at high doses they are absorbed less efficiently (91). Furthermore, the synthesis of vitamin A from β-carotene and other provitamin A carotenoids is strictly regulated in the body. Hypervitaminosis A has never been reported as a result of provitamin A supplementation.

<sup>1</sup> Elemental iron is used because more soluble iron compounds would give the spices a black colour.

## Summary: vitamin A fortification

- A variety of oily and dry forms of the retinol esters, retinyl acetate and retinyl palmitate, are available for food fortification purposes. The dry forms are usually gelatin-, starch- or gum-coated and all forms contain antioxidants.
- Absorption of all forms is good (around 90%) but losses of vitamin A during processing, storage and food preparation may be high.
- Vitamin A fortification of margarine and sugar has been shown to be efficacious. Vegetable oils and cereal flours are also considered to be useful fortification vehicles.
- Adverse health effects have been associated with acute and chronic high intakes of retinol (mainly through supplementation) but not with high intakes of the pro-vitamin A carotenoids.

TABLE 5.6

### Iodine fortificants: chemical composition and iodine content

Fortificant	Formula	Iodine content (%)
Calcium iodide	$\text{CaI}_2$	86.5
Calcium iodate	$\text{Ca}(\text{IO}_3)_2 \cdot 6\text{H}_2\text{O}$	65.0
Potassium iodide	KI	76.5
Potassium iodate	$\text{KIO}_3$	59.5
Sodium iodide	$\text{NaI} \cdot 2\text{H}_2\text{O}$	68.0
Sodium iodate	$\text{NaIO}_3$	64.0

## 5.3 Iodine

### 5.3.1 Choice of iodine fortificant

There are two chemical forms of iodine that are suitable for use as food fortificants, namely, iodate and iodide. They are usually added as the potassium salt, but sometimes as the calcium or sodium salt (**Table 5.6**).

Potassium iodide has been used as an additive in bread and salt for about 80 years, and potassium iodate for about 50 years. Iodates are less soluble in water than the iodides, more resistant to oxidation and evaporation, and being more stable under adverse climatic conditions, do not require the co-addition of stabilizers. Although more expensive, potassium iodate is thus preferred to potassium iodide, especially in hot and humid climates, and is recommended as an additive for many foods, including salt (282,283). For historical reasons, however, countries in Europe and North America still use potassium iodide, while most countries with tropical climates use potassium iodate. Losses of

iodine because of iodide oxidation are increased by moisture, humidity, exposure to heat and sunlight, or by impurities in the salt to which it is added.

### 5.3.2 Experience with iodine fortification of specific foods

#### 5.3.2.1 Salt

Salt is the most widely used food vehicle for iodine fortificants. Indeed, universal salt iodization (USI), that is, the iodization of all salt for human (food industry and household) and livestock consumption, is the strategy recommended by WHO for the control of iodine deficiency disorders (284). The choice of this strategy is based on the following factors:

- salt is one of the few commodities consumed by everyone;
- salt consumption is fairly stable throughout the year;
- salt production is usually limited to a few geographical areas;
- salt iodization technology is easy to implement and available at reasonable cost throughout the developing world (0.2–0.3 US cents/kg, or 1 US cent per person/year);
- the addition of iodine to salt does not affect its colour, taste or odour;
- the quality of iodized salt can be monitored at the production, retail and household levels.

The mining of solid rock deposits is the main source of salt in Australia, Europe and North America. Elsewhere, i.e. in Africa, Asia and South America, solar evaporation of either sea water, lake or underground brines is the main source. After extraction, crude salt is refined so that its purity increases from 85–95% NaCl to 99% NaCl. Specifications for the physical characteristics and chemical composition required for food grade salt are laid down in the Codex Alimentarius (285).

Iodine is usually added to salt after the salt has been refined and dried, by one of two main techniques. In the wet method, a solution of potassium iodate ( $\text{KIO}_3$ ) is either dripped or sprayed at a uniform rate onto salt passing by on a conveyor belt. The technique is particularly cost-effective. For instance, in Switzerland, a single conveyor belt and sprayer produces enough salt for 6 million people at a cost of 1 US\$ per 100 kg salt or 7 US cents per person per year (286). The alternative method, the dry method, involves sprinkling potassium iodide powder (KI) or potassium iodate ( $\text{KIO}_3$ ) over the dry salt. This technique is more demanding, in that it requires a salt made of small homogeneous crystals and the thorough mixing of the salt after addition of the iodine compound to ensure an even distribution of iodine. Poor mixing is a major cause

of inappropriate salt iodization. Technical information on the salt iodization process is available elsewhere (287).

The stability of iodine in salt depends on the water content, acidity and purity of the salt to which it is added. In order to reduce iodine losses during storage, the iodized salt must be as pure and as dry as possible, and it must be appropriately packaged. Iodine tends to migrate from the top to the bottom of a container when the water content is too high. It will evaporate if the acidity is too high. Losses also tend to occur when packaging with impervious linings is used; as the packaging becomes damp, the iodide migrates from the salt to the fabric, and then evaporates. This is less likely to happen with potassium iodate because the iodates are less soluble and more resistant to oxidation. Types of packaging that help to prevent iodine losses include high density polyethylene bags that are either laminated with low density polyethylene or lined with a continuous film that is resistant to puncture. In a multi-country study of iodine losses from salt, high humidity combined with porous packing (such as jute bags), caused a 30–80% loss of iodine over a period of 6 months (288).

Because salt iodization is cheap and easy to implement, great strides in salt iodization programmes have been made in a relatively short period of time (Table 5.7). During the 10-year period, 1989 to 1999, the proportion of households consuming iodized salt increased from 10% to 68% and by 1999, of 130 countries affected by iodine deficiency, 98 had in place legislation requiring the iodization of salt (284). Several factors have limited progress towards the goal of USI; these include difficulties in enforcing legislation on iodized salt; problems caused by having a high number of small-scale salt producers and the absence of an operational monitoring system. The existence of pockets of populations living in remote areas that cannot easily access iodized salt is another factor which can hinder the effective implementation of salt iodization programmes and their sustainability in some countries. In order to assist countries

TABLE 5.7

**Progress towards universal salt iodization in WHO regions, status as of 1999**

WHO region	Coverage (% of households)	No. of countries with legislation on iodized salt
Africa	63	34
Americas	90	17
South-East Asia	70	7
Europe	27	20
Eastern Mediterranean	66	14
Western Pacific	76	6
Total	68	98

Sources: adapted from references (284,289).

develop and sustain effective salt iodization programmes, several international organizations, including WHO, have jointly established a mechanism for strengthening national capacity in activities that support salt iodization, in particular, quality assurance and monitoring. The work of the International Resource Laboratory for Iodine network (IRLI), which includes training and technology transfer and information sharing, is outlined in more detail in **Annex B**.

#### 5.3.2.2 *Bread*

From a technical point of view, bread is a good vehicle for iodine and has been shown to be an effective way of ensuring a constant supply of dietary iodine. It has been used in a few European countries where bread is a staple food, such as Russia (290,291), and in Tasmania. The main carrier for iodine in the Netherlands is the salt added to bread, i.e. baker's salt, which has been enriched with iodine since 1942. In recent years, the potassium iodide content of Dutch baker's salt has been increased.

#### 5.3.2.3 *Water*

Because water is consumed daily, it too has the potential to be a useful vehicle for iodine fortification. Its major limitation, compared with salt, is that sources of drinking water are so numerous and ubiquitous that iodization would be difficult to control. Moreover, iodine has limited stability in water (no longer than 24 hours) such that continuous daily dosing of the water supply would be necessary. Although the use of water as a vehicle for iodine fortification is technically more difficult than the use of salt, there are certain conditions where water iodization could be a suitable method for the correction of iodine deficiency.

The simplest way of fortifying water with iodine is to add a concentrated iodine solution (as potassium iodide or iodate) in a dropwise fashion until a specified concentration in the water contained in a given vessel is reached. This method is widely used in schools in northern Thailand (292). Alternatively, in the case of hand pumps and open wells, iodine in porous polymer containers can be introduced into the water supply. The porous containers allow the slow release of potassium iodide solution into the water supply. However, such containers have a limited shelf-life and must be changed every year. Such practices have been successful in several parts of the world; in Africa, in the Central African Republic, Mali (293) and Sudan (294), in Asia, in the central Asian republics, Malaysia (295) and Thailand and in Europe, in Italy (Sicily). In most settings, the limiting factor, especially in terms of cost-effectiveness, is that the whole population and the livestock need to use the iodized water supply point to benefit from iodization (296). A third option, which is suitable for piped water supplies, is to divert some of the piped water through a canister packed with

iodine crystals, and then reintroduce this iodized water back into the main water supply. The direct addition of an iodine solution to freshwater supplies has also been attempted. For instance, a 5% potassium iodate solution was introduced into the single river which supplied water to an isolated population in China for a period of 12–24 days (297). The result was an improvement in urinary iodine of children, and a relatively stable increase in soil iodine.

A review of the efficacy and cost-effectiveness of the different procedures used to iodize water concluded that while efficacious for the most part, there is no doubt that the cost, and the monitoring systems needed, are more problematic than those required for iodized salt (296).

#### 5.3.2.4 *Milk*

Iodine-enriched milk has been instrumental in the control of iodine deficiency in several countries. However, this has been largely a consequence of the use of iodophors by the dairy industry rather than the result of a deliberate addition of iodine to milk. Iodine-enriched milk has become a major adventitious source of iodine in many countries in northern Europe, as well as in the United Kingdom (298) and the United States. Use of iodized bread in Tasmania was discontinued when other sources of iodine, notably milk (consequent to the use of iodophors by the dairy industry), became available.

#### 5.3.2.5 *Other vehicles*

The feasibility of using sugar as a vehicle for iodine fortification has been assessed in pilot studies in Sudan (299), and that of fish sauce in south-east Asia where it is a major source of dietary sodium (i.e. salt). Besides fortifying table salt (300). Finland fortifies its animal fodders and as a result the iodine content of foods derived from animal sources has increased.

### 5.3.3 *Safety issues*

Iodine fortification is generally very safe. Iodine has been added to salt and bread for more than 50 years without any notable toxic effects (301). At its fifty-third meeting in 1999, the Joint FAO/WHO Expert Committee on Food Additives concluded that potassium iodate and potassium iodide could continue to be used to fortify salt for the prevention and control of iodine deficiency disorders (238). Because the synthesis and release of thyroid hormones is usually well regulated, through mechanisms that enable the body to adjust to a wide range of iodine intakes, intakes of up to 1 mg (1 000 µg) per day are tolerated by most people.

Nevertheless, an acute, excessive increase in iodine intake can increase the risk of iodine toxicity in susceptible individuals, that is, those who have had chronic iodine deficiency. This condition is known as iodine-induced

hyperthyroidism (IIH) and it is the most common complication of iodine prophylaxis. Outbreaks have been associated with almost all iodine supplementation programmes (302); it tends to occur in the early phase of programme implementation and mainly affects the elderly who have long-standing thyroid nodules. IIH is, however, usually transitory in nature and its incidence rate reverts to normal levels after 1–10 years of intervention.

Outbreaks of IIH, which were subsequently attributed to the sudden introduction of excessively iodized salt in populations who had been severely iodine deficient for very long periods, have recently been reported from the Democratic Republic of the Congo (303) and Zimbabwe (304). Such reports would appear to indicate that IIH could occur if salt is excessively iodized (305). If an outbreak of IIH was to occur following the introduction of iodized salt, it would be expected to follow a similar pattern to that observed during iodine supplementation programmes, that is, manifest early on in the history of the programme and predominantly among the elderly. IIH prevention requires the monitoring of salt iodization levels and the iodine status of the population, coupled with proper training of health staff in the identification and treatment of IIH (306).

Iodine-induced thyroiditis is another condition that can be aggravated or even induced by increasing iodine intakes (307). To date, there have been no large-scale investigations of the impact of iodine intervention programmes on iodine-induced thyroiditis.

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### Summary: iodine fortification

- Universal salt iodization, that is, the iodization of all salt for both human and animal consumption, is the strategy recommended by WHO to correct iodine deficiency.
  - Potassium iodate is preferred to potassium iodide for salt iodization because it is more stable.
  - The benefits of correcting iodine deficiency far outweigh the potential risks of fortification. Iodine-induced hyperthyroidism and other potential adverse effects can be almost entirely avoided by adequate and sustained quality assurance and monitoring of iodine fortification.
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## CHAPTER 6

# Zinc, folate and other B vitamins, vitamin C, vitamin D, calcium, selenium and fluoride

## 6.1 Zinc

### 6.1.1 Choice of zinc fortificant

Zinc compounds that are suitable for use as food fortificants include the sulfate, chloride, gluconate, oxide and the stearate. All of these compounds are either white or colourless, but have varying water solubilities; some have an unpleasant taste when added to certain foods. Although it is only poorly water soluble, zinc oxide is the cheapest of the zinc fortificants and therefore tends to be the preferred choice. Recent studies have shown that the absorption of zinc from cereal products fortified with zinc oxide is as good as that from those fortified with the more soluble zinc sulfate (308,309), presumably because the oxide is soluble in gastric acid. However, zinc absorption from the oxide may be poor in individuals with low stomach acid secretion.

### 6.1.2 The bioavailability of zinc

Zinc absorption from food is dependent on the amount of zinc consumed and the ratio of phytate to zinc in the meal being consumed. According to recent estimates by the International Zinc Nutrition Consultative Group (IZiNCG), when zinc intake is just adequate to meet the physiological requirements for absorbed zinc, in adult men about 27% of the zinc content is absorbed from diets having a phytate:zinc molar ratio of less than 18, which drops to about 19% when the phytate:zinc molar ratio is greater than 18 (i.e. high phytate). The corresponding zinc absorption rates for adult women are 35% and 26%, respectively (109). When zinc intake is greater than the critical level needed to meet requirements, the fractional absorption becomes progressively less, although the net absorption of zinc increases slightly. In one study involving healthy, well-nourished adults from the United States, zinc absorption from the sulfate (or the oxide) added to a low-phytate bread meal was about 14% (total zinc content, 3.1–3.7 mg per meal) compared with around 6% from the same fortificants added to a high-phytate wheat porridge meal (total zinc content, 2.7–3.1 mg per meal) (309).



### 6.1.3 Methods used to increase zinc absorption from fortificants

In light of the above findings, and given the similarities to iron (see *section 5.1.2*), it is reasonable to assume that reducing the phytic acid content of food will increase the absorption of zinc from fortificants, at least in the case of adults (310). Whether the same applies to infants and young children is uncertain. A lower extraction rate will result in a reduced phytate content of cereals but also a reduced zinc content, so the net effect on zinc supply tends to be minimal. Alternatively, the phytate content can be reduced by activating the phytases that are naturally present in most phytate-containing foods (through germination, fermentation and/or soaking) or by adding microbial or fungal phytases. Including sources of animal protein in the diet has also been shown to be an effective way of improving zinc absorption from high-phytate diets (93).

Absorption enhancers equivalent to ascorbic acid for iron, do not exist for zinc. However, according to the results of one study conducted in adult women, the addition of NaFeEDTA as a fortificant can increase zinc absorption from the diet, in this case from about 20% to 35%; 1% of the additional amount of zinc absorbed was excreted in the urine (311). This finding has yet to be confirmed in other studies. However, if, as reports suggest, the addition of Na<sub>2</sub>EDTA or NaFeEDTA to cereal flours inhibits the action of yeast during the bread-making process, these compounds would be of limited use, at least in cereal flours.

### 6.1.4 Experience with zinc fortification of specific foods

Hitherto, fortification with zinc has been fairly limited, and is generally confined to infant formula milks (with zinc sulfate), complementary foods and ready-to-eat breakfast cereals (in the United States). In Indonesia it is mandatory to add zinc to wheat noodles. More recently, several Latin American countries have expressed some interest in fortifying cereal flours with zinc.

Several studies have demonstrated the benefits of zinc supplementation on the growth rate of children (see *section 4.1.3*). However, very few trials have assessed the efficacy or effectiveness of zinc fortification. Although the addition of zinc oxide to breakfast cereals increased plasma zinc concentrations in pre-school-aged children in the United States, there was no evidence of concomitant increases in growth rates or in food intake (312). However, in Turkey, zinc fortification of bread did increase the growth rates of schoolchildren who initially had low plasma zinc (313).

Little is known about the effects of added zinc on the sensory properties of foods. The fortification of wheat flour with relatively high levels of zinc (as zinc acetate) did not affect the baking or organoleptic properties of the bread dough (313). Likewise, the addition of 60 or 100 mg zinc/kg wheat flour (as zinc sulfate or zinc oxide) did not change the acceptability of bread (314). Encapsulation of

zinc compounds is possible but has not been considered to date. This would, however, be a convenient way to mask the unpleasant taste of some zinc compounds.

## 6.2 Folate and other B vitamins

The B-complex vitamins are considered as a group in this chapter, as not only do they share some similar characteristics when used as food fortificants but they also tend to be added to the same foods. Members of the group of B vitamins covered here include folate/folic acid (vitamin B<sub>9</sub>), thiamine (vitamin B<sub>1</sub>), riboflavin (vitamin B<sub>2</sub>), niacin, pyridoxine (vitamin B<sub>6</sub>) and vitamin B<sub>12</sub> (cobalamin).

### 6.2.1 Choice of vitamin B fortificants

The characteristics of the vitamin B compounds that are suitable for adding to foods are summarized in **Table 6.1**. In general, the B vitamins are relatively stable, with thiamine being the most labile to heat. Synthetic folate, i.e. folic acid (in the form of pteroyl monoglutamic acid) is moderately heat stable (315), but is susceptible to the effects of oxidizing and reducing agents (316).

Some fortificant loss is inevitable, the degree of loss being dependent on factors such as the temperature used during food processing or preparation, the moisture content, extrusion temperatures and pressures, the presence of other micronutrients (in the premix and in the fortified food), the nature of the packaging, and the anticipated shelf-life of the fortified product. Vitamin recoveries in bread made from fortified flour range from about 70% to 95% for niacin, and from 75% to 90% for thiamine and pyridoxine. About 70% of any added thiamine, pyridoxine and niacin is retained when enriched flour is used to prepare pasta, even after drying and cooking. On this basis, and assuming that any added B vitamins are 100% absorbed, in flour an overage of approximately 20–30% is thus usually sufficient to provide the desired amount in food products such as breads and cereals.

Folic acid has a light yellow colour, which does not carry over to fortified foods because it is added at such low levels, typically between 1.5 and 2.4 ppm. There is some loss of the vitamin on exposure to light, and during cooking and baking. The biggest losses tend to occur from biscuits and pasta, but even these are probably no more than 20%. As folic acid concentrations in foods are difficult to measure, reported levels in fortified flour and baked products are often subject to considerable assay error.

TABLE 6.1

**Vitamin B fortificants: physical characteristics and stability**

<b>Vitamin</b>	<b>Fortificant compound</b>	<b>Physical characteristics</b>	<b>Stability</b>
Thiamine (B <sub>1</sub> )	Thiamine hydrochloride	More soluble in water than the mononitrate form White or almost white	Both salts are stable to oxygen in the absence of light and moisture but are unstable in neutral or alkaline solutions and in the presence of sulfites.
	Thiamine mononitrate	White or almost white	Losses during leavening and baking are estimated to be 15–20%. Available in a coated form. The mononitrate is preferred for dry products.
Riboflavin (B <sub>2</sub> )	Riboflavin	Relatively water insoluble Yellow	Very unstable in light. Rapid loss from milk on exposure to light but stable in white bread.
	Sodium salt of riboflavin 5'-phosphate	Soluble in water Yellow	
Niacin	Niacin (nicotinic acid)	Soluble in alkali, sparingly soluble in water White	Very stable to oxygen, heat and light, both in the dry state and in aqueous solution.
	Niacinamide (nicotinamide)	Water soluble White	
Pyridoxine (B <sub>6</sub> )	Pyridoxine hydrochloride	Water soluble White or almost white	Stable in oxygen and heat, but relatively sensitive to UV light.
			Available in a coated form.
Folic acid (B <sub>9</sub> )	Pteroyl monoglutamic acid	Sparingly soluble in water, soluble in dilute acid and alkali Yellow-orange	Moderately stable to heat. Stable in solution at neutral pH but increasingly unstable at higher or lower pH.
			Unstable in UV light.
Vitamin B <sub>12</sub> (cobalamin)	Cyanocobalamin	Pure vitamin B <sub>12</sub> is sparingly soluble in water; the diluted forms are however completely soluble	Relatively stable to oxygen and heat in neutral and acid solution, but unstable in alkali and strong acids, in strong light, and in alkaline solutions at >100°C.
		Dark red, often supplied diluted on a carrier (0.1%)	

## 6.2.2 Experience with vitamin B fortification of specific foods

There is a long history of experience of adding B vitamins to cereals (including wheat and maize flours) and rice grains, in both industrialized and developing countries. The benefits of restoration of thiamine, riboflavin and niacin in cereals and flours, 65–80% of which are removed by milling, have long been recognized. Indeed, the enrichment of flours and cereals has made, and continues to make, a major contribution to meeting the recommended intake of these vitamins even in the industrialized countries (317). The amount of niacin added to wheat flour typically ranges from 15 to 70 mg/kg (178); thiamine (vitamin B<sub>1</sub>) addition levels range from 1.5 to 11 mg/kg, and those for vitamin B<sub>12</sub>, from 1.3 to 4 mg/kg (318).

About 75% of the folate in whole wheat is also lost during milling, but folic acid has been included in cereal fortification programmes only relatively recently. In 1998, it became mandatory to fortify grain products with folic acid in the United States, the rationale being that it would lower the prevalence of neural tube defect births. The required fortification level is 154 µg/100 g flour (Mandate 21 CFR 137.165). According to one assessment the impact of this measure has been a 26% reduction in the incidence of neural tube defects (48). Mandatory folate acid fortification has also quite rapidly lowered the prevalence of low plasma folate concentrations in adults from around 22% to almost zero, and reduced the prevalence of elevated plasma homocysteine by about 50% (49). In addition to the United States, some 30 countries now add folic acid to flour, including Canada (150 µg/100 g), Chile (220 µg/100 g wheat flour), Costa Rica (180 µg/100 g), Dominican Republic (180 µg/100 g), El Salvador (180 µg/100 g), Guatemala (180 µg/100 g), Honduras (180 µg/100 g), Indonesia (200 µg/100 g wheat flour), Mexico (200 µg/100 g wheat flour), Nicaragua (180 µg/100 g) and Panama (180 µg/100 g) (318).

The B-complex vitamins are added directly to flour as single nutrients or as a premix (which usually also contains iron), or they are diluted with a small amount of flour at the mill before being added to the bulk. In the case of ready-to-eat breakfast cereals, the B vitamins can either be added to the dry mix prior to extrusion or other processes, or a vitamin solution or suspension can be sprayed onto the cereals after they have been toasted. Riboflavin has a strong yellow colour and slightly bitter taste, but at the levels that are typically added to white flour any colour or taste problems are likely to be minimal. Coated forms of the water-soluble vitamins, such as thiamine and vitamin B<sub>6</sub>, are available if off-flavours or other problems arise (Table 6.1).

## 6.2.3 Safety concerns

### 6.2.3.1 Thiamine, riboflavin and vitamin B<sub>6</sub>

As toxicity is not a problem, the United States Food and Nutrition Board has not defined upper intake limits (ULs) for thiamine and riboflavin. In the case

of vitamin B<sub>6</sub>, sensory neuropathy has been linked to high intakes of supplements but according to the findings of the United States Food and Nutrition Board, “No adverse effects associated with vitamin B<sub>6</sub> from food have been reported. This does not mean that there is no potential for adverse effects resulting from high intakes. Because data on the adverse effects of vitamin B<sub>6</sub> are limited, caution may be warranted”. A UL of 100 mg for adults and 30–40 mg for children has thus been set (128). These levels are very unlikely to be obtained from fortified foods.

#### 6.2.3.2 *Niacin (nicotinic acid and niacinamide)*

Vasodilation or flushing (i.e. a burning or itching sensation in the face, arms and chest) has been observed as a first adverse effect in patients given high doses of nicotinic acid for the treatment of hyperlipidemia. Based on such evidence, the United States Food and Nutrition Board has defined a UL of 35 mg/day for nicotinic acid (128). Intakes of niacinamide have, however, not been associated with flushing effects.

Bearing in mind the different characteristics of the two forms of niacin, the Scientific Committee for Food in the European Union has proposed a UL for nicotinic acid of 10 mg/day and a separate, much higher, UL for niacinamide of 900 mg/day (319). The latter thus poses no safety limitations in common food fortification practice.

#### 6.2.3.3 *Folic acid fortificants*

The consumption of folic acid in amounts normally found in fortified foods has not been associated with adverse health effects. However, there has been some concern that high folic acid intakes could mask or exacerbate neurological problems, such as pernicious anaemia, in people with low intakes of vitamin B<sub>12</sub> (128). This has led to a reluctance to fortify with folic acid in some countries. This concern is particularly pertinent to those individuals who derive folic acid from both supplements and a range of fortified foods, as it is the case in many industrialized countries. In this situation, some people may exceed the UL for folic acid, which has been set at 1 mg/day (128)(129 old 110). An obvious solution to this potential problem is to fortify foods with both vitamin B<sub>12</sub> and folic acid.

To avoid any possible risk of adverse effects, folic acid fortification programmes should be designed so as to limit regular daily intakes to a maximum of 1 mg. In addition, measures which require folic acid-containing supplements and fortified foods to also contain vitamin B<sub>12</sub> could be considered, especially in the case of products consumed by older citizens who are at greater risk of vitamin B<sub>12</sub> deficiency and its associated conditions, in particular, pernicious anaemia.

## 6.3 Vitamin C (ascorbic acid)

### 6.3.1 Choice of vitamin C fortificant

Ascorbic acid and ascorbyl palmitate are often added to oils, fats, soft drinks and various other foods as a way of improving the stability of other added micronutrients (e.g. vitamin A) or as an iron absorption enhancer (see *section 5.1.2.1*). However, ascorbic acid is itself relatively unstable in the presence of oxygen, metals, humidity and/or high temperatures. To retain vitamin C integrity (especially during storage), foods must therefore be appropriately packaged, or the ascorbic acid encapsulated.

### 6.3.2 Experience with vitamin C fortification of specific foods

As a general rule, foods that are not cooked are better vehicles for vitamin C fortification. Blended foods, such as those used for feeding programmes in emergency situations, were often fortified with vitamin C as this was believed to be the most efficient way of delivering this nutrient to populations likely to be deficient. However, a trial with PL-480 cereals found that although almost all of the encapsulated fortificant ascorbic acid was retained during transit from the United States to Africa, it was rapidly destroyed when the cereal product was cooked for 10 minutes (270). On the other hand, the addition of vitamin C to commercially processed foods such as dry milk, infant formulas, cereal-based complementary foods, chocolate drink powders and beverages has been found to be successful in increasing intakes of this nutrient. As sugar helps to protect the ascorbic acid in soft drinks, sugar has been proposed as a possible vehicle for the vitamin (184).

## 6.4 Vitamin D

### 6.4.1 Choice of vitamin D fortificant

Either vitamin D<sub>2</sub> (ergocalciferol) or D<sub>3</sub> (cholecalciferol) can be added to foods. The two forms have similar biological activities and both are very sensitive to oxygen and moisture, and both interact with minerals. A dry stabilized form of vitamin D, which contains an antioxidant (usually tocopherol) that protects activity even in the presence of minerals, is generally used for most commercial applications.

### 6.4.2 Experience with vitamin D fortification of specific foods

Milk and other dairy products, including dried milk powder and evaporated milk, are often fortified with vitamin D. Many countries also fortify margarines with this vitamin.

Low exposure to sunlight is a risk factor for vitamin D deficiency and can be a problem among those who live in the more northerly or southerly latitudes

where UV radiation levels are lower during the winter months, and among women who, for cultural reasons, spend a large proportion of their time indoors or covered with clothing. In such situations, vitamin D fortification of milk and margarine have been found to be useful strategies for increasing intakes; the goal is to supply up to 200 IU/day in the total diet.

## 6.5 Calcium

Compared with other micronutrients, calcium is required in relatively large amounts. A heightened awareness of the need to increase intakes of calcium for osteoporosis prevention has meant that calcium fortification has attracted a good deal of interest in recent years.

### 6.5.1 Choice of calcium fortificants

Calcium salts suitable for use as food fortificants are listed in **Table 6.2**. Bioavailable forms recommended for the fortification of infant formulas and complementary foods include the carbonate (it can liberate  $\text{CO}_2$  in acid systems), the chloride, the citrate and the citrate malate, the gluconate, the glycerophosphate, the lactate, the mono-, di- and tribasic phosphates, the orthophosphate, the hydroxide and the oxide (320). All of these salts are either white or colourless. Most are bland although the citrate has a tart flavour, the hydroxide is slightly bitter, and high concentrations of the chloride and the lactate can be unpleasant. The cost of calcium carbonate is very low, usually less than that of flour.

As the daily amount of calcium required is several thousand times higher than that of most other micronutrients, it tends to be added separately (as opposed to part of a premix). The calcium content of commercially available salts ranges from 9% (the gluconate) to 71% (the oxide) (**Table 6.2**). Salts with lower concentrations will have to be added in larger amounts, a factor that may affect the final choice of fortificant.

There is little reason to believe that low solubility is a major constraint to the bioavailability of fortificant calcium. In general, absorption of added calcium is similar to that naturally present in foods, which ranges from about 10% to 30%. However, high levels of calcium inhibit the absorption of iron from foods and so this too is something that needs to be taken into consideration when deciding how much calcium to add. The co-addition of ascorbic acid can help overcome the inhibitory effect of calcium on iron absorption.

### 6.5.2 Experience with calcium fortification

Wheat flour was first fortified with calcium in the United Kingdom in 1943 in order to restore the calcium lost during milling. Today, it is compulsory to add 940–1560 mg calcium carbonate/kg to white and brown (but not wholegrain)

TABLE 6.2

**Calcium fortificants: physical characteristics**

Compound	Calcium content (%)	Colour	Taste	Odour	Solubility (mmol/l)
Carbonate	40	Colourless	Soapy, lemony	Odourless	0.153
Chloride	36	Colourless	Salty, bitter	–	6712
Sulfate	29	Varies	–	–	15.3
Hydroxyapatite	40	–	–	–	0.08
Calcium phosphate dibasic	30	White	Sandy, bland	–	1.84
Calcium phosphate monobasic	17	Colourless	Sandy, bland	–	71.4
Calcium phosphate tribasic	38	White	Sandy, bland	Odourless	0.064
Calcium pyrophosphate	31	Colourless	–	–	Insoluble
Glycerophosphate	19	White	Almost tasteless	Odourless	95.2
Acetate	25	Colourless	–	–	2364
Lactate	13	White	Neutral	Almost odourless	0.13
Citrate	24	Colourless	Tart, clean	Odourless	1.49
Citrate malate	23	Colourless	–	–	80.0
Gluconate	9	White	Bland	Odourless	73.6
Hydroxide	54	Colourless	Slightly bitter	Odourless	25.0
Oxide	71	Colourless	–	–	23.3

Source: adapted from reference (320).

flours milled in the United Kingdom. In the United States, the addition of calcium to flour has been optional since the early 1940s. Calcium sulfate, carbonate, chloride, phosphate, acetate or lactate are all suitable for fortification of wheat flours, but the oxide and hydroxide may require alterations in the pH of the dough for successful bread-making (321).

The range of foods that are fortified with calcium has steadily grown over the years as it became increasingly clear that intakes were low in many populations. The more soluble calcium salts, such as the citrate malate or the gluconate, are generally used to fortify juices and other beverages. Tribasic calcium phosphate, and sometimes calcium carbonate or lactate, is used to fortify milk, to which gums (e.g. carrageenan, guar gum) must also be added to prevent the calcium salt from sedimenting. Yoghurt and cottage cheese can also be fortified with these calcium compounds. In industrialized nations and in some Asian countries, soya beverages are marketed as a replacement for cow's milk in which case these too should be fortified with calcium. Stabilizers such as sodium hexametaphosphate



or potassium citrate can improve the quality of soya beverages fortified with calcium gluconate or lactogluconate.

The addition of calcium salts to some foods can cause undesirable changes in colour, texture and stability by increasing the cross-linking of proteins, pectins and gums. Calcium fortificants can also darken the colour of chocolate beverages.

## 6.6. Selenium

### 6.6.1 Choice of fortificant

For food fortification purposes, the sodium salts are generally considered to be the most suitable source of selenium. The selenite is a white, water-soluble compound, from which absorption is about 50%. It is readily reduced to unabsorbable elemental selenium by reducing agents, such as ascorbic acid and sulfur dioxide. Sodium selenate is colourless, and is less soluble in water and more stable than the selenite, especially in the presence of copper and iron. It has the better absorption (nearly 100% from the fortificant alone or 50–80% depending on the food vehicle to which it has been added), and also increases the activity of the enzyme, glutathione peroxidase, more effectively. When tested in milk-based infant formulas, more selenium was absorbed from the selenate (97% versus 73%), but as more selenium was excreted in the urine with the selenate (36% versus 10%), the net retention of selenium appears to be similar regardless of which chemical form is used (322). The relative retention of selenium from other fortified foods, including salt, has not been investigated. Organic forms of selenium, such as selenomethionine, are absorbed as well as the selenate, but remain longer in the body and thus theoretically pose a higher risk of toxicity. They have not been widely used for food fortification for this reason.

### 6.6.2 Experience with selenium fortification of selected foods

In regions of China where selenium deficiency is endemic, salt has been fortified with sodium selenite (15 mg/kg) since 1983. This measure increased average daily selenium intakes from 11 µg to 80 µg and has effectively reduced the prevalence of Keshan disease (see also *section 4.8.3*).

Sodium selenate is currently used to fortify a range of foods in various parts of the world. In Finland, for example, sodium selenate is added to fertilizers applied in areas having low soil selenium; measurable increases in the selenium content of milk, meat and cereals grown on these soils were observed within 6 months (217). Sodium selenate is an ingredient in some sports drinks (around 10 µg/l) and in the United States is used to fortify infant foods. Until 1985, bread supplied about half of the selenium intake for the United Kingdom population, but after 1985, when European wheat was replaced by Canadian wheat this dropped to about 20%.

## 6.7 Fluoride

### 6.7.1 Choice of fortificant

There are a number of ways in which fluoride intakes can be increased: fluoride can be added to water supplies at the point of supply or added to toothpaste. Hexa-fluoro-silicate acid (HUSIAC) is the most commonly used fluoride compound for large-scale water fortification. It is added as a concentrated aqueous solution. The fluoridation of salt and the enrichment of milk with fluoride are alternative options that have been used in some parts of the world.

### 6.7.2 Experience of fluoridation

The introduction of a salt fluoridation programme in Jamaica was associated with a large reduction in dental decay in children, when assessed after 7 years (323). However, a smaller trial in Hungary indicated that residence during early infancy in an area where salt was fluoridated was not associated with a reduced risk of later caries (324). In Costa Rica, a national fluoride salt fortification programme, requiring the addition of 225–275 mg fluoride/kg salt, became mandatory in 1989. There then followed a very substantial and progressive reduction in tooth decay, and in 1999, based on measurements of urinary fluorine excretion rates, the level of fluoride in salt was lowered to 175–225 mg/kg (325). However, it is possible that other sources of fluoride (i.e. toothpaste) may have contributed to the observed reduction in the prevalence of tooth decay in Costa Rica.

Where it is impractical or unacceptable to fluoridate water or salt, the addition of fluoride to milk is an alternative approach for preventing dental caries. Generally speaking, the level of fluoridation is best governed by the usual volume of milk consumed by young children. Guidelines for fluoride fortification of milk and milk products are available elsewhere (326).

A recent evaluation of the feasibility of adding fluoride to school milk in the United Kingdom concluded that fortification was both feasible and desirable (327). In rural Chile, preschool-aged children received 0.25–0.75 mg fluoride per day in fortified, powdered milk for a period of 4 years. The rate of decayed, missing and filled teeth declined substantially compared with a control community, and the percentage of children who remained caries-free doubled (328). Favourable results have also been reported from Beijing, in children who consumed 0.5 mg fluoride in milk each day at kindergarten and 0.6 mg fluoride in milk at home on weekend days (329). Similarly, in Scotland schoolchildren who consumed 1.5 mg fluoride daily in 200 ml milk had a significantly lower prevalence of caries than a control group after 5 years (330). However, these results were not replicated in a more recent study conducted in another region of the United Kingdom (331).