FORTIFICATION HANDBOOK

VITAMIN AND MINERAL FORTIFICATION OF WHEAT FLOUR AND MAIZE MEAL

L'Initiative pour les micronutriments
The Micronutrient Initiative

Contributors

Quentin Johnson
Venkatesh Mannar
Peter Ranum

Editors

Annie Wesley
Peter Ranum

This manual is based on current information as of June 2004. It will be periodically reviewed and updated to include new or revised data, costs, standards, QC methods, technologies and guidelines on fortification. Any suggestions for changes should be sent to The Micronutrient Initiative at the following email address: awesley@micronutrient.org

Table of Contents

Tables

Section 1 INTRODUCTION

This manual was prepared to serve as a guide in the development and implementation of fortification programs of wheat flour and maize meal. Though the intended audience consist of policy makers, millers and program managers, particularly in the developing countries, the information will be relevant for anyone interested in fortification of cereal staples. More than a decade of experience in advocating, designing and implementing successful flour fortification programs have contributed to the information presented in the manual.

In general, flour fortification is technically simple requiring only minor modifications in most of the modern flour mills. However, discussions with the milling industry, government and non-government partners have pointed to a need for an easy-to-use, comprehensive document to provide specific information relevant to flour fortification, and to guide choices related to premixes, fortification methods, equipment, quality control, etc.

Covering the full vitamin and mineral fortification of wheat and maize (corn) flours, this manual is intended to serve as a complementing document to the recent *Food Fortification Guidelines* by WHO and the Manual for *Wheat Flour Fortification with Iron by USAID – MOST*. More details on the scope of this manual are given at the end of this chapter.

Micronutrient Malnutrition

An alarming proportion of world's population suffers from 'hidden hunger', the term used for micronutrient deficiencies because the symptoms often cannot be seen or felt. Deficiencies in micronutrients - small quantities of vitamins and minerals that the body needs for physical and mental development - are widespread affecting more than a third of the world's population. In their absence, individuals and families suffer serious consequences including learning disabilities, impaired work capacity, illness and death. Collectively, the micronutrient deficiencies damage health, cause death, harm reproduction, reduce intelligence, educability and academic achievement, and lower work productivity and occupational choices. Of special concern, micronutrient deficiencies interfere with child growth and development, sometimes permanently.

Important micronutrients with public health significance include vitamin A, iron, iodine, B. complex vitamins and zinc. More than two billion people or a third of the world's population are unable to enjoy their maximum physical and mental potential owing to an inadequate intake of these nutrients. The prevalence of the problem is much higher in developing countries relative to developed countries. Even in developed countries like the USA and Canada, the rates of iron deficiency may reach the levels of public health significance and nearly every country is deficient in folic acid.

Fortification as an intervention strategy

There are many ways to increase micronutrient intake - by taking supplements regularly or through dietary measures that promote the regular consumption of micronutrient rich foods and improve their absorption in the diet. Technology is now available to improve the micronutrient content of cereal crops through selective plant breeding. However, in many situations these interventions are either not available or inaccessible by those who need them the most. Fortification of commonly eaten foods, including cereals, offers a low-cost and simple way of delivering micronutrients to a large number of people who need them.

Fortification is the process of adding vitamins and/or minerals to foods to increase its overall nutritional content. Fortification when imposed on existing food patterns may not necessitate changes in the customary diet of the population and does not call for individual compliance. It can often be dovetailed into existing food production and distribution systems. For these reasons, fortification can often be implemented and be sustained over a long period of time, making it to be the most cost-effective way to overcome micronutrient malnutrition.

Fortification has been recognized by many national governments as an important strategy to help improve the health and nutrition status of millions of people on a continuous and self-sustaining basis. The work productivity and learning ability of the nation as well as the cognitive capacity of the next generation could substantially be improved through fortification.

Milled cereal as a Vehicle for Delivering Deficient Micronutrients

Cereals are important food vehicles for fortification. Though several foods could be used for carrying micronutrients, wheat flour and maize meal are excellent vehicles because they are staple foods in many parts of the world and key ingredient in so many food preparations. When micronutrient deficiencies are population-wide and result from a combination of low intake and/or low bioavailability, fortification of commonly consumed cereal flours with iron, folic acid and other vitamins offers a number of strategic advantages. In many situations, cereals flours are the best choice for fortification because they are widely and regularly consumed, and mostly processed in centralized facilities with established distribution and marketing capacity. Due to these reasons, cereal fortification has played a major role in improving the health of the world populations at large. Currently, nearly 40 countries now fortify flour. With the advocacy and technical assistance from the Micronutrient Initiative (MI) and other partner organizations, several additional countries have either recently started or are seriously considering flour fortification.

Advantages of Flour and Maize Meal as Fortification Vehicles

There are a number of good reasons why wheat flour and maize meal are fortified with deficient micronutrients.

1. They are food staples, consumed in significant quantities by all age groups and economic classes at nearly every meal. This makes them ideal vehicles for getting deficient nutrients to the general population.

2. Most of the micronutrients being added are naturally present in the whole grain but greatly reduced by the milling refinement process. Many fortification programs simply call for restoring deficit nutrient levels to that contained in the whole grain, often called *enrichment* or *restoration*.

3. Fortification at the flour or maize mill is fairly simple and easy to control and regulate.

4. The mills producing the bulk of the flour and maize meal are large, modern and centrally located.

5. Some micronutrients, like folic acid and other B vitamins, are ideally suited for addition to milled cereals. There is no other food staple as well suited for B vitamin fortification.

6. Flour and maize meal have been fortified now for sixty years, so the concept, technology and sustainability are well established.

7. The milling equipment, design and quality control procedures for flour fortification have all been developed and are readily available.

8. There are a number of commercial concerns operating worldwide that supply fortification premix and mill equipment at reasonable prices due to heavy competition.

9. Fortification of wheat flour and maize meal is an established and proven public health measure with widespread support by the medical and milling communities.

10. Cereal fortification is safe because a person cannot eat enough fortified flour or maize meal to exceed the upper safety levels of micronutrient intakes.

11. Fortification at the mill is relatively inexpensive and affordable. It will not noticeably impact the cost of the food to the consumer; yet the public will eventually pay for it with a small, overall price increase.

12. There are a number of groups - including the Micronutrient Initiative, UNICEF, USAID/MOST, ADB and GAIN - available to provide technical, promotional and financial support for establishing cereal fortification programs.

The Flour Fortification Initiative

The Flour Fortification Initiative is a collective network of individuals and organizations dedicated to making micronutrient fortification of flour standard practice. The network is an interactive infrastructure of people in the public, private and civic sectors who are working on behalf of fortification and who leverage their collective resources and relationships to do so more effectively. The FFI promotes:

- Fortification of all wheat flour and maize meal with deficit micronutrients where feasible and efficacious.
- Allowing every person to have access to fortified wheat or maize products where such products are part of their regular diet.
- Having every large size flour mill (>50 MT/day capacity) fortify some or all of their refined white flour (< 83% extraction or < 0.70% ash).

This involves accelerating and expanding ongoing efforts along with initiating new programs. The approach of FFI is to involve the private sector right from the beginning as part of Private-Public-Civic sector collaboration. While governments and NGOs can recommend flour fortification, only mills can actually do it. FFI recognizes that flour fortification alone will not solve the world's malnutrition problem. It must be done as part of a wider program that includes supplementation, education and other food based interventions.

Commercial or state run flour mills are urged to voluntarily fortify their flour if not currently required to do so, while recognizing that mandatory programs are preferable and more effective than voluntary ones. Each country or region can decide what types and levels of micronutrients to include in their fortification program based on their particular consumption and nutritional situation, hopefully using guidance from the WHO and this manual. But a minimum fortification in new programs is thought to be the addition of 2.4 ppm folic acid and restoration levels of iron using guidelines on the types and levels of iron compounds to use on different types of flours.

The web site (http://www.sph.emory.edu/wheatflour/Main.htm) of the Flour Fortification Initiative provides up to date information on its activities and data related to flour fortification.

Fortification at Different Size Mills

Many of the technical, economic and operational parameters of fortification are dependent on the size of the mill. Mill size is determined by its production capacity, either in the amount of grain it can process per unit time or in the amount of products (flour, meal, bran, etc.) it can produce. Capacity is often smaller than what a mill actually produces. Ideally, a mill should run over 90% of capacity but many mills in the world run at much less than that.

Table 1.1 compares mills of three different sizes: large, medium and small. Section 8 of the manual describes the fortification methods used on the large mills while Section 9 shows those for small mills. Medium size mills differ in many respects from both of these, but the fortification techniques they employ generally resemble those of the large size mills.

Table 1.1 Comparison of Different Size Mills

Parameter	Large scale	Medium scale	Small Scale
Milling capacity (wheat or maize)	> 48 MT per day or > 2 MT/hr	24 to 72 MT/day or 1 to 3 MT/hr	< 24 MT per day or < 1 MT/hr
Yearly operation	> 200 days/year	100 to 300 days/year	100 to 300 days/year
Daily operation	24 hours/day	8 to 24 hours/day	4 to 12 hrs/day
Flour or meal production	> 40 tons per day	5 to 50 MT/day	< 5 tons per day
Process	Continuous,	Usually continuous	Batch or continuous
Flow	Usually long flow, gradual reduction,	Often short flow reduction	Short, agressive reduction
Equipment - grinding	Multiple roller mills	More than one roller or non-roller (pin, hammer) mills	Single roller mill or pin, stone or hammer mill
Equipment - other	Free swinging sifters, purifiers	Screen sifters	May have screen sifter
Grain cleaning	Multiple, continuous equipment	Simple screening	Hand cleaning if any
Investment in equipment	High, > one million $	Medium	Low, < ten thousand $
Mill structure	Concrete, 4 or more stories adjoining silos	Varies, Can be modular unit in sheds	Sheds or none
Mill location	Usually near seaport or rail line in industrial area.	Usually in populated areas or near areas of heavy cereal production	Anywhere but typically in market area or near to where people shop and live.
Ownership	Company or government	Generally private	Generally one individual
Number of Employees	Varies greatly	3 to 10	One or two
Miller training	Some outside	On the job	On the job
QA/QC Manager	Yes	Usually not separate position	No
QC laboratory	Generally yes	Generally no	No
Wheat flour extraction rate	60 to 100%	78 to 95%	85 to 100%
Flour treatment	May add enzyme, ascorbic acid, bleach, bromate	None	None
Number of mill products	Six or more	A couple	One
Shelf life of flour, meal	6-9 months	1 to 6 months	1 - 3 month
Market for products	National and export	Regional	Local
Fortification premix used	Concentrated (50 – 200 g/MT)	Varies – may make diluted preblend with flour	Diluted preblend

Scope of the Manual

This manual covers different aspects of the fortification of wheat and maize (corn) flours. Each section of the manual is written to be self-sufficient so that the sections could be used as independent documents as necessary. However to obtain maximum benefit, it is recommended to consider all the sections together especially when starting or implementing a flour fortification program.

This manual will serve as a useful tool in introducing and accelerating flour fortification wherever wheat flour or maize meal is milled in large (> 4 tons/hour) and medium (1 to 4 tons/hr) quantities. The manual also covers in Section 9 the fortification of cereal flours processed at small (< 1 ton/hour), decentralized mills in rural communities of many developing countries. More details on fortification at small mills will be published as a separate manual.

This manual is based on the current information at the time of writing and will be updated periodically to include new data, technologies and guidelines on fortification. Advancements in the science and technology may permit things that are not currently possible, such as the addition of vitamin C to bread for nutritional reasons, or may lead to new recommendations on the types and levels of micronutrients to add.

Micronutrients covered in the manual

This manual covers the following nine vitamins and five minerals that are either currently being added to cereal staples somewhere in the world, or have been suggested to be added.

- **Vitamins**: *Folic acid, thiamin, riboflavin, niacin, pyridoxine, vitamin B12, vitamin A, vitamin D and vitamin C.*

- **Minerals**: *Iron, zinc, selenium, calcium, iodine*.

There are other micronutrients not covered, such as magnesium and pantothenic acid, which are being added to blended and complementary foods, such as corn soy blend (CSB), but there has been no interest in adding them to cereal staples at this time. It is not the intent of flour fortification to create the perfect food or to provide all of mankind's micronutrient needs, but rather to serve as a public health measure helping to alleviate chronic micronutrient malnutrition in regions where it exists. This does not require adding all of these micronutrients, but only the ones in greatest need.

Normal addition of these micronutrients can be categorized as being:

a) *Enrichment* or *restoration* when added to whole grain levels. (Typically done with zinc, iron, thiamin, riboflavin, niacin, pyridoxine, riboflavin, and selenium)

b) *Fortification* when added to well above whole grain levels (folic acid, calcium) or because they are not naturally present in grain (vitamin A, vitamin B_{12}, vitamin D).

However, to keep things simple, this manual will refer to any addition of micronutrients as *fortification*.

Cereal fortification not covered in this manual

1. **Rice** – The fortification of rice involves a different technology than that used on wheat flour or maize meal since rice is used as a whole grain rather than a flour or meal. The one exception to this is rice flour produced by a dry milling process, which can be fortified with the same methods used in flour but this has very limited production. Countries that depend on rice as the main cereal staple have many small rice mills making the control, logistics and economics of fortification quite different and much more difficult than with a few, large, centrally located flour mills. This is not to discourage the fortification of rice or to say it cannot be done. Rice is currently being fortified in the U.S. and with some open-market and targeted feeding programs. There are now some good methods to keep the added micronutrients with the rice using new coating and extrusion technologies so that they are not removed by washing and draining or lost on cooking. Technical difficulties remain, particularly in the color and appearance of the fortified rice kernel, which is added to rice at 1 to 2%, causing them to be picked out and discarded when the fortified rice is manually cleaned.

2. **Prepared Foods** – This manual is on the fortification of dry flour and meals done at the mill. It does not cover fortification performed at the bakery or food production plant. However, if those plants use fortified flour they clearly produce a fortified product as well.

3. **Non-Staple Cereal Foods** – Included with the prepared cereal foods are many branded ready-to-eat snacks, breakfast cereals and other cereal based foods. Many of these are fortified, some quite extensively. This type of fortification, called open market fortification by WHO, is done to improve the health image and increase the market demand for a company's products and is not a

general health program. The methods, strategies and economics applicable in this type of fortification are quite different from those discussed in this manual.

4. **Targeted Foods –** Foods targeted to special groups, such as those used to feed infants (complementary foods), the aged or people with special dietary needs have a distinct fortification technology that is not covered in this manual.

Manual Sections

1. Introduction
2. Micronutrient Malnutrition – Severity and Consequences (in preparation)
3. Cereal Staples
4. Milling and Processing
5. Types and Levels of Micronutrients to Add
6. Properties of Minerals
7. Properties of Vitamins
8. Fortification Methods – Large mills
9. Fortification Methods – Small mills
10. Quality Assurance and Control

<u>The following sections are in preparation and will be added as they are completed</u>:

11. Regulations and Enforcement
12. Economics of Fortification
13. Communications and Advocacy
14. Evaluating Effectiveness of Fortification Programs

Appendices and Additional Materials

A. Acknowledgements (in preparation)
B. Abbreviations and Glossary of terms (in preparation)
C. Guidelines on Design and Procurement of Premixes for the Fortification of Milled Cereals
D. Fortification premix suppliers
E. Mill equipment suppliers (in preparation)
F. Examples of fortification premix composition (in preparation)
G. Fortification Declarations (in preparation)
H. Examples of actual regulations (in preparation)
I. Analytical methods

Table 1.2 Review of Micronutrients Covered in this Manual

Mineral	*Physiological Functions*	*Deficiency Problems*	*Good Food Sources*	*Status in Cereal Fortification Programs*
Iron	Hemoglobin formation, oxygen transport, cellular oxidation	Iron Deficiency Anemia (IDA): impaired development and mental and physical work capacity	Meat, dried beans. Some foods are high in iron but it is poorly absorbed due to presence of inhibitors	Has always been included
Zinc	Growth, wound healing, taste acuity, insulin function.	Retarded growth (dwarfism) and sexual development, reduced resistance to disease	Dairy, meats, shellfish	Usually included in new programs for developing countries
Calcium	Bone and teeth structure, nerve and muscle action	Osteoporosis, increased risk of bone fractures, associated with increased risk of hypertension, preeclampsia, and colon cancer	Dairy products, tofu, dried beans	Often optional but seldom practiced
Selenium	Cofactor in enzymes involved in antioxidant protection and thyroid hormone metabolism. fat metabolism, peroxide neutralization,	Characteristic signs of selenium deficiency have not been described in humans, but very low selenium status is a factor in the etiologies of a juvenile cardiomyopathy (Keshan Disease) and a chondrodystrophy (Kashin-Beck Disease) that occur in selenium-deficient regions of China.	Meats, fish and grains except those grown in selenium-deficient soils.	Possible and suggested, but yet to be practiced
Iodine	Thyroid function, regulation of cell oxidation	Goiter	Seafood, iodized salt	Possible but yet to be practiced

Vitamins	***Physiological Functions***	***Deficiency Problems***	***Good Food Sources***	***Status in Cereal Fortification Programs***
Folic acid	Synthesis of RNA, DNA and protein	Neural tube birth defects	Green-leafy vegetables, citrus fruits	Usually included
Thiamin	Cofactor for several enzymes involved in carbohydrate catabolism	Beriberi: cardiovascular and nervous system disorders	Pork, legumes, yeast, whole grains	Often included, good for rice eating countries
Riboflavin	Participates in oxidation-reduction reactions in numerous metabolic pathways and in energy production	Ariboflavinos: characterized by weakness, sore throat, hyperemia and edema of the pharyngeal and oral mucous membranes, dermatitis	Dairy, eggs, widely distributed in meats and plants	Usually included
Niacin	Cofactor in many oxidation-reduction reactions	Pellagra: bilateral dermatitis, diarrhea, and dementia.	Widely distributed in meats and plants, whole grains	Often included, good for maize eating countries
Pyridoxine	Cofactor in many enzyme reactions	Severe deficiencies rare: may produce seizures, dermatitis anemia, and other problems	Widely distributed in meats and plants, whole grains	Newly suggested but included only in South Africa
Vitamin B12	Co-factor for two enzyme reactions, one involved with folic acid.	Pernicious anemia, severe deficiencies rare	Found only in animal products	Suggested but yet to be included
Vitamin A	Vision mechanism, gene expression, growth & development, maintenance of epithelial cellular integrity and immune functions	Night blindness and xerophthalmia leading to permanent blindness	Preformed vitamin A only present in animal products, beta- carotene, a vitamin A precursor, in colored grains and vegetables.	Included in a few programs
Vitamin D	Role in bone formation and calcium absorption and control.	Rickets	Dairy	Optional in U.S. but rarely practiced
Vitamin C	Multiple metabolic roles, enhances iron absorption	Scurvy (rare), increased susceptibility to infection	Fruits, potatoes	Not feasible with current technology but desirable.

Section 3 CEREAL STAPLES

This section provides data on the production, consumption, trade and nutritional composition of wheat flour and maize meal, showing why they make good vehicles for delivering deficit vitamins and minerals to the general population.

Cereal Production and Consumption

Cereal grains are the fruits of cultivated grasses. They provide mankind more nourishment than any other food class, and about half of all calories consumed. The most important cereals are wheat, maize (corn) and rice, which are grown in nearly equal amounts, as shown in Table 3.1. Most of the wheat and rice goes to direct human feeding after removing the outer layers, or bran, which is generally used as animal feed. Maize and barley are used as foods, animal feed, brewing and non-food applications. Rice and wheat provide the world about the same amount of calories, but wheat provides more protein since it is higher in protein content. Millet and sorghum are minor cereals that do not find much direct human consumption outside of Africa and some part of India.

Table 3.1 Worldwide Production and Consumption of Cereals (FAO)

	2000 World Crop Production	*2000 World per capita consumption* as Food	as Energy	as Protein
Cereal	***million metric tons***	***kg/person/year***	***kcal/person/day***	***grams/person/day***
Maize	593	19.1	157	3.8
Wheat	585	69.4	535	15.8
Rice	*400	57.6	576	10.7
Barley	140	1.1	8	0.2
Millet	29	3.4	20	0.7
Sorghum	58	4.2	35	1.0
All cereals	1,862	157.2	1356	32.7

***** milled equivalents

There are major regional preferences in the type of cereal consumed. Asia and the Far East predominantly consume rice but the consumption of wheat is significant and growing. Maize is the main staple in Central America, Mexico and sub Sahara Africa. The Middle East, North America, countries in the former Soviet Union and Europe consume mostly wheat.

Worldwide production of cereals has more than kept up with population growth thanks to the development of the higher yielding varieties and improved agronomic practices of the *green revolution*. This is expected to continue. Over the past 20 years, worldwide wheat consumption has been growing on average at a steady 1% per annum.

As the world's population became increasingly dependent on cereals for food, cereals gradually replaced the more expensive but nutrient rich animal food sources rich in iron and vitamin A. This has resulted in an actual decrease in the consumption of most micronutrients in many populations. Not only do cereals have lower levels of some micronutrients than animal foods, but some nutrients, like iron, are not as well absorbed from cereals as they are from animal products, while others, like vitamin B_{12}, are missing altogether, reinforcing the old adage that man cannot live by bread alone.

Global Cereal Production and Trade

The world produces nearly 600 million metric tons of wheat a year. Wheat can be grown in most countries but it is mainly grown in the temperate, drier climates. China and India grow the most wheat of any country. They usually consume as much as they grow but lately have started to export some wheat. The world's five major wheat exporters are the U.S.A., the E.U., Canada, Australia and Argentina, which have accounted for approximately 50% of the 100 metric tons of wheat traded internationally each year.

From 175 to 200 million tons of wheat is held over each year, which assures adequate supplies and reasonable prices in case of droughts.

Table 3.2 Major Wheat Exporting Countries

(million metric tons/year)

Country	*Production*		*Exports*	
	1995-2000 FAO average	*02/03 projected**	*1995-2000 FAO average*	*02/03 projected**
U.S.A.	64	46	29	24
Canada	26	18	18	12
Australia	22	20	17	16
E.U.-15		108	15	15
Argentina	15	14	9	9
Ukraine	14	17	2	8
Kazakhstan	8	12	3	5
Russia				5
India		72		4
China		92		1

*USDA Foreign Agricultural Service

Table 3.3 Major Wheat Importing Countries

(FAO 1995-2000 average in million metric tons/year)

Country	*Imports*
Brazil	7.1
Egypt	6.3
Iran	5.2
Algeria	4.3
Indonesia	3.8
Korea, Republic of	3.2
Morocco	2.6
Mexico	2.5
Pakistan	2.3
Philippines	2.2
Iraq	2.2
Yemen	2.0
Bangladesh	1.5
Nigeria	1.5

Wheat and maize are major global commodities and are traded openly throughout the world. The price will vary depending on world stocks, weather, and the dynamics of the world commodity trading markets and futures options. The *Chicago Board of Trade* sets prices and is a good benchmark for international prices. Government subsidies to farmers and economies of scale in major wheat growing countries mean these commodities may be available to many other countries cheaper than if they grew it locally. Shipping costs depend upon many factors related to port handling and freight movement. Grain storage and handling in ports is a significant cost-determining factor for wheat delivered to the mill. Most large mills are located close to large ports to reduce transport costs of wheat in and flour out.

The trade in milled wheat flour and maize meal is far less than that in whole grains. The world total in wheat flour trade is around 8.7 million tons of wheat equivalent. The major flour exporters are shown in Table 3.4. Most of the exported wheat flour is not fortified. Africa gets a lot of flour imports, about 3 million tons per year. Major flour importing countries are Yemen, Indonesia, Hong Kong and Libya, but these change from year to year depending on building new mills.

Governments also donate these commodities as food aid through the World Food Program or various NGOs. The average annual world cereal donation is 8.5 million tons of which 5.1 million tons are wheat or flour. Not all of this ends up as direct food assistance. Much of it is monitized, meaning it is sold on the open market. The aid organizations then use the proceeds to provide other types of assistance. Most of the wheat flour and maize meal provided in food aid programs is fortified, often including vitamin A.

Table 3.4 Leading Wheat Flour Exporting Countries[1]

Country	***1999-2000 exports 1000 tons wheat equivalents***
E.U.	4,312
U.S.	1,294
Japan	478
Argentina	470
Turkey	382
China	300
Australia	181
Canada	127

Flour production and consumption by countries

Table 3.5 shows the countries with the highest wheat flour production and whether or not the flour is fortified. Iran, Iraq and Pakistan are other major wheat flour producing countries, but for which there is no current production data. The large amount of atta flour produced by small neighborhood *chakki* mills in India and nearby countries is not reflected in this data.

Table 3.5 Leading Wheat Flour Producing Countries[2]

Country	***1999 Wheat Flour Production (1000 tons)***	***Flour Fortification***
Algeria	3,000	No
Argentina	3,563	Yes
Australia	1,984	Some
Brazil	6,789	In process
Canada	2,332	Yes
China	78,750	In process
Egypt	5,209	Some
France	4,780	No
Germany	4,118	No
India	4,145	No
Indonesia	2,074	Yes
Japan	4,627	No
Mexico	2,490	Yes
Morocco	2,360	In process
Romania	2,005	No
Russia	11,094	No
Spain	2,643	No
Ukraine	3,354	No
U.K.	4,498	Yes
U.S.A	18,686	Yes

[1] Based on data from the International Grains Council reported by Milling & Baking News, March 13, 2001, page 18.
[2] Based on information compiled by the International Grain Council reported in the Milling & Baking News, Aug. 27, 2002, page 32.

Estimates of wheat flour and maize meal consumption for each country are shown in Table 3.6. These estimates were made by taking 75%, a standard extraction rate, of the 1997-2000 average of wheat and maize consumption from the FAO food balance sheets, except for Latin American countries where the figures were provided by the milling industry. The maize consumption is given only for selected countries where maize is an important food staple. One should be cautious in using the maize data since some of the supply may go to non-food uses, such as brewing. These figures can be used as an initial guide to identify cereal fortification opportunities, to help calculate the amount of micronutrients to add to achieve a desired public health impact, and to estimate the impact of an existing fortification program. Some of these values have questionable accuracy and care should be taken in their application if not corroborated by other data.

Cereals as Food Staples

Cereals are common food staples because they are versatile, tasty, readily available on the market, affordable and culturally acceptable. They are consumed everyday, and often at every meal, by all age groups including infants. People like to eat cereal products, at least the ones they are accustomed to eating. There are no cultural or religious restrictions to their use. The basic cereal staples of wheat flour and maize meal are very inexpensive, even when they are imported. A person can get their 2000 kcal energy requirement from $0.14 worth of wheat flour or $0.10 worth of maize meal. That's an important consideration when one makes only $1.00 per day, as is the case with many people in the developing world.

Wheat and maize are milled into flour and meals by similar processes, as described in Section 4, which makes them very conducive to fortification. Rice, on the other hand, is milled by quite a different process and is not easily fortified. Wheat flour is used in a wide variety of foods, the most common ones being breads and noodles. Maize meal has far fewer applications. The most common are *tortillas, gruels* and *pastes* (called by different names including *grits, ugali, mealy meal* and *pap*). These are the cereal foods that people actually eat – no one eats dry flour, but it is the flour or meal that is fortified since it is generally produced at a large, centrally located mill where the micronutrients can easily be added. It is also possible to fortify at small mills, as discussed in Section 9, but it is generally not cost effective to do it at the bakery or cereal processing plant except for special dietary products, like cereal based complementary foods for infant feeding.

Fortification of Cereal Staples

When flour or maize meal is fortified at the mill, it causes all the many products made from that flour at hundreds or thousands of bakeries and food preparation sites to be fortified as well. That makes mill fortification much more efficient than bakery fortification, or even distribution of supplements. Some companies may choose to make a specially fortified, proprietary, cereal-based product, such as a ready-to-eat breakfast cereal, cake or an extruded snack, called *open market foods* by the WHO. There is no problem with their doing this as long as it is legal do to so in the country it is being sold and conforms to the applicable food regulations. But those types of products are not cereal staples and their fortification does not constitute a public health program. This type of proprietary fortification rarely reaches the general public and can be discontinued at any time the producer feels they no longer enjoy a marketing advantage. The most effective food fortification programs are ones where the consumer does not have to continually make a conscience effort to buy a fortified product, often for a slightly higher price. Rather, they are ones where food staples, like wheat flour and maize meal, are routinely fortified, called *mass fortification* in WHO parlance.

Table 3.6 Estimated consumption of wheat flour and maize meal by country and region

Country	Flour g/day	Maize g/day
Armenia	269	
Afghanistan	208	
Albania	319	
Algeria	391	
Angola	49	72
Antigua and Barbuda	137	
Argentina	176	
Australia	137	
Austria	147	
Azerbaijan	337	
Bahamas	78	
Barbados	137	
Belgium-Luxembourg	192	
Bangladesh	39	
Belarus	143	
Belize	108	33
Benin	21	126
Bermuda	127	
Bolivia	99	91
Botswana	64	95
Bosnia and Herzegovina	160	
Brazil	85	35
Brunei Darussalam	80	
Bulgaria	243	
Burundi	5	43
Cambodia	4	
Cameroon	27	96
Canada	177	
Cape Verde	107	125
Central African Rep.	19	42
Congo, Dem. Rep.	14	43
Chad	13	24
Chile	216	27
China	162	38
Colombia	54	77
Costa Rica	93	
Cote d'Ivoire	33	
Croatia	177	
Cuba	72	
Cyprus	207	
Czech Republic	197	
Denmark	173	
Dominican Republic	63	
Ecuador	52	31
Egypt	281	114
El Salvador	68	
Eritrea	118	
Ethiopia	54	
Estonia	154	
Fiji Islands	191	
Finland	140	
France	187	
French Polynesia	146	
Georgia	218	137
Gabon	101	16
Gambia	60	19
Germany	139	
Ghana	23	81
Greece	273	
Grenada	110	
Guatemala	55	186
Guinea	22	21
Guyana	105	
Haiti	63	53
Honduras	72	
Hungary	216	
Iceland	142	
India	118	
Indonesia	35	
Iran	314	
Iraq	223	
Ireland	196	
Israel	254	
Italy	294	
Jamaica	118	
Japan	86	
Jordan	280	
Kazakhstan	384	
Kenya	46	
Kiribati	110	
Korea, DPR	49	
Korea, Republic of	100	
Kuwait	159	
Kyrgyzstan	398	
Latvia	173	
Laos	4	
Lebanon	239	
Lesotho	88	286
Liberia	78	
Libya	323	
Lithuania	241	

Country	***Flour g/day***	***Maize g/day***
Madagascar	12	
Macedonia	236	
Malawi	15	
Malaysia	53	267
Maldives	147	
Mali	11	62
Malta	296	
Mauritania	165	
Mauritius	185	
Mexico	79	254
Mongolia	221	
Morocco	339	
Mozambique	28	108
Moldova	142	
Myanmar	7	
Namibia	50	139
Nepal	70	86
Netherlands	121	
Netherlands Antilles	140	
New Caledonia	179	
New Zealand	138	
Nicaragua	46	99
Niger	10	
Nigeria	26	54
Norway	194	
Pakistan	261	
Panama	71	60
Papua New Guinea	54	
Paraguay	60	97
Peru	77	25
Philippines	59	
Poland	212	
Portugal	193	
Romania	313	
Rwanda	7	49
Russian Federation	258	
Saint Kitts and Nevis	120	
Saint Lucia	192	
Saint Vincent/Grenadines	139	
Sao Tome and Principe	96	
Saudi Arabia	198	
Senegal	49	19
Seychelles	143	
Sierra Leone	28	54
Slovenia	176	
Slovakia	213	
Solomon Islands	41	
Somalia	12	41
South Africa	123	204
Spain	177	
Sri Lanka	92	
Sudan	69	
Suriname	124	
Tajikistan	306	
Swaziland	68	149
Sweden	156	
Switzerland	183	
Syrian Arab Republic	323	
Turkmenistan	413	
Tanzania	17	
Thailand	19	
Togo	20	129
Trinidad and Tobago	173	
Tunisia	409	
Turkey	371	
United Arab Emirates	113	
Uganda	7	61
United Kingdom	184	
Ukraine	255	
U.S.A.	171	
Uruguay	147	48
Uzbekistan	324	
Vanuatu	43	
Venezuela	79	
Viet Nam	14	
Yemen	247	
Zambia	22	263
Zimbabwe	50	242

Region		
North America Developed	172	25
Central America	72	223
South America	110	44
North & Central America	138	80
Oceania	124	7
Developed Countries	194	23
Developing Countries	124	42
Africa	91	84
Europe	213	13
Africa South of Sahara	32	79
Asia	138	27
World	140	37
Low-Income Food Deficit	124	39

Grain Structure and Cereal Composition

All cereal grains consist of three main components. These are:

Bran
The bran consists of several layers of fibrous material that surround the endosperm and protect the germ component. These layers are known as the *pericarp, testa* and *aleurone.* The outer bran layers are rich in dietary fiber and have high levels of minerals. The aleurone layer contains high levels of vitamins and phytic acid.

Endosperm
The endosperm contains starch and protein. The protein present in the endosperm in wheat becomes the gluten matrix with the addition of water. This matrix provides the structure for breads, cookies, biscuits, noodles and other processed foods made from wheat flour. Wheat is the only plant that has this gluten forming protein.

Germ
The germ component is the embryo of the plant. It is high in protein and fat. In addition the germ contains the highest level of group B vitamins.

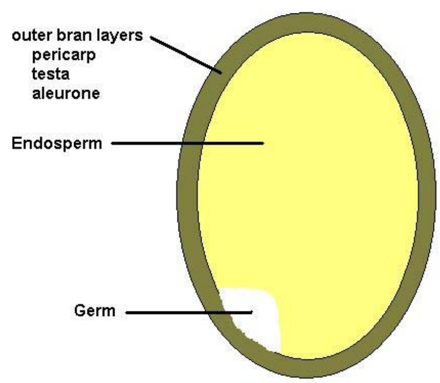


Figure 1 Stylized Grain Structure Diagram

Table 3.7 Percentages of Major Components for Different Cereals.

Grain	*Endosperm %*	*Bran %*	*Germ %*
Wheat	83	14	3
Maize	82	6	12
Sorghum	82	7	11
Millet	76	19	5

Nutritional Composition of Cereals

Wheat provides significant levels of calories, protein, carbohydrates and dietary fiber. While whole grain wheat is a good source of many minerals and vitamins, milling reduces the levels of micronutrients contained in the whole cereal grain, as shown in Tables 3.8 and 3.9. For example, 100 grams/day of whole wheat flour supplies 22% of the United States RDA for iron. However, 100 grams/day of refined white flour supplies only 6%. Fortification of flour with 38 ppm iron restores the original level that would be provided by whole wheat.

Table 3.8 Nutrient Composition of Whole and Refined Wheat

Nutrient	*Level units*	*Whole Wheat**		*Wheat Flour**		*Percent Retention*
		Level	*INQ*	*Level*	*INQ*	
Calories	kcal/100g	339	1.0	364	1.0	105%
Protein	%	13.7	1.2	10.3	0.9	80%
Calcium	ppm	340	0.2	150	0.1	44%
Iron -men	ppm	54	3.9	12.0	0.8	22%
-women			1.8		0.4	
Zinc	ppm	35	2.0	7.0	0.4	20%
Thiamin	ppm	4.1	2.0	2.0	0.9	49%
Riboflavin	ppm	1.1	0.5	0.4	0.2	37%
Niacin	ppm	48	1.9	10	0.4	21%
	NE	83	3.3	32	1.3	
Pyridoxine	ppm	3.8	1.7	1.0	0.4	24%
Folates	ppm	0.41	0.6	0.25	0.3	61%
Phosphorus	mg/100g	346	2.7	108	0.9	31%
Phytic acid	mg/100g	800		280		35%

***** Whole wheat protein and micronutrient levels can vary widely. Iron, for example, can range from 30 ppm to over 100 ppm. The values shown here are averages taken from USDA Food Composition Tables.
*** ***Normal white, non-fortified, all-purpose flour with 75% extraction

Table 3.9 Nutrient Composition of Whole and Refined Maize

Nutrient	*Level units*	*Whole Maize*		*Maize Meal**		*Percent Retention*
		Level	*INQ*	*Level*	*INQ*	
Calories	kcal/100g	362	1.0	371	1.0	102%
Protein	%	8.1	0.7	8.8	0.7	108%
Calcium	ppm	60	0.0	30	0.0	50%
Iron - men	ppm	35	2.4	5	0.3	14%
- women			1.1		0.1	
Zinc	ppm	18	1.0	9	0.7	49%
Thiamin	ppm	3.9	1.8	2	0.9	52%
Riboflavin	ppm	2.0	0.9	0.3	0.1	15%
Niacin	ppm	36	1.3	6	0.2	17%
	NE	46	1.7	17	0.6	
Pyridoxine	ppm	3.0	1.3	1.5	0.6	48%
Folates	ppm	0.25	0.3			
Phosphorus	mg/100g	241	1.9	84	0.7	
Selenium	ppm	0.16	1.6			

* Degerminated 63% extraction with 0.55% ash

The *Index of Nutritional Quality* or INQ shown in the above tables is a convenient way of assessing the micronutrient content of a food in relationship to the dietary requirement (RDA). An INQ of 1.0 means that if you get your caloric requirement of 2000 kcal from wheat, you will also achieve 100% your requirement for that micronutrient, while an INQ of 0.2 means you will only get 20% of your dietary requirement. The following observations can be made from this data:

- The milling refinement of both wheat and maize causes little change in caloric or protein levels but large losses of most vitamins and minerals.
- Whole wheat is good source of protein, thiamin, niacin, pyridoxine and phosphorus.

- Whole wheat appears to be a good source of iron and zinc, but low absorption from whole-wheat products due to high phytic acid levels may negate some of that.
- Wheat flour is a good source of protein, thiamin and phosphorus.
- Neither whole wheat nor wheat flour are good sources of calcium or riboflavin.
- Maize is even a worse source of calcium than wheat. However, nixtamalized masa flour used to make tortillas has calcium added in processing making it a good source of calcium.
- Maize is lower in protein and niacin than wheat and nixtamalized products have even less niacin.
- Maize is not only lower in zinc and iron than wheat, but it has more phytic acid and is not normally fermented (which removes much of the phytic acid), making it a very poor source of absorbable iron.
- The maize levels of the B vitamins are similar to wheat for both the whole grain and the refined milled product.

Phytic acid

Whole cereal grains contain phytic acid (inositol hexaphosphoric acid), which forms insoluble compounds with minerals, particularly calcium, iron and zinc. These insoluble compounds make these minerals unavailable for absorption by the body. Much of the phytic acid is localized in the aleurone, the outer layer between the endosperm and bran. Since most of the phosphorus found in wheat is in the form of phytates, the phosphorus level, as well as the ash content, is closely related to the phytic acid content. Phytic acid levels in wheat and flour are shown in Table 3.8. Refined white flour contains reduced levels of phytic acid compared to the whole grain. As a result, inhibition of iron absorption by phytic acid is less of a problem with more refined milled white flour.

Yeast and flour provide the enzyme *phytase*, which destroys most of the phytic acid during dough fermentation in the breadmaking process. Over 70% of the phytic acid is estimated to be hydrolyzed, the longer the fermentation and lower the pH, the more phytic acid that is removed.

Phytic acid becomes a serious problem only when high extraction flour is used to make unleavened bread. This, unfortunately, is a major use of wheat in many countries of the world. For example, in India and surrounding countries high extraction whole-wheat *atta* flour is used to make *chapattis* and other flat breads made with little or no fermentation. Addition of iron, zinc and calcium to atta flour would have reduced benefit because of the minerals being tied up by phytic acid making it unavailable for absorption. See Section 4 for a discussion on methods to reduce phytic acid in cereals.

Section 4 MILLING AND PROCESSING

This section describes the milling and processing of wheat and maize into flours and processed products made from them.

Wheat Milling

Brief History

In prehistoric times wheat was dehusked and ground using mortars to produce a meal of ground grains. Rotary grain mills such as stone mills were thought to have been invented by the Romans. Even today in parts of the world many different types of grains are processed using mortars and stone mills.
The development of rollermills occurred in the 19th century in Europe. The principle of this method of milling, especially for wheat, has not changed since that time. Rollermills have allowed for the processing of wheat into white flour whereas using stone mills and mortars results in whole grain flours.

Objectives of Rollermill Process

The objectives for the milling of white flour using rollermills are:

1. To separate as much as possible the endosperm from the bran and germ fractions in the whole grain. The endosperm is required for the manufacture of white flour and it should be free from bran and germ particle contamination. This will result in a product with improved palatability and lengthened shelf life.
2. To reduce the maximum amount of endosperm into fine particles (flour) and to maximize the amount of white flour from the wheat. (In conventional milling practice flour is defined as that material which will pass through a sieve with an aperture size of 140 microns.)

Milling Process – Rollermills

In rollermills milling consists of three basic processes:

1. *Grinding:* breaks the grain and parts of the grain with some separation of the individual parts of the grain.
2. *Sieving:* classifies mixtures of grain particles of different sizes into fractions of narrower particle size ranges.
3. *Purifying:* separates mixtures of bran and endosperm particles using air currents and sieves.

Gradual Reduction Process

The milling process for rollermills is defined as a gradual reduction process. This breaks down the grain in a series of grinding stages using a succession of grinding rolls of different types. Each grinding stage produces a blend of coarse, medium and fine fractions including flour. These mixtures are then sieved and purified to allow for a good separation of bran and endosperm. The Gradual Reduction Process consists of two main systems that are interconnected: These are:

Break System

The Break System consists of 4 or 5 rollermill stages. The rolls are corrugated and set in pairs. The objective of the break system is to remove as much of the endosperm from the wheat berry and to separate the endosperm particles from the bran and germ particles using sifters and purifiers.

Reduction System

The Reduction System consists of 8 to 16 grinding stages depending upon the type of wheat to be milled. The objective of the reduction system is to grind the endosperm into finer particles producing flour using rollermills and sifters.

Flour Collection System

Flour is produced at every grinding stage; each flour is known as a "flour stream". The proportion of each flour stream as a percentage of the total flour produced will vary considerably depending upon its origin in the mill. For example most of the flour originates from the first few grinding stages at the start of the reduction system.

All the individual flour streams are transferred from the sifters through spouts connected to a flour collection conveyor. The flour collection conveyor is a screw conveyor that blends all the individual flour streams together producing the final flour. In some mills there may be 2 or 3 flour collection conveyors depending upon the commercial requirements and the marketplace.

Milling Process – Single Stage Milling

Unlike the Rollermill process single stage milling is a rapid single milling method that converts wheat, maize and any other cereal grain into flour made up of all the components of the grain berry. In some cases the grain may be dehulled (maize) or decorticated (sorghum) prior to milling. The equipment for these mills is very simple and can be powered by hand, water, animals, electricity or diesel engine. The mills can be of the following types:

Stone mill

The grain passes between a pair of stones, one of which is turning while the other is stationary. The stones may be vertical or horizontal.

Hammermill

Hammermills consist of swiveled metal blades that grind the grain by smashing it against a metal screen. These mills run at very high speed and the fine meal is forced through the screen by air due to the high speed of the hammers.

Plate mill

The grain is passed through a pair of metal plates one of which is running and the other is stationary. The plates are usually in the vertical position.

Pin mill

The grain is passed through a pair of plates which have pins protruding from them. One plate runs against a stationary plate. The fine meal is then forces through the screen by air due to the high speed of rotation of the plates.

Milling Products

The following table illustrates the types of products produced from the different types of milling process.

Table 4.1 Basic Mill Products

Milling Process	Grain Types	Milled Products
Rollermill	Wheat Maize Rye	White Flours Whole Grain Flours Bran Germ
Single Stage	All cereals	Whole Grain Flours

Production Capacity

Rollermills

By convention *production capacity* is defined as the amount of wheat that can be processed in a rollermill in a 24 hour period. It is usually expressed in terms of Metric Tonnes (MT)of wheat that can be cleaned and ground in 24 hours assuming that the mill operates for a full 24 hours[3]. In many countries flour mills do not always operate for 24 hours per day but will operate between 8 to 24 hours a day depending upon power availability and market conditions. Typical production capacities range from 40 to 500 MT. Flour production can be estimated based on the Extraction Rate (see below).

Single stage mills

These mills run at much lower capacities than rollermills. These mills run for 8 to 12 hours per day. The milling capacities range from 0.5 to 1.5 MT per hour depending upon size and power source and availability.

Extraction Rate

Extraction Rate is defined as the amount of flour produced by weight from a known weight of wheat. This is known as "flour yield" or percentage extraction rate. This is sometimes calculated from dirty wheat as received and sometimes from cleaned, tempered wheat. The latter can produce slightly higher values.

The source of white flour in wheat is the endosperm, which represents about 82% of the total wheat berry (the germ is about 2% and bran is about 16%). Therefore the theoretical maximum amount of white flour that can be obtained is 82%. These figures differ depending on the size of the wheat kernel, with the larger, plumper kernels capable of producing more flour. In actual practice the maximum amount of white flour is about 80%.

White and brown flours

With increasing extraction the flour contains higher amounts of bran, alerone (the layer between the endosperm and the bran) and germ fractions along with increased levels of most micronutrients. Flours with extraction rates above roughly 83% are considered to be *high extraction flours*. If little or no bran or germ is removed then the resulting flour is considered to be whole wheat flour. Flours with extraction rates below 83% are *white flours* while *brown flours* have extractions between 83% and 95%.

Ash content

The most common and sensitive method used for assessing the degree to which wheat has been refined into flour is its *ash content*. This is measured by incinerating a weighed sample of flour in a furnace at a very high temperature for a set period of time. The resultant ash is weighed and expressed as a percentage of the flour weight. Ash content is a measure of mineral matter in the grain and flour. The bran coat contains about 5% ash compared to 0.3% for pure endosperm. Therefore ash content is a measure of bran contamination in white flour and hence extraction rate. The higher the extraction, the higher the ash content, the greater the bran contamination and the higher the mineral content in that flour.

Table 4.2 Extraction Rates and Ash Contents of Different Grades of Flours

Flour Type	Ash Content %	Extraction Rate
Top Patent	0.40	50%
Patent	0.50	70-75%
Straight Grade	0.55-0.75	78-83%
High Extraction (Brown)	0.75-1.10	83-95%
Whole Wheat flour	1.1-1.5	95-100%

[3] Some mills may report production capacity as the amount of flour that can be produced in 24 hours. These figures are typically 20% lower than the amount of wheat that can be processed.

Wheat Based Foods

Wheat flour cannot be consumed without first being processed. The main processes employed are leavening, extrusion and baking. For the purposes of mass cereal fortification, the following processes and resultant wheat based foods have great importance:

- Yeast leavened: bread, crackers
- Chemically leavened: flat breads, biscuits (cookies), cakes, pancakes, muffins
- Non-leavened: pasta, couscous, noodles (wet, dry and instant), flat breads

These foods are important because they are widely consumed so they reach the majority of the general population, including the at-risk population groups and the food is affordable.

Bread

Bread has been widely consumed in many parts of the world for thousands of years. The very earliest breads were unleavened, that is they were made from water and cereal flours. The resulting dough was mixed, divided into pieces, shaped and then baked on hot stones over a fire. The cereals used in this process were wheat, rye, corn and barley and blends of these cereals.
Bread can be defined in two ways, leavened and unleavened:

Yeast raised (leavened) bread

Yeast leavened bread is defined as bread that has undergone some degree of fermentation. Fermentation results in the development of an aerated product through the development of carbon dioxide and modification of the protein structure (gluten development) of the dough. Fermentation is achieved by the addition of yeast to the dough system. Historically fermentation was thought to have been discovered by the Egyptians. They discovered that if the dough is left for a long time it would begin to rise due to wild yeasts. Baking this rising dough resulted in aerated bread. Yeast raised breads may be baked in a pan giving a soft crust or on a hearth giving a crustier product.

One of the benefits of yeast fermentation is that it reduces the level of phytic acid in the bread. Phytic acid is a known inhibitor of minerals and is present in all cereals. Fermentation therefore increases the bioavailability of minerals in bread.

Chemical leavened bread

In some countries yeast is replaced or augmented by chemical leavening agents, such as *baking powder* or *baking soda*. In this case the dough is mixed, formed and then baked with no fermentation step. The aeration of the dough is achieved by the development of carbon dioxide through the chemical reaction of the baking powder and baking soda as the dough is baking.

Unleavened bread

Unleavened bread is made from just the cereal flour and water mixed together into a dough. The dough is allowed to rest and then pieces of the dough are flattened and baked either in a hot oven or on a stone in a simple fire. This type of bread is usually made from whole grain flours and may contain mixtures of cereal flours. Examples are *chapattis* and *roti* use in India and wheat tortillas used in the Americas.

Table 4.2 Types of Breads used in Various Regions of the World.

Regions/Countries	Leavened Bread types	Unleavened Products
Africa	Pan, crusty and flat breads	Flat breads, porridge
Americas	All types	Tortillas, biscuits (cookies), muffins, cakes
Asia – Central, Russia Asia – Middle East Asia – India subcontinent	Pan, crusty and flat breads Flat (Arabic) breads, nan	Pancakes, biscuits Couscous, biscuits Chapatti, roti, biscuits
Australia	Pan and crusty breads	Cakes, biscuits
Europe	All types	Cakes, biscuits

Noodles and Pasta

Noodles are made from a dough of flour or semolina (granular endosperm, usually from durum wheat), water, salt and sometimes contain egg. The dough is mixed, extruded or rolled and cut into strands or extruded into different shapes. Some noodles are cooked fresh but most are dried. Instant noodles are fried in oil and baked. Dried and instant noodles are cooked by boiling in water and then eaten. The cooking water may be flavored and is often eaten with the noodles, but may be discarded. With pasta (macaroni and spaghetti) the water is always discarded causing some of the added micronutrients to be lost.

Table 4.4 Noodles and Pasta

Regions/Countries	Noodles	Flour Types
Africa Middle East	Macaroni, Spaghetti, Couscous	Durum Semolina Semolina
Americas	Macaroni, Spaghetti Chinese style Instant Noodles	Durum Semolina Wheat Flour Wheat Flour
Asia	Chinese style Instant Noodles	Wheat flour Wheat Flour
Europe	Macaroni, Spaghetti Chinese Style Instant Noodles	Durum Semolina Wheat Flour Wheat Flour
Australia	Macaroni, Spaghetti Chinese Style Instant Noodles	Durum Semolina Wheat Flour Wheat Flour

A yellow or creamy color is desired in noodles and most pasta, while Japanese noodles are white. Noodles can be made from durum semolina, wheat semolina, and wheat flour depending on the type of noodle. They can be fortified by adding the micronutrients to these flours at the mill, or, in the case of instant noodles, by adding them to the flavoring (spice) sachet.

Maize (corn) Milling

White and yellow maize can be milled in two different ways, dry milling and wet milling. For the purpose of this manual only dry milling will be discussed since wet milled maize products are not normally fortified. Maize can be milled using both a rollermill and a single stage process (as described above for wheat milling).

Rollermilling of Maize

The rollermilling process for maize is similar to that of wheat with the following exceptions:

- The maize kernels have to be de-germed and de-hulled prior to grinding on rollermills.
- The maize is transformed into grits (endosperm particles larger than flour) with the amount of flour minimized by the milling process.

Maize milling produces a number of products differing in particle size, composition and application. Many of these products are not suitable for mass fortification in that they go to non-food products (wallpaper paste, corn oil extraction, brewing) or special use foods (snacks, breakfast cereals, ingredients, fillers). Only those maize grits and meal products intended for direct food consumption are considered in this manual for fortification.

Single Stage Milling

Single stage maize mills are found extensively throughout Africa and Central America. These are usually stone mills, hammer mills or plate mills. These mills are used in rural and peri-urban areas. The maize is produced by subsistence farmers who take the grain to the mill for processing. The miller takes cash payment or withholds a portion of the finished flour as payment. The finished flours are whole grain maize flours or maize flour milled from de-hulled maize. In this case the maize is dehulled manually at the household level or with a separate dehulling machine at the mill and then ground into meal. In some parts of Africa and Central America the maize is soaked and then processed.

Maize Products and Food Uses

The milled maize products intended for direct human consumption may be made from white or yellow corn and may differ in particle size, extraction and whether or not the germ has been removed. The following table shows the composition of maize meals available in South Africa. All but the unsifted products are intended to be fortified.

Table 4.5 Maize Meals Produced in South Africa

Maize Meal Product Name	*Ash content target*	*Fat target*	*Extraction target*	*Percent of market*
Super	0.55 %	1.5%	63 %	36
Special	0.85 %	2.7%	79 %	36
Sifted	1.1 %	3.7%	89 %	12
Unsifted	--	--	~100%	--

Other maize products that are being or could be fortified include:

- ***Corn Masa Flour*** – A lime-treated (nixtamalized) whole grain maize flour used to make corn tortillas in Latin and North America.
- **Arepa** - A degermed, precooked maize flour used in Venezuela.

Fortification Effects on Product Quality and Acceptance

As a guiding principle it is essential that the fortification of wheat flour and maize meal will not change consumer acceptability of the fortified food. It would be ideal for the fortification to be invisible to the consumer. That is, there would be no detectable difference in the appearance, sensory properties or even the price of the fortified product, but this is not always achievable.

Colour and Appearance

The visual appearance of any food is the first of the organoleptic senses that a consumer experiences. Therefore if the fortified food has had any changes in colour and appearance the consumer is more likely to reject the product. At the current fortification levels normally found in wheat and maize there is no adverse impact on colour or appearance. Elemental iron powders may cause a slight darkening of flour, while high levels of riboflavin and folic acid can cause a slight yellowing, but these changes are accepted once all flour is similarly treated. Ferrous sulphate does not cause any colour problems in the dry flour/meal but could lead to off-colours in cooked maize products.

Flavour and Aroma

The same criteria for colour and appearance apply for flavour and aroma. The consumer must not be able to detect a discernable difference. Any detectable change in flavour and aroma caused by fortification is unacceptable.

Shelf Life

As a general rule of thumb the addition of micronutrients to produce a fortified flour of wheat or maize must not reduce the normal or expected shelf life of the flour. Any reduction of shelf life will result in lost products and reduced consumer acceptance of the food.

The usual cause of reduced shelf life is due to the development of rancidity in the flour caused by soluble iron and zinc salts. This is particularly true for high extraction and whole grain flours.

Taste and Mouthfeel

There must be no change in product texture and mouthfeel. Rancidity will affect the taste and mouthfeel as well as aroma and flavour of both the flour and the finished products as consumed. Rancid products have a slightly soapy mouthfeel and a distinctive unpleasant odour.

Sensory Testing

There has been extensive testing and experience to show that fortification can be accomplished without adversely affecting any of these sensory properties in standard products such as flour, maize meal, bread, cakes, instant noodles and pasta. However, there are some cereal-based foods unique to different regions of the world that have not been tested. These should be tested prior to starting a general fortification program.

Reducing Phytic Acid

High levels of phytic acid in grains are a problem because they inhibit the absorption of iron, zinc and other minerals. Whole grain wheat and maize contain nearly 1% phytic acid. Reducing this level acid can improve the absorption of these minerals. Milling refinement lowers the amount of phytic acid in flour by 60% to 90% depending on the extraction rate, but the remaining phytic acid is still strongly inhibitory. The final level of the phytic acid is closely related to ash content - the lower the ash , the lower the phytic acid.

Common practices of fermentation, soaking and germination further reduce the level through the action of *phytase*, which is naturally present in cereals. One potential solution is to add the phytase enzyme (from Aspergillus Niger), but the dough must be allowed to sit for some time prior to baking for the enzyme to have any effect. Yeast fermentation during bread baking lowered the phytic acid from added bran by 60% after two hours and up to 85% for longer periods (Navert 1985). The lower the pH during fermentation, the more phytic acid that is removed (Fretzdorff 1992). However, the molar ratio of phytic acid to iron should be decreased to at least 1:1 and optimally less than 0.5:1 to achieve a meaningful increase in iron absorption (Hurrell 2002). Getting this low a molar ratio is difficult. In order to reach a final molar ratio below 1.0 from wheat containing 0.9% phytic acid, one needs at least 50 ppm iron, a 75% reduction in phytic acid on milling and a 75% reduction on baking. Unfortunately, non-fermented products like noodles and pasta will never achieve this low a ratio.

Literature Cited

Fretzdorff, B. and Brummer, J. M. (1992). “Reduction of phytic acid during breadmaking of whole-meal breads.” Cereal Chem. **69**: 266-270.

Hurrell, R. F. (2002). "Fortification: overcoming technical and practical barriers." J Nutr **132**(4 Suppl): 806S-12S.

Navert, B., B. Sandstrom, et al. (1985). "Reduction of the phytate content of bran by leavening in bread and its effect on zinc absorption in man." Br J Nutr **53**(1): 47-53.

Section 5 TYPES AND LEVELS OF MICRONUTRIENTS TO ADD

This section provides data on the types, levels and costs of micronutrients that can be added to wheat flour and maize meal in cereal fortification programs and gives the cereal fortification standards used throughout the world. It shows how fortification standards and premix composition can be calculated. It also discusses the stability and safety of micronutrients in flour and baked products.

Definitions: Restoration, Enrichment and Fortification

Restoration is the replacement of nutrients lost during food processing. The milling refinement of wheat into flour or maize into meal results in the concentration of vitamins and minerals in the mill feed or bran products and a corresponding lowering of the micronutrient content, from that contained in the whole kernel, in the flour and meal products that go to direct human consumption. The degree of these "losses" is dependent on the *extraction rate*. The lower the extraction rate, the greater the loss. In the United States and Canada, the enrichment standards are based on restoring flour to original levels present in whole wheat. In the UK standards are based on restoring levels to that of a 90% extraction flour.

Enrichment is a special term coined for the practice of adding back only those micronutrients that are lost during milling and for which there is good evidence that a deficiency exists within the general population.

Fortification is adding nutrients whether or not they are present in the food, or adding levels that are much higher than any natural content. For example, adding vitamin A to wheat flour is fortification rather than enrichment because wheat does not contain vitamin A in its original state. The level of folic acid now being added to flour in the U.S. is also a type of fortification since it is much higher than the level normally found in wheat. This manual will use the general term, *fortification*, to describe any addition of micronutrients to cereals.

Types of Nutrients to Add

The micronutrients that are currently being added, or have been suggested to be added are listed in Table 5.1. Each is discussed in greater depth in Sections 6 and 7. As a general rule, only those vitamins and minerals should be added for which there is a clear and demonstrated need by the population consuming the flour to be fortified. Other factors that have restricted the micronutrients to add are:

- ***Cost*** – As can be seen in Tables 5.1 and 7.1, the costs of some micronutrients relative to their dietary requirement are far more expensive than others. For example, it costs more to add vitamin A than it does to add all of the ten cheapest micronutrients. High cost is also a constraint with calcium, vitamin C, magnesium, biotin, vitamin E and pantothenic acid.
- ***Stability –*** Stability is mainly a problem with vitamin C, which can be lost entirely in fermented baked products. All of the vitamins except for niacin will experience some loss of activity, while minerals will have no activity losses. See Table 5.4 for estimated retention of micronutrients.
- ***Safety -*** Safety concerns have limited the inclusion of certain micronutrients such as copper and selenium.
- ***Product acceptability*** – Problems with product acceptance due to undesirable flavors have restricted the use of magnesium and certain zinc salts.
- ***Trade harmonization*** – It is often desirable for countries to adopt the fortification standards used by other countries in the region in order not to interfere with the free trade of flour and baked products.

The most commonly added vitamins and minerals are shown in Table 5.1. The oldest cereal fortification programs involve the mandatory addition of iron and three B vitamins, thiamin, riboflavin and niacin, with calcium often optional. Folic acid and zinc have been included recently with emerging evidence of their need. A new program in South Africa requires addition of eight micronutrients including vitamin A and vitamin B_6. Israel is the first country to require vitamin B_{12}.

Table 5.1 Micronutrients used in Cereal Fortification

Nutrient	*RDA**	*EAR***	*Chemical Sources used in cereal fortification*	*Activity or Concentration*	*Amount of source needed to supply 100% of RDA per kg*	*Cost (2003 estimates with added processing factor)*	
Units:	mg/day			%	ppm or mg/kg	$/kg active component	$ per 100,000 RDA
Iron	8, 18	10.3, 13.2	Elemental Reduced Iron	97	10.3	2.2	2.2
			Electrolytic Reduced Iron	98	10.2	2.8 - 7.1	2.8 - 7.1
	10#		Ferrous Sulfate	32	31	4.9	4.9
			Ferrous Fumarate	32	31	11.1	11.1
			Sodium Iron EDTA	13.5	74	78.6	78.6
Zinc	11, 8	3.6, 2.6	Zinc Oxide	80	12.5	2.3	2.3
	10#		Zinc Sulfate	36	28	13	13.0
Calcium	1000	855	Calcium Sulfate	23	4350	0.60	60
			Calcium Carbonate	40	2500	0.62	62
Selenium	0.055	.028, .021	Sodium Selenate	42	0.13	52	0.3
Iodine	0.15	0.095	Calcium Iodate	62	0.24	34	0.4
Folate	0.4	0.32	Folic acid	87	0.46	36	1.4
Vitamin B_1	1.2	1.0, 0.9	Thiamin Mononitrate	103	1.2	17	2.0
			Thiamin Hydrochloride	100	1.2	18	2.2
Vitamin B_2	1.3	1.1, 0.9	Riboflavin	100	1.3	28	3.6
Niacin	16, 14	12, 11	Niacin (Nicotinic Acid)	100	15	7	11
	15#		Niacinamide	100	15	7	11
Vitamin B_6	1.3	1.1	Pyridoxine Hydrochloride	83	1.6	44	5.7
Vitamin B_{12}	0.0024	0.002	Cyanocobalamine, 1%	1	0.24	15,200	3.6
Vitamin A	0.9 or 3000 IU	0.42 or 1390 IU	Vitamin A Palmitate, 250 SD	250 IU/mg	12		42
Vitamin D	0.005 or 200 IU	0.005 or 200 IU	Vitamin D3, 100 SD	100 IU/mg	2		8
Vitamin C	90, 75 75#	54, 44	Ascorbic Acid	100	75	8.7	65

* Recommended Dietary Allowances (RDA of the Food and Nutrition Board of the U.S. Institute of Medicine, 2001,
** Estimated Average Requirement from RNIs of FAO/ WHO. If two numbers shown for RDA, first is for males and second is for females.
Both RDA and EAR values for females are for non-pregnant, non-lactating adults (19-50 years). # RDA used for cost calculation purposes.

Levels of Nutrients to Add

There are different strategies and issues to consider when deciding the levels of nutrients to add to milled cereal staples. These include:

- **Restoration** - The level of each nutrient in the unprocessed food must be known if the criteria is based wholly or partially on restoring lost nutrients, which was the original criteria for cereal enrichment in the United States and Canada. Natural levels for wheat and maize are given in Tables 3.8 and 3.9.

- **Balancing dietary requirements** - It is desirable to have a balance in the levels of nutrients contained in the fortified product and the dietary requirements. Folic acid is an exception to this in that higher levels relative to the other micronutrients are normally added in order to insure prevention of neural tube defects.

- **Making up for dietary deficiencies** – This strategy is to make up for all or part of the difference between the dietary requirement for a nutrient and its average consumption by the general or target population. This calculation depends on which dietary requirement values are used. It can also be difficult to find good data on micronutrient intakes for some target populations.

- **Upper level quality constraints** - Due to effects on color, taste, smell or baking characteristics of the flour, there may be a maximum level of a particular nutrient that can be added without adversely affecting product quality or acceptance. Iron, zinc and riboflavin are the micronutrients most commonly affected by this constraint.

- **Upper level safety constraints** - The amount of nutrient added should not exceed safe limits. This can be assessed from the total amount consumed in relation to published safe upper levels, as shown in Table 5.3.

- **Cost** - Vitamin A and calcium are very expensive relative to their RDA compared to other micronutrients, so their inclusion or level may be reduced from ideal in order to make the program economically acceptable. See Tables 5.1 and 7.1 for relative costs.

Dietary Requirements -The latest Recommended Daily Allowances (RDAs) established by the National Academy of Sciences, Food and Nutrition Board (see Table 5.1) are used in this manual. Some countries may have different values for dietary requirements and the values will change with age and sex, which is a particular problem with iron. Some nutritionists may prefer to use the WHO Recommended Dietary Intakes (RNI) or the Estimated Average Recommendation (EAR), which are also shown in Table 5.1.
Micronutrient Activity - The amount of a nutrient source to add to meet a particular standard has to be adjusted on the basis of the concentration or activity of the nutrient in the chemical compound, as shown in Table 5.1. In the case of vitamins, molecular weight adjustments must be made if the chemical source of the nutrient used differs from the reference vitamin standard. Some vitamins, like folic acid, must also be adjusted for moisture content and others, like vitamin B_{12}, are sold in diluted forms to make it easier to weigh.
The activities of fat-soluble vitamins (vitamin A and D) are given in International Units (IU) but more recently in weight equivalents.

- One microgram (μg) of vitamin A (one Retinol Activity Equivalent or RAE = 3.33 IU
- One RAE is equivalent to 12 μg of β-carotene and 24 μg of other provitamin A carotenoids.
- One μg of vitamin D as cholecalciferol = 40 IU.

Average Flour Intake Capable of Fortification

Some estimate of the flour or maize meal intake, or usual daily consumption, must be used when devising fortification levels to add on the basis of making up for dietary deficiencies. This can be difficult since consumption will vary across the population depending on sex, age, race, geography and cultural factors. Table 3.6 provides estimates of wheat flour and maize meal consumptions in different countries, but these figures, based mainly on FAO data, can be inaccurate for some countries and should be verified by information from other sources, such as the milling industry.

Also, the figure used should be what can realistically be fortified, which is a percentage of the total production ranging from nearly 100% for countries like Indonesia that have no small mills down to 50% or less for countries like India that have many small mills. South Africa and six countries in Central Asia calculated their fortification standards using an average intake figure. South Africa used 200 grams/day from either wheat flour or maize meal, while Central Asia used a figure of 260 grams/day for wheat flour. Clearly, the flour consumption in some Central Asian countries is greater than that, but that was the amount that was believed to be capable of fortification.

Fortification Standards

The amounts of the vitamins and minerals to be added determine the actual improvement in the micronutrient levels in the cereal foods as well as the premix composition. Most countries adopt fortification standards as a way of regulating fortification. These standard are the minimum levels of the micronutrients that are required to be in the dry cereal product once it leaves the mill. They are generally different values from the levels added because they must take into account the natural levels of the micronutrients in the flour. However, the standards can be calculated from the levels added, and vice versa, using the following formulas.

$S = R(AF + N)$ $\qquad$ $A = (S/R - N)/F$

Where:
S = Minimum Standard
A = Level to Add
N = Natural level in the flour
R = Retention ratio during milling (see Table 5.4)
F = Overage factor, a commonly used 5% overage gives an overage factor of (100-%overage)/100 = 0.95.

Example with thiamin in wheat flour
S = 6 ppm
N = 2 ppm (from Table 3.8)
R = 0.95 (from Table 5.4)
F = 0.95 with a 5% overage

Then the level to add (A) = ((6/0.95)-2)/0.95 = 4.5 ppm

Or, if the amount desired to be added (A) was 4 ppm
Then a proposed minimum standard (S) = 0.95 x (4 x 0.95 + 2) = 5.5 ppm

The levels to add can be determined from the standards with the formula shown above, which takes into account the natural level in the unfortified flour and a normal processing loss for each micronutrient, while providing an overage to allow for process variation and analytical error. The standard would become the regulation, whether mandatory or voluntary. Clearly, the values for N, R and F may vary depending on the type of flour and processing, but the values mentioned here provide starting points for calculating possible standards or levels to add. Note that "R" is only the retention of the micronutrient during milling and not the retention during storage or baking of the flour.

Maximum Standards

Some countries may desire to have a maximum along with a minimum standard, giving an allowable range into which the final level should fall. This would seem to be a prudent thing to do. However, experience has shown that having maximum standards provide little additional safety and can be difficult to comply with if the range is set too narrow (e.g. 25% or less of the minimum). The United States used to have minimum-maximum levels for all cereal fortification standards, but the U.S. Food and Drug Administration chose to replace the maximum with a general statement of "overages left to Good Manufacturing Practices". Millers will not deliberately add excessive amounts of premix since there is considerable expense and no advantage in doing so.

The WHO Fortification Guidelines (in print) provides procedures for determining minimum and maximum levels of fortification. The first value is based on increasing the intake of a micronutrient a desired percentage of the EAR based on the usual daily consumption of the food to be fortified. They then calculate the *Maximum Micronutrient Content* based on the lowest of three criteria: cost, safety and technological limits. This value may lower the minimum standard and help determine the maximum. If they are sufficiently high, the maximum, if used, is set at 50% above the minimum.

Fortification Premixes

Except for calcium, which is normally added to flour separately because of the large bulk involved, all other micronutrients are added as a single mixture or *premix*. This is much more convenient, accurate and economical than trying to add the individual nutrients separately. Appendix C gives a list of commercial premix suppliers. Premixes are designed to meet specific fortification standards when used in a particular type of flour or meal at a stated addition rate. Their composition is normally not regulated but determined by the experience of the premix manufacturer and the needs of the miller to meet a particular minimum standard.

A fortification premix is typically designed to be added at a rate between 50 and 300 grams per metric ton of flour. Addition rates lower than that are too difficult to control accurately, even for large mills. Small mills may require a more dilute premix that can be added at rates higher than 300 g/MT. In that case the mill may mix the premix with flour to create a ***preblend.*** Mills normally make sufficient preblend for no more than a couple days run to avoid problems with infestation or off-flavor development. Flour improvers such as enzymes and oxidants may be included in a preblend.

Fortification premixes generally include a diluent (such as starch, calcium salts, maltodextrose or other bulking agents) along with free-flow agents (e.g. tricalcium phosphate, silica) in order to prevent flow problems and make it blend well with the flour. Each lot of a fortification premix should have a certificate of analyses proving that its micronutrient content is as specified. Each container of premix should be packaged so that it is protected from air, light and water. The label on each premix container should show the ingredients and the level of each micronutrient that would be added at the indicated addition rate.

Table 5.2A Wheat Flour Fortification Standards in Different Countries[4]

Country	*Type* of Program*	*Vit. B_1 ppm*	*Vit. B_2 ppm*	*Folic Acid ppm*	*Niacin ppm*	*Zinc ppm*	*Iron/ Type** ppm*	*Ca g/kg*	*Notes on others*
Argentina	M	6.3	1.3	2.2	13		30 –FS		
Australia	M[6]	6.4							
Azerbaijan	V	3.3	2.8	1.5	18	25	55 –E		
Bahrain	M			1.5			60	(2.1)	
Bangladesh	P	6.4	4.0	1.5	53	33	66 –E		5
Belize	M	4.0	2.5	1.5	45		60		
Bolivia	M	4.45	2.65	1.5	35.6		60		
Brazil	P,M			1.5			42		
Canada	M	6.4	4.0	1.5	53		44	(1.1)	6
Chile	M	6.3	1.3	2.2	13		30		
Columbia	M	6.0	4.0	1.54	55		44		
Costa Rica	M	5.4	3.6	1.8	45		45 –FF		
Cuba	M	7.0	7.0	2.5	70		45		7
Dominican R.	V	6.0	4.0	1.5	55		60		
Ecuador	M	4.0	7.0	0.6	40		55		

[4] As of June, 2003.
[5] Project for atta flour. Includes 10,000 IU/kg vitamin A.
[6] Optional standards on pyridoxine (3.1 ppm) pantothenic acid (13 ppm) and magnesium (1.9 ppm). Calcium (1.4 g/kg) required in Newfoundland.
[7] Includes 6 ppm pyridoxine

Country	*Type* of Program*	*Vit. B_1 ppm*	*Vit. B_2 ppm*	*Folic Acid ppm*	*Niacin ppm*	*Zinc ppm*	*Iron/ Type** ppm*	*Ca g/kg*	*Notes on others*
El Salvador	M	4.0	2.5	1.5	45		55		
Fiji	P	6.0	2.0	1.5	55	30	60		
Guatemala	M	4.0	2.5	1.5	35		55	1.1	
Haiti	V	6.4	4.0	1.5	53		44		
Honduras	M	4.4	2.6	1.5	35.6		55		
Indonesia	M - LA	2.5	4.0	2.0		30	50		
Iran	P			1.5			30 –FS		
Israel	P - LA	5.8	4.0	1.5	46		37.5		[8]
Jordan	M			1.5			30 –FS		
Kazakhstan	V	3.3	2.8	1.5	18	25	55 –E		
Kuwait	M – LA	6.4	4.0	1.5	53		60		
Kyrgyz Rep.	V	3.3	2.8	1.5	18	25	55 –E		
Mexico	M	4.0	2.4	1.6	28	16	24		
Mongolia	V	3.3	2.8	1.5	18	25	55 –E		
Morocco	P - LA	4.5	2.8	1.5	36		45		
New Zealand	M[9]	6.4							
Nicaragua	M	6.0	3.5	1.5	40		60		
Nigeria	M	6.2	3.7		49.5		40.7	(1.1)	[10]
Oman	M			1.5			30		
Panama	M	6.0	4.0	1.5	55		60		
Paraguay	M	4.5	2.5	3.0	35		45		
Peru	M						28		
Qatar	M			1.5			60		
Russia	P	4.5	2.0	0.4	40		30		
Saudi Arabia	M	6.38	3.96	1.5	52.9		36.3	(2.1)	
South Africa	M -LA [11]	1.94	1.78	1.43	23.7	15	35 –E		[12]
Switzerland	V	4.4			50		29		
Tajikistan	V	3.3	2.8	1.5	18	25	55 –E		
Trinidad Tobago	M						30		
UAE	M			1.5			30		
UK	M	2.4			16		16.5	(2.35)	
United States	R	6.4	4.0	1.5	53		44	(2.1)	
Uzbekistan	V	3.3	2.8	1.5	18	25	55 –E		
Venezuela	M	1.5	2.0		20		20		
Zambia	V		3.3		35.5		28.9		

CODE:

* P = Proposed, V = Voluntary, M = Mandatory, R = Required for specific regions or states, LA = Level Added, otherwise value gives minimum level standard required in fortified flour.

** Iron types specified under regulations: FS = Ferrous Sulfate, E = Electrolytic reduced iron, FF = Ferrous Fumarate, R = Reduced iron.

[8] Includes 0.01 ppm Vitamin B_{12}.
[9] Bread flour only.
[10] Includes 30,000 IU/kg vitamin A.
[11] South Africa (RSA) has separate standards for the final level of micronutrients in wheat flour and brown flour.
[12] Includes 2.63 ppm pyridoxine and 1786 mcg/kg (5947 IU/kg) vitamin A. RSA previously had voluntary standard of 2.5 ppm riboflavin and 25 ppm niacin in flour.

Table 5.2B Maize Meal Fortification Standards in Different Countries

Country	*Type* of Program*	*Vit. B_1 ppm*	*Vit. B_2 ppm*	*Folic Acid ppm*	*Niacin ppm*	*Zinc ppm*	*Iron/ Type** ppm*	*Vit. A IU/kg*	*Others ppm*
Brazil	P-M			1.5			42		
Costa Rico	M	4.0	2.5	1.3	45		22 –FB		
Mexico	V	4.0	2.4	0.4	28	24	16 –R		
South Africa	M - LA[13]	2.19	1.69	2.0	25	15	35 –E	6,943	3.1 vit. B_6
United States	R	4.4	2.65	1.54	35		28.7		1100 Ca
Venezuela	M	2.9	2.5		48		46 –FF/R	9,000	
Zambia	V	2.4	2.0	0.4	22.4	12	12	5,661	2.4 vit. B_6

Hazard Assessment In Flour Fortification

There are two types of safety concerns in fortifying flour. The first is that the standard be set at a level low enough to guarantee that no one will consume chronically dangerous levels of any micronutrient over the long run, while still being set high enough to provide beneficial intake levels. One way of assessing this is from the new Upper Levels (UL) set by the National Academy of Sciences, shown in Table 5.3. This UL is set at a level lower than the *No Observed Adverse Effect Level,* so it should not be interpreted as meaning that higher levels are unsafe. It does mean that any intake below it is safe.

The amounts of fortified flour or maize meal that anyone person could consume on a regular basis will vary greatly depending on the person's size and what other food is available. But it can be surmised that hardly anyone will consume his or her caloric requirement of 2000 kcal/day entirely from flour or meal, which amounts to 540 grams. Table 5.3 shows the level of each micronutrient that would have to be in flour for the UL to be exceeded at that consumption on a regular basis corrected for the highest retention given in Table 5.4. A comparison of that to the highest fortification standard for each nutrient from Table 5.2 shows that the UL is exceeded only by the level of folic acid used in Paraguay and the vitamin A level in Nigeria.

Table 5.3 Maximum safe levels in flour

Nutrient	***Upper Level (UL)*** ***From the Food and Nutrition Board of the U.S. Institute of Medicine, 2001*** mg/day	***Amount needed in flour for 540 grams (2000 kcal) to exceed UL*** ppm
Iron	45	83
Zinc	40	74
Calcium	2500	4630
Selenium	0.4	0.74
Iodine	1.1	2.0
Folate	1	2.6
Vitamin B_1	none	
Vitamin B_2	none	
Niacin	35[14]	72
Vitamin B_6	100	206
Vitamin B_{12}	none	
Vitamin A	3.0 or 10,000 IU	6.2 mg/kg or 20,600 IU/kg
Vitamin D	0.050 or 2,000 IU	3,700 IU/kg

[13] South Africa has separate standards for the final level of micronutrients in different types of maize.
[14] The UL for niacin is based on its vasodilatation effect, which is less of a problem with niacinamide. The Scientific Committee for Food in the European Union has proposed a UL for nicotinic acid of 10 mg and a separate UL for niacinamide of 900 mg. Thus the later form poses no safety limitations in common food fortification practice.

The other safety concern regards the chance of acute toxicity if flour was accidentally over fortified. Because of the high cost of fortification premixes, no mill could tolerate having excessively high amounts used over extended periods. Normal QC testing would quickly show a problem. Any over-fortification would, thus, be for a short period and involve a small amount of flour. This could happen if the fortification feeder was kept running after the flour stopped flowing. Equipment and procedures for preventing that will be discussed in later sections of this manual. Also, there are some built in mechanisms that would prevent such flour from actually being sold or consumed. Highly overfortified flour would show an off-color, from the iron and riboflavin as well as an off-flavor making it unacceptable to the ultimate consumer.

A fifty-year experience with flour fortification has proven it to be very safe with hazards that can be easily prevented by established QA and QC procedures.

Losses of Added Micronutrient

Some of the added micronutrients are lost during the milling process due to a combination of exposure to heat, oxygen and light. Some of the very light or small particle size materials with large surface area may be physically removed with the dust during pneumatic suction, while larger particles may be removes during sieving. Column A in Table 5.4 gives estimates of how much of each nutrient is retained during typical milling practices. Higher losses might be expected in mill products with larger particle size, such as semolina and farina. As mentioned previously, these milling losses should be factored in when calculating how much of each nutrient to add to meet a minimum standard.

Table 5.4 Retention of micronutrients in cereal foods

	A	*B*	*C*	*D*	*E*	
Micronutrient	***During Milling %***	***During baking* %***	***During cooking* %***	***Pasta Noodles* %***	***% Retentions Used in RSA***	
					Wheat	**Maize**
Vitamin C	80	0	50	50	--	--
Thiamin	95	80	80	65	90	80
Riboflavin	95	90	80	75	90	80
Niacin	98	90	90	65	95	80
Vitamin B_6	95	90	90	80	80	70
Folacin	95	70	70	70	70	50
Vitamin A	60 to 90	90	90	95	80	60
All Minerals	98	100	100	75 Fe	100	100

* USDA data

A second type of nutrient loss occurs during food preparation. Column B gives USDA values of nutrient retention in *baked* products, such as bread and biscuits. Column C gives USDA retention values in *cooked* products, such as porridges and gruels and column D shows pasta and noodles. The losses shown for pasta occur when the cooking water is discarded. These food preparation losses affect how much of each micronutrient would be actually consumed, but they are not normally considered in establishing fortification standards. The one exception to this was the fortification standards developed for South Africa (RSA) in which the retention values shown in column E were used in the calculations.

Section 6 PROPERTIES OF MINERALS USED IN CEREAL FORTIFICATION

The different essential minerals fall in one of the following classifications that depend on their concentration in the body and dietary requirement. This difference in the quantity needed has a major impact on cost and other aspects of mineral fortification.

- Major elements - calcium, phosphorus, sodium, potassium, chloride and magnesium.
- Elements needed in small amounts - iron and zinc.
- Trace elements needed in tiny amounts - includes iodine, copper and selenium.

For a more comprehensive source on mineral fortification consult *The Mineral Fortification of Foods* (Hurrell 1999)

Iron

There is a great need to increase the level of absorbable dietary iron due to the extremely high incidence of iron deficiency anemia (IDA) in many countries. Iron has the greatest complexity and produces more problems in fortification than any other micronutrient. Creating an effective iron fortification program for cereals can be very challenging. Iron is included in all the cereal fortification programs in the world to date (see Table 5.2). Despite lingering questions on its effectiveness, it would not be wise to exclude it from cereal fortification programs in countries with high levels of IDA.

Because of the importance and difficulty in iron fortification, a number of good publications have already been made on this topic, which can be consulted for additional information. A major concern is which iron source to use. Guidelines on iron in cereal fortification programs have been prepared by SUSTAIN (SUSTAIN 2001), WHO (Allen 2003) and PAHO (PAHO 2002) that can be consulted. The key recommendations of WHO are given in Table 6.1.

Table 6.1 Suitable Iron Fortificants for Cereals

According to WHO Guidelines

Cereal Product	***Fortificant***
Low extraction (white) wheat flour or degermed maize meal or flour	Ferrous sulfate, dried (bakery flour only) Ferrous fumarate Electrolytic iron (2X amount)* Encapsulated ferrous sulfate
High extraction wheat flour, maize meal or flour, corn masa flour	NaFeEDTA Ferrous fumarate (2X amount) Encapsulated ferrous sulfate (2X amount) Encapsulated ferrous fumarate (2X amount)
Pasta or semolina	Ferrous sulfate, dried

* If X is the amount of iron that would be normally added in an ideal situation (e.g. 30 ppm), 2X means that twice that amount of iron should be used (e.g. 60 ppm) to account for a lower bioavailability, either due to the nature of the added iron source or the absorption inhibiting properties of the food to be fortified

The main criteria in choosing an iron source are bioavailability, effect on product quality and cost.

Bioavailability

Bioavailability, or the degree, to which the body can utilize or absorb a particular mineral source, is a particularly important factor with iron since it varies greatly with different iron sources. Ferrous sulfate and ferrous fumarate are considered to have good bioavailability while that of the elemental (reduced) iron powders is believed to be lower. There are many factors in the meal, diet and the way the food is processed that will affect the ability of people to absorb different forms of iron. There is ongoing research on the bioavailability of the different forms of iron from different products made from wheat flour and maize that will hopefully provide answers on which type of iron is best to use.

Organoleptic changes

Ferrous sulfate is a pro-oxidant that can accelerate rancidity development in unsaturated lipids. Because flour contains small amount of fats, the addition of ferrous sulfate can reduce its acceptable shelf life. This is not normally a problem with flour that is used within a month after milling, such as flour for commercial bakeries (bakery flour), but it can cause unacceptable flavor developments in household flour after months of storage. Reduced elemental iron is considered safe in any type of flour, even those requiring extended storage periods and flours of higher extractions and therefore higher levels of fat.

Iron fortificants that can be added to flour without causing adverse sensory changes in one situation do not necessarily work with the same food product in another situation. One example is ferrous sulfate, which is added as a fortificant to wheat in Chile but could not be used for this purpose in Central America. This may have been due to differences in climate, type and extraction of the wheat flour, or the quality of the ferrous sulfate purchased from different suppliers.

The extent to which fat oxidation has occurred in cereal flours can be determined by measuring hexanal (2,4-dimethy-2-pentene) levels with gas chromatography. This can be used as a rapid estimate (within hours) of the propensity of a flour going rancid by comparing a fortified to an unfortified flour (Bovell-Benjamin 1999). Others have preferred to use more long term storage of cereal products in cans at 37°C for 4 months, followed by measurement of pentane in the headspace (Hurrell 1989). Both chemical detection methods agree well with taste panel evaluations of rancidity. Trained sensory evaluation panels should determine if there are changes in texture, flavor, color or aroma in the fortified food compared to an unfortified control food, as a result of processing or storage (including simulated shelf-life conditions), and during food preparation (Bovell-Benjamin 2000). The amount of detectable rancidity correlates closely with pentane and hexanal production, which can increase ten fold.

Color

A potential problem with iron fortification is development of unwanted color. These include a green or bluish color when free iron interacts with cereals and a gray color when it interacts with chocolate or cocoa. Dried ferrous sulfate is a light tan powder and adds no color to flour, but it can react with other compounds and ingredients (i.e. bananas) to cause noticeable color changes in dough. Ferrous sulfate added to maize meal can cause undesirable blue or green colors in cooked products made from maize meal. Encapsulated forms of ferrous sulfate are recommended for maize. Large particle size ferrous sulfate can cause black spots on bread crust. Hydrated ferrous sulfate is blue-green in color and will cause color problems in the fortified flour and bread. As a result, hydrated ferrous sulfate should never be used in flour fortification.

Ferrous fumarate is dark red in color and can be noticed in white flour or white maize meal if used at high levels. It is not as soluble or reactive in dough as is ferrous sulfate. Elemental iron powders are black in color. They add no color to maize meal or wheat flour but have a slight darkening effect, which is considered acceptable. They produce no known color reactions in dough.

The color of fortified flour and baked products can be assessed by both visual and instrumental methods. The latter are very sensitive to small changes that are not visually noticeable. The potential of an iron fortificant to cause color changes can sometimes be assessed by the “blue banana test”, in which the iron fortificant is added to a hot cereal porridge mixed with puréed bananas. Soluble iron compounds such as ferrous sulfate will rapidly turn the porridge a deep blue.

Cost

The relative cost of the different iron sources are shown in Tables 5.1 and 7.1. The cheapest form of iron to add is elemental reduced iron followed by ferrous sulfate and then ferrous fumarate.

Sources of Iron

Encapsulated iron salt

There are coated forms of ferrous sulfate and ferrous fumarate available. The encapsulates used include hydrogenated vegetable oils, mono- and diglycerides, maltodextrins, and ethyl cellulose. The best products have a fat coating that protects them from chemically reacting with unsaturated fats in the flour or meal, but which will melt on baking and/or be degraded by lipases in the gut so that the ferrous salt is available for absorption. These products may have a large particle size causing them to be removed from flour during final (rebolt) sifting. The products are fairly expensive, costing 4 to 8 times that of the uncoated product on an equal iron basis. But preliminary studies have shown them to be well absorbed, even with high extraction flour, and costs may drop if their use becomes more prevalent.

Iron EDTA

A proposed solution to the problem of phytic acid inhibiting the bioavailability of added iron (discussed in Section 3) is to use iron-EDTA (ferric sodium edetate) (INACG 1998). The iron in this compound is chelated with EDTA, a commonly used food additive. This prevents the iron from being bound to phytic acid making it more easily absorbed by the body. In the human gut the iron is released from the EDTA allowing it to be absorbed. There is little advantage in using EDTA in low extraction white flour used in a yeast-leavened breadmaking process since the final level of phytic acid is very low. However, there does appear to be good justification to use it in high extraction flours, such as *atta* used in South Asian counties to make unleavened *chapattis*. The main drawbacks with iron-EDTA are it's much higher cost compared to the other iron sources and its tendency to cause color changes in some foods, but unlike other soluble iron compounds it does not promote lipid oxidation in stored cereals.

Elemental iron powders

These powders, called *reduced iron* or *ferrum reductum*, are the most common iron sources used in cereal fortification because they have the least detrimental effect on product quality and shelf-life and the lowest cost. There are five types commercially available, shown in the following table, which differ in their method of manufacture and physical properties, which in turn affects their bioavailability. The current thinking is that electrolytic, carbonyl reduced and hydrogen reduced forms have about half the bioavailability of ferrous sulfate, so they should be used at twice the level to achieve the same effect, a strategy that was first adopted by WHO/EMRO for flour fortification in the Middle East. Other forms of elemental iron powders, or forms with large particle size (> 44 microns) should not be used.

Table 6.2 Types of Elemental Iron Powders used in Fortification

Elemental iron product type	***Use in fortification***	***Cost***	***Bioavailability***[15]
Hydrogen reduced	Common	Low	Medium
Carbon monoxide reduced	Seldom	Low	Poor
Atomized	Common	Low	Uncertain
Electrolytically reduced	Occasional	High	Good
Carbonyl reduced	Never	Very High	Uncertain

Magnets and metal detectors

All the elemental iron powders are attracted to a magnet, whereas the iron salts are not. Many mills and bakeries use magnets to remove tramp iron from the flour in order to prevent equipment damage and maintain food safety. There is sometimes a concern that the magnets will remove elemental iron powders when added in a fortification program. Ferrous sulfate and ferrous fumarate will not be attracted to a magnet, so there is no problem in having a magnet in the line when these two iron salts are used.

[15] Studies are being conducted by SUSTAIN (www.sustaintech.org) to better assess the bioavailability of these products.

There are three types of magnets in common use: iron, ceramic and rare earth. Only the rare earth magnet, the strongest and most expensive, can pull reduced iron out of flour. The iron magnet type is the cheapest and the weakest. Ceramic types fall in between these two other types of magnets and are the most common in mills but the rare earth types are generally used in new equipment. When using a magnet with flour that has been fortified with reduced iron, the problem is not that the magnet will remove the iron from the flour but that the magnet will become clogged with iron causing it to lose its effectiveness in removing tramp iron. This problem can be solved by a self-cleaning magnet or by directing the flour at the magnet surface so that it continually cleans it of reduced iron.

There is no evidence of separation of any of the enrichment components added to flour on a continuous basis at the flour mill, during transport and storage, at the bakery and in the final bread. Studies (Fortmann 1974) on flour passing by a magnet show no difference in iron content before and after the magnet. There was also no evidence of flour streaking, which might be expected if large clumps of reduced iron were falling off the magnet. Alternatively, to ensure uniformity and minimize separation, sieves can be used in conjunction with magnets. Reduced iron has a very small particle size (<325 mesh) and will easily go through the finest rebold or final sifter (100 mesh), which will remove, all tramp iron or any ferrous or nonferrous metals of a large enough size to be dangerous. A mill can then use magnets at the start of the milling process before the iron is added, and rely on the final screen to remove any tramp metal.

Food manufacturers often use metal detectors to ensure that no large clumps of iron are in the final food product. These detectors may respond slightly to elemental iron powder added to the flour, but they can be calibrated so that they ignore the added iron and still detect larger iron particles that would be noticeable or possibly harmful to the consumer.

Zinc

While zinc deficiencies are not as obvious and measurable as IDA, they often accompany those of iron. Zinc and iron are similar in their dietary requirements, their levels in cereals and having their absorption inhibited by phytic acid, so any dietary deficiency in iron usually means there will be one for zinc as well. Based on estimates of zinc intake and bioavailability from FAO's food balance data, it is estimated that about 20% of the world is at risk of zinc deficiency. Zinc only started to be included in cereal fortification programs in the 1990s after recognizing this situation and the serious problem with its deficiency, particularly in children, where it can result in stunting and increased risk of disease. It is now being added to wheat flour in Mexico, South Africa, Central Asia and Indonesia, and to maize meal in South Africa and Mexico. The levels added are typically 20 to 30 ppm zinc, or restoration levels.

Zinc sources

Unlike the iron sources used in cereal enrichment, all of the zinc sources are white in color, so inherent color is not a problem. There is a potential problem in some of the more soluble sources causing color changes in certain food ingredients, such as chocolate. All zinc salts have undesirable flavors. For example, zinc oxide has a bitter taste while zinc sulfate is very astringent. It does not appear that these inherent flavors carry over to the fortified foods at the levels used in fortification. As with ferrous sulfate, there is both a dried and hydrated form of zinc sulfate. The hydrated form is reported to cause problems with caking, giving a preference to the dried form.

Perhaps the most important difference in the zinc sources is their solubility, since it relates to both bioavailability and effects on food quality. Zinc oxide is insoluble in water, but soluble in dilute acid. This implies it will be inert in dry foods but should be available for absorption following exposure to stomach acid. Zinc acetate, zinc gluconate and zinc sulfate are soluble in water and the chloride is very soluble.

Zinc oxide is the most commonly used zinc source in the fortification of cereal-based foods, followed by zinc sulfate and, to a very limited extent, zinc gluconate. Zinc sulfate is specified for use in the blended foods corn soy blend (CSB) and wheat soy blend (WSB) produced for the U.S. Food for Peace Program. It is also used in similar weaning or complementary foods made throughout the world. Zinc acetate and zinc gluconate find use only in dietary supplements and some weaning foods. There is a wide range in the cost.

The least expensive source is zinc oxide, costing approximately one-third that of zinc sulfate, the next cheapest source.

Bioavailability of zinc sources

The absorption of zinc from foods is similar to that of iron. Approximately 15% of a zinc fortificant will be absorbed on average. This percentage will be lower from high phytate foods such as whole maize (closer to 5-10%), and higher from refined or low phytate cereals (10 to 40%) (Sandstrom 1989, Sandstrom 1997). One study on bread (Ranhotra 1977) showed little difference in absorption in rats of the different sources. Absorption of zinc carbonate was poor, but absorption of zinc oxide was nearly as good as the more soluble forms. Zinc absorption from the oxide is as good as from zinc sulphate when used to fortify tortillas in Mexico (Diaz 2001), or low- or high-phytate wheat-based meals in the United States (Lopez de Romana 2002), presumably because it is soluble in gastric acid. Zinc absorption from the oxide may be poor in individuals with low stomach acid secretion. In healthy, well-nourished adults in the United States, zinc absorption from the sulfate or oxide added to a low-phytate bread meal was about 14%, compared to around 6% when either fortificant was added to a higher-phytate wheat porridge meal(Lopez de Romana 2003). Studies in Turkey (Saldamli 1996) reported that bread fortified with zinc acetate had acceptable quality and was effective in preventing zinc deficiency in children.

Effect on product quality

A number of studies have shown zinc fortification to have few detrimental effects on flour, bread and noodle quality, even at levels several times higher than that normally used (Ranhotra 1977, Kilic 1998, Lopez de Romana 2002).

Selenium

Selenium functions as a component of enzymes involved in antioxidant protection and thyroid hormone metabolism. Selenium deficiency (Keshan and Urov diseases) is rare and found mainly in areas where the soil is very low in selenium. This includes regions in China, Siberia, Finland and New Zealand. Finland has tried adding selenium to fertilizers where it increased selenium levels in milk, meat and cereals within six months (Aro 1995). Asian wheat tend to have lower selenium content than that in North America, which has higher soil selenium levels. The greatest interest in selenium fortification of flour is in Asia and countries in the former Soviet Union for that reason, as well as the belief that selenium helps provide protection against radiation damage, such as occurred after the Chernobyl nuclear disaster.

Salt has been fortified with sodium selenite (15 mg/kg) since 1983 in regions of endemic selenium deficiency in China. Sodium selenate is the most common form used in food fortification. It has been added to infant formula and sports drinks. Sodium selenate is colorless, less soluble in water, and more stable than the selenite, especially in the presence of copper and iron. There are high selenium forms of inactive yeast available that could be used to fortify baked products.

When tested in milk-based infant formulas, more selenium was absorbed than the selenate (97% vs, 73%) but more was excreted in the urine (36% vs. 10%), so the net retention of selenium was similar from both Sources (Van Dael 2002). Most cooking procedures cause relatively little loss of selenium from foods.

No country currently fortifies flour or any cereal staple with selenium, but there has been some interest in doing so, particularly in Russia. The very small amounts (0.1 to 0.2 ppm) of selenium that would be required to provide a major portion of the RDA would not be expected to have a detrimental effect on the color, baking properties or consumer acceptance of wheat flour. The cost of adding selenium is very low.

Calcium

Calcium is essential for the formation of bones and teeth and is important in maintaining a healthy skeleton. Calcium also helps regulate the acid-base balance of the body, the heartbeat, and the irritability of the neuro-muscular system (tetany). Inadequate calcium consumption is associated with risk of bone fracture and osteoporosis (bone softening) but is not the only cause of these conditions, which are most prevalent in elderly women.

There is a clear need for additional calcium in many populations, particularly in developing countries that have limited intakes of milk and dairy products, the primary dietary source of calcium in most diets. Wheat and maize are very poor sources of calcium, as shown in Tables 3.8 and 3.9. Most of the calcium provided by cereal foods comes from the calcium containing ingredients that are added to bread and biscuits as functional ingredients, such as calcium propionate, calcium phosphates and whey. These ingredients are not normally added to bread in developing countries, however.

Dried, nixtamalized maize flour, called *masa flour*, used to make tortillas is a common product in Mexico and Central America. This product has high calcium content due to the addition of calcium carbonate in the nixtamalation process, so there is no need for additional calcium. *Self-rising flour* also contains high levels of calcium due to the addition of the chemical leavening ingredients. All other cereal products are very low in calcium.

History of calcium fortification

Calcium has a long history of being added to flour and bread, originally as ground bone meal but now as calcium salts. During the Second World War the UK Government decreed that the milling industry should produce flour milled to an 85% extraction to conserve wheat supplies. The Medical Research Council recommended that calcium carbonate be added to this flour to counteract the effect of phytic acid. In 1942 the UK government ordered the addition of calcium carbonate at the rate of 156 mg per 100 g of flour, which was increased to 235-390 mg per 100 g of flour in 1946. As Newfoundland was part of the UK in 1942, the mandatory addition of calcium to wheat flour was in effect. When Newfoundland joined the Canada in 1948, wheat flour continued to be fortified with calcium, while in the rest of Canada calcium fortification was voluntary. Many other Commonwealth countries including India, Pakistan, Kenya, Uganda, and Nigeria adopted the UK flour fortification regulations that included calcium on a voluntary basis. While a number of other countries permit the addition of calcium to flour (see Table 5.2 for voluntary standards), no country requires it. However, because of the positive marketing and cost advantages, some millers find it advantageous to fortify some of their flour brands with calcium without being required to.

Calcium sources -The main calcium sources used in cereal fortification are calcium sulphate (gypsum) and calcium carbonate (limestone). Both are white and bland in flavour. Calcium sulphate is produced from mined gypsum by a precipitation process and is available either as the dihydrate with 23% calcium or the anhydrous form with higher calcium content (27%) but generally a higher price as well. Calcium carbonate used in cereal fortification is normally made by grinding limestone mined from very pure deposits. There is a considerable variation in the particle sizes available, from very fine to coarse. Manufacturers can recommend which of their products is best for flour fortification. All the calcium sources are added as is and can experience packing and flow problems if too fine a product.

There are many other calcium salts (such as calcium phosphates, calcium lactate and calcium citrate) that are used to fortify different types of foods, but they are much more expensive and offer no real additional benefits over the sulphate and carbonate in cereal fortification. They may differ somewhat in bioavailability, but that is not considered as critical an issue with calcium as it is with iron. Tricalcium phosphate is currently used to fortify complementary blended foods (CSB and WSB) since it provides both phosphorus and calcium, and is believed to help prevent infestation of the food on storage. Neither of these is particularly important with flour or maize fortification. While the body needs a balanced intake of both calcium and phosphorus, cereals already contain a high level of phosphorus, as shown in Table 3.8, so it is not necessary to add more.

Methods and economics of calcium fortification - Because of the large bulk of the calcium source that needs to be added, it is not normally included in the fortification premix and its addition is done separately from the rest of the fortification. The amount of calcium sulphate that needs to be added to flour to meet the U.S. optional standard of 2.1 g/kg is 8.7 kg/MT, which is 58 times greater than a normal fortification premix addition rate of 150 g/MT. As a result, it would make no economic sense to combine these, so a separate feeder with a higher capacity will be required if calcium is to be added.

The ingredient cost of adding calcium is about $1 per metric ton of fortified flour, but it could be higher depending on the local availability of the calcium compounds. This would appear to make it expensive compared to the rest of the micronutrients (see table 7.1), but that is misleading since the calcium source replaces an equal amount of flour, which effectively lowers the cost. If the cost of the replaced flour is higher than the cost of the added calcium, the ingredient cost to the miller can even be negative, meaning the mill can actually save money by adding calcium. This savings is possible but not likely in most countries. The value of the flour to the miller ranges from $150 to $250 per MT while the cost of the calcium source can range from $125 to $300 per MT, with shipping and tariffs largely accounting for difference. The cost of adding calcium can be calculated by the formula:

$C = P + A\,(C_c - C_f)/1000$
Where:
C = Cost of calcium fortification in $/MT of fortified flour
P = processing cost (~0.10 $/MT of flour)
A = calcium source addition rate in kg/MT
Cf = cost or value of wheat flour in $/MT
Cc = cost of calcium source in $/MT

Calcium fortification has no effect on the color or taste of flour or bread, even at the high levels used. Calcium carbonate has a slight pH raising and buffering action on flour. Added calcium is generally believed to be beneficial for yeast-leavened bread baking. Calcium fortification will greatly increase the flour's ash content, making ash levels unusable as a way of measuring flour quality or extraction.

Iodine

Iodine fortification is usually reserved for salt. There are no countries that currently require iodine to be added to flour. However, there can be situations where salt iodization is not adequate and additional measures are needed to prevent the occurrence of IDD. If flour or maize meal is being fortified in these countries, iodine can be included for hardly any additional cost.

Iodates function as oxidative bread improvers and have been added to flour and bread dough for that reason, typically at levels up to 15 ppm, which is ten times the amounts that would be added for nutritional reasons. An addition of 10 ppm calcium iodate would add 412 ug of iodine per 100 grams of bread or 110% of the RDA for iodine per slice serving size.

Bread is normally made with 2% salt on a flour basis. This has been reduced in recent years due to concerns on high sodium levels, but it normally stays above 1.5%. If the salt is iodized to U.S. standards of 77 mg/kg, 2% salt addition would add 96 ug/100g iodine to bread or 25% of the RDA per serving, assuming no loss during baking. That would make bread an important source of iodine. However, if non-iodized salt is used in baking, as may often be the case, this source of iodine is lost.

Iodized bread has been used in some countries. This can be achieved either by adding special iodized bakers' salt, as done in the Netherlands, or deliberately adding iodine to bread, the preferred source being calcium iodate. The level of iodine fortification suggested is 0.2 to 0.4 ppm iodine, which would put it in line with the levels of other micronutrients added. At this addition, it would be insufficient to have either a safety problem or much of an oxidative improving effect, and would add very little cost to the premix.

Literature Cited

Allen, L., De Benoist, B., Dary, O. & Hurrell, R. (2003). Guidelines on food fortification with micronutrients for the contol of micronutrient malenutrition. Geneva, Department of Nutrition for Health and Development, World Health Organization.

Aro, A., Alfthan, G. & Varo, P. (1995). "Effects of supplementation of fertilizers on human selenium status in Finland." Analyst **120**: 841-843.

Bovell-Benjamin, A. C., Viteri, F. E. & Allen, L. H. (1999). "Sensory quality and lipid oxidation of maize porridge as affected by iron amino acid chelates and EDTA." J Food Sciences and Nutrition **64**(371-376.).

Bovell-Benjamin, A. C., Viteri, F. E. & Allen, L. H. (2000). "Iron absorption from ferrous bisglycinate and ferric trisglycinate in whole maize is regulated by iron status." Am J Clin Nutr **71**: 1563-1569.

Diaz, M., Rosado, J. L., Salas, R., Munoz, E. C. & J. E. Westcott (2001). "Bioavailability of zinc sulfate and zinc oxide added to corn tortilla." FASEB J. **15**: A578.5 (Abstract).

Fortmann, K. L. Joiner, R. R. and Vidal, F. D. (1974). "Uniformity of Enrichment in Baker's Flour Applied at the Mill." Bakers Digest **48**(June): 42.

Hurrell, R. (1999). The Mineral Fortification of Foods. London, Leatherhead Intn. Ltd.

Hurrell, R., Furniss, D.E., Burri, J.,Whittaker, P.,Lynch, S. R., Cook, J. D (1989). "Iron fortification of infant cereals: a proposal for the use of ferrous fumarate or ferrous succinate." Am J Clin Nutr **49**(6): 1274-82.

INACG (1998). Iron EDTA for food fortification, International Nutritional Anemia Consultative Group (INACG)., Washington, D.C.

Kilic, I., Ozalp, I., Coskun, T., Tokatli, A., Emre, S. Saldamli, I., Koksel, H. & Ozboy, O. (1998). "The effect of zinc-supplemented bread consumption on school children with asymptomatic zinc deficiency." J Pediatr Gastroenterol Nutr **26**: 167-171.

Lopez de Romana, D., Brown, K. H. & Guinard, J. X. (2002). "Sensory trial to assess the acceptability of zinc fortificants added to iron-fortified wheat products." J Food Science **67**: 561-465.

Lopez de Romana, D., Lonnerdal, B. & Brown, K. H. (2003). "Absorption of zinc from wheat products fortified with iron and either zinc sulfate or zinc oxide." Amer J Clin Nutr in press.

PAHO (2002). "Iron compounds for food fortification: Guidelines for Latin America and the Caribbean ." Nutr Rev **60**(7): S50 - S61.

Ranhotra, G. S., Loew, R. J. & Puyat, L. V. (1977). "Bioavailability and functionality (breadmaking) of zinc in various organic and inorganic sources." Cereal Chemistry **54**: 496.

Saldamli, I. (1996). "Zinc-Supplemented Bread and Its Utilization in Zinc Deficiency." Cereal Chemistry **73**(4): 424-427.

Sandstrom, B. (1989). Dietary pattern and zinc supply. Zinc in Human Biology. C. F. Mills. New York, Springer-Verlag.

Sandstrom, B. (1997). "Bioavailability of zinc." Eur J Clin Nutr Hosp **51**(Suppl 1): S17-19.

SUSTAIN (2001). Guidelines for Iron Fortification of Cereal Food Staples, http://www.micronutrient.org/frame_HTML/resource_text/publications/fe_guide.pdf.

Van Dael, P., Davidsson, L., Ziegler, E. E., Fay, L. B. & and Barclay, D. (2002). "Comparison of selenite and selenate apparent absorption and retention in infants using stable isotope methodology." Pediatr Res **51**: 71-75.

Section 7 PROPERTIES OF VITAMINS USED IN CEREAL FORTIFICATION

Vitamins are essential organic compounds that the body requires to maintain life and which it cannot synthesize itself, meaning they have to be in the food supply. Vitamins are either water-soluble (vitamin C and all the B vitamins) or fat soluble (vitamins A, D, E and K).

Folic Acid

Folic acid has been included in cereal fortification programs for only the last ten years, but has proven so successful that now it is considered a leading reason to fortify cereal staples, even in developed countries with few overt micronutrient deficiency problems. The initial reason to include folic acid was to prevent the neural tube birth defects (NTD) of *spina bifida* and *anencephaly*. These can occur if the mother has insufficient stores of folic acid during the first few weeks of pregnancy. Folic acid supplements taken prior to pregnancy will help prevent this but many women are not aware they are early on in pregnancy when it is needed or they simply fail to take supplements.

It is nearly impossible to get adequate intakes of folate from natural sources, particularly so since the natural folates have only 60% of the vitamin activity of synthetic folic acid. The level of folates in cereals is low, even in whole grain products. The surest way to get folic acid to the whole population is to add it to a food staple, with wheat flour and maize meal being the preferred vehicles, particularly if it can be included with an existing or planned fortification program. A standard recommendation in the prevention of NTDs is to have women consume 0.4 mg/day of synthetic folic acid, not including natural folate sources, and this is only achievable with mass fortification of a cereal staple such as flour.

Folic acid fortification of flour has proven to be highly effective. Studies have shown a three-fold increase in serum folic acid after cereals were fortified in North America with 1.5 ppm folic acid (Lawrence 1999) and a 19% decrease in NTDs (Honein 2001). Canadian studies showed similar results in reducing serum folate insufficiency (Ray, Vermeulen 2002) and a 50% reduction in NTDs (Persad 2002) (Ray, Meier 2002) .

There is growing evidence that folic acid fortification will reduce the incidence of elevated homocysteine levels (Jacques 1999), considered a major factor in cardiovascular disease and strokes. A study in Chile showed that the national folic acid fortification program reduced serum homocysteine levels in the elderly (Hirsch 2002). While this could lead to a reduction in the incidence of heart disease and strokes, the extent to which it can do this has yet to be established. With the benefits of folic acid fortification now firmly established, over thirty countries are now adding to flour, as listed in Table 5.2. An expert panel convened by the Micronutrient Initiative recommended a 2.4 ppm folic acid addition level to all flours.

There has been concern that high folic acid levels could mask neurological problems in people with low intakes of vitamin B_{12}, and this has led to reluctance to fortify with folic acid in some countries. This is more of a worry in developed countries where people may get multiple sources of folic acid from supplements and other fortified foods. It may be possible then for some people to exceed the upper safety limit of folic acid of 1mg/day, which is based on this vitamin B_{12} interaction. An obvious solution to this is to add vitamin B_{12} along with folic acid.

Folic acid has a light yellow color, which does not carry over to the flour or cereal food because it is added at such low levels, typically from 1.5 to 2.4 ppm. There is some loss of the vitamin on exposure to light and during cooking and baking, but not as much as originally supposed. Yeast leavened breads will be actually higher in folic acid than what is contributed by the fortified flour since yeast has a significant level of folate activity. The biggest loss of folate will occur in cookies and pasta, but this is probably no more than 20%. Folic acid is difficult to analyze, so levels reported in fortified flour and baked products can have considerable assay error.

Riboflavin (Vitamin B_2)

Cereals are not very good sources of riboflavin, so people dependent on wheat, rice or white refined maize are likely to be deficient in this vitamin. This can result in a variety of skin and mucous membrane problems, that, while not life threatening can be very unpleasant. Riboflavin deficiency has been implicated with elevated serum homocysteine levels along with some of the other B vitamins (Jacques 2001).

There can be a large loss of the vitamin when the food is exposed to sunlight or UV light. Its bright yellow color, while desirable in some cereal foods, like pasta and yellow corn meal, may cause problems in products where whiteness is preferred, such as rice and white corn meal. Riboflavin has been part of most cereal fortification programs. Its cost is higher than folic acid and thiamin, but not excessive. There is good reason to include riboflavin in most cereal fortification programs.

Thiamin (Vitamin B_1)

Thiamin has been included in cereal fortification programs since their inception in the 1940s. The levels in wheat and maize are not particularly low, even in the refined products, but the level of the vitamin that makes it through to the final food product that is actually consumed is much reduced due to its poor stability, particularly under alkaline (high pH) conditions. The thiamin level in white rice is quite low, causing more of a problem with thiamin deficiency (beriberi) in rice eating populations. There is also concern about thiamin deficiency causing Wernicke-Korsakoff syndrome (WKS) in alcoholics (Yellowlees 1986). Australia and New Zealand started fortifying bread flour with thiamin for this reason. It has led to a significant reduction in the prevalence of WKS in these countries (Harper 1998).

Thiamin can be added as either thiamin mononitrate or thiamin hydrochloride. The mononitrate form is preferred because it is considered more stable. Both are white powders and add no color to the flour. There are no known functional problems in adding thiamin to flour and the cost of thiamin fortification is not very high.

Vitamin B_{12}

Cereals contain no vitamin B_{12}. It is present only in animal products. Deficiencies occur mainly in the elderly. The main justification for adding vitamin B_{12} is so that high levels of folic acid can be added without risk of masking B_{12} deficiencies (Ray 2000) but it is also implicated along with some other B vitamins in reducing serum homocysteine levels (Bower 1995). Currently only one country, Israel, has included vitamin B12 in their cereal fortification program but there have been increasing calls that it be included (Quinlivan 2002).

Vitamin B12 (cyanocobalamin) is a complex molecule and difficult to produce. Its cost is very high but the fortification cost is reasonable since it is needed in such small amounts. It is a dark red compound, typically sold in a 1% dilution to make it easier to handle. It is relatively stable, but its stability in baking has not been tested. It is one of the most difficult vitamins to analyze for in foods, with a microbiological procedure being the preferred method. There have been no reports of its addition adversely affecting the color or baking properties of wheat flour.

Pyridoxine (Vitamin B_6)

There is some suggestion that pyridoxine, along with folic acid and vitamin B_{12}, can help lower homocysteine levels and thereby reduce the incidence of heart disease and stroke (Duell 1997; Jacques 2001; Kelly 2003). While whole grains are good sources of this vitamin, refined wheat flour and maize meal are not. But because it is found in a variety of foods, overt B_6 deficiency is uncommon.

Pyridoxine hydrochloride is a white powder and is not known to cause any problems when added to cereals. Its cost of fortification is similar to that of riboflavin. There is some loss of pyridoxine on exposure to UV light. Currently, only South Africa includes vitamin B6 in their cereal fortification program.

Niacin (Vitamin B_5)

Niacin is low in refined flours, and much of the niacin in whole maize, while fairly high, is unavailable since it is in a bound form. The bound niacin is released and made available in the nixtamalation process of making tortillas, but other types of maize staples will be low in available niacin. This helped cause a high incidence of pellagra in the Southeastern United States, where maize was the main food staple, resulting in thousands of deaths each year. It was for this reason that niacin was included in the original cereal fortification program, which proved very successful in preventing pellagra. There is good reason to include it with any maize eating population, particularly those that do not use a nixtamalation process, such as sub-Sahara Africa and South America. However, it is relatively expensive and could be added at a lower rate to wheat flour, or even excluded from flour fortification programs in wheat consuming populations.

One reason for excluding Niacin is tryptophan, an amino acid in proteins that acts as a niacin precursor. 60 mg of tryptophan = 1 mg of niacin = 1 Niacin Equivalent or NE. The RDA for niacin is now given in NE units. When tryptophan content is considered even refined wheat flour becomes a fairly good source of niacin but maize meal is still inadequate, as shown in Tables 3.8 and 3.9.

Niacin comes in two chemical forms: niacinamide and nicotinic acid. The latter is normally referred to as niacin so as not to be confused with nicotine, a totally different compound. Niacinamide is slightly more expensive but it has the advantage of not be a vasodilator as is nicotinic acid, which results in a flushing and skin reddening reaction in those handling the fortification premix. Both are white powders and have no detrimental effects on taste or flour functionality. Niacin is very stable and has no problem with cooking or baking losses.

Vitamin A

Deficiencies of vitamin A are considered a serious health problem in many parts of the world. It affects visual function leading to night blindness and xerophthalmia. WHO estimates about 3 million pre-school children show ocular signs of vitamin A deficiency and that 254 million preschool children throughout the world are subclinically deficient.

Cereals contain no natural vitamin A and very low levels of beta-carotene, a vitamin A precursor. They are, however, potential vehicles for vitamin A fortification in deficient populations. Flour and maize meal are not usually fortified with vitamin A in developed countries, where margarine is often fortified, and vitamin A deficiency is not a problem. The U.S. Title II (Food for Peace) Food Program provides wheat flour and maize meal fortified with vitamin.

Vitamin A has been added to precooked maize flour in Venezuela since 1993. At the fortification level of 2.7 mg/kg and an intake of 80 g flour/day, it supplies about 40% of the recommended intake of the vitamin (Chavez 1997). South Africa adds it to flour (1.6 mg/kg) and maize meal (1.9 mg/kg). Nigeria started requiring the addition of vitamin A to flour at a very high level of 9 mg/kg in 2002. In the Philippines, wheat flour was fortified with 4.5 mg/kg so that the level in the bread was 2.2 ug/g. This supplied about 33% of the Filipino RDA for the vitamin for school children, and increased their liver stores of retinol significantly by the end of a 30-week efficacy trial (Solon 2000).

Several forms of vitamin A are available for food fortification. These include retinyl acetate, retinyl palmitate, and provitamin A (β-carotene). β-carotene has an intense orange color that makes it unsuitable as a fortificant for many foods, but it is used to give an orange-yellow color to margarines and beverages. The retinyl esters are available in an oil-soluble form (for fortification of oils and fats), spray-dried (for flours and powdered milk) and as water-dispersable beadlets (for fortification of sugar and other water-soluble foods). A special coated, protected form of retinyl palmitate, often generically referred to as *SD250*, is the recommended form of vitamin A for flour fortification because it is considered to be the most stable in this application. This product contains encapsulates and antioxidants that differ between manufacturers, making it impractical to specify its exact composition. Alternatively, the USDA specifies that the product used in PL480 (Food for Peace) commodities retain at least 80% of its activity under defined storage conditions. The stability of vitamin A in these commodities was found to be surprisingly good, with over 95% retained after nine months (Ranum, 1999). There were additional losses during milling and baking, so that about 80% of the vitamin A added is actually consumed. Lower retentions, even down to 50%, can occur for non-bread baked products and maize meal.

It would be feasible to add vitamin A to any kind of flour or maize meal, including the high extraction or whole wheat (atta) flours prevalent in some countries. The primary constraint is the cost (see Table 7.1). Inclusion of vitamin A can double or triple the cost of a cereal fortification program. Vegetable oil may be better carrier because the form of vitamin A that can be used in oil is cheaper and the stability is somewhat better. However, in many countries, wheat flour or maize meal may be the only processed food consumed widely enough to deliver vitamin A to at-risk populations through food fortification.

Vitamin D

Vitamin D is an important regulator of calcium metabolism and can help prevent rickets in breast-fed infants and osteomalacia in the elderly. People who are exposed to sunlight will not need much vitamin D, but adult deficiencies have been shown to exist in northern latitudes, with a higher prevalence in older subjects (Yan 2000). Low levels of exposure to UV light in urban settings, clothing practices and air quality can lead to an increased need for dietary vitamin D.

Milk, including dry and evaporated milk, is the preferred vehicle to fortify with vitamin D. It was included as optional in the early cereal fortification programs in the U.S., but it was never practiced and has since been removed. No country currently adds vitamin D to cereal staples, but it has been proposed for Mongolia. It is often added to complementary foods targeted for children, such as CSB, and to margarine.

Vitamin D is a fat-soluble compound. Either vitamin D2 (also called cholecalciferol or ergocalciferol) or D3 (cholecalciferol or 7-dehydrocholesterol) can be added to foods, and have a similar biological activity, but the D3 form is preferred for cereals. One International Unit (IU) of vitamin D is equivalent to 0.025 ug of the vitamin. Both forms are very sensitive to oxygen, moist air and minerals. Dry stabilized vitamin D is available, and contains an antioxidant (usually tocopherol) that protects potency for much longer, even in the presence of minerals. The form commonly used in cereals contains 100,000 IU or 2.5 mg of D3 per gram.

Vitamin C

Ascorbic acid or vitamin C provides a number of important nutritional benefits (Bendich 1995), but the one considered most desirable for cereal products is its ability to enhance the absorption several fold of both native and added iron.

Ascorbic acid is routinely added to bread flour around the world at levels from 15 to 100 ppm to improve the flour’s bread baking properties. Enzymes in the flour quickly convert it to dehydroascobic acid, which acts as an oxidative improving agent during fermentation giving a larger loaf volume and a lighter crumb. Unfortunately, further oxidation during and after baking destroys any remaining vitamin C activity. Foods prepared from maize meal retain more added vitamin C than with bread. About half of the ascorbic added to CSB, which is mainly maize meal, was retained when it was prepared as a paste (ugali or pap), commonly used in Africa (Ranum 1998). The ascorbic acid added to CSB is lightly coated with ethyl cellulose (4%), but this has little benefit in preventing loss during cooking. Greater cooking stability is possible with more heavily coated products.

Unfortunately, the cost of adding the levels of ascorbic acid necessary to improve iron absorption may be prohibitive. It could be cheaper to use iron EDTA. This is an area that needs more research and development before it can be effectively applied to cereal fortification.

Literature Cited

Bendich, A. & L. Langseth (1995). "The health effects of vitamin C supplementation: a review." J Am Coll Nutr 14(2): 124-36.

Bower, C. & N. J. Wald (1995). "Vitamin B12 deficiency and the fortification of food with folic acid." Eur J Clin Nutr 49(11): 787-93.

Chavez, J. F. (1997). Enrichment of precooked corn flour and wheat flour in Venezuela: A successful experience. Food Fortification to End Micronutrient Malnutrition. State of the Art. Ottawa, Canada., The Micronutrient Initiative: 62-65.

Duell, P. B. & M. R. Malinow (1997). "Homocyst(e)ine: an important risk factor for atherosclerotic vascular disease." Curr Opin Lipidol 8(1): 28-34.

Harper, C. G., Sheedy, D. L., Lara, A. I., Garrick, T. M., Hilton, J. M.& Raisanen, J. (1998). "Prevalence of Wernicke-Korsakoff syndrome in Australia: has thiamine fortification made a difference?" Med J Aust 168(11): 542-5.

Hirsch, S., de la Maza, P., Barrera, G., Gattas, V., Petermann, M. & Bunout, D. (2002). "The Chilean flour folic acid fortification program reduces serum homocysteine levels and masks vitamin B-12 deficiency in elderly people." J Nutr 132(2): 289-91.

Honein, M. A., Paulozzi, L. J., Mathews, T. J., Erickson, J. D. & Wong, L. Y. (2001). "Impact of folic acid fortification of the US food supply on the occurrence of neural tube defects." Jama 285(23): 2981-6.

Jacques, P. F., Bostom, A. G., Wilson, P. W., Rich, S., Rosenberg, I. H.& Selhub, J. (2001). "Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort." Am J Clin Nutr 73(3): 613-21.

Jacques, P. F., Selhu, J., Bostom, A. G., Wilson, P. W. F. & Rosenberg, I. H. (1999). "The Effect of Folic Acid Fortification on Plasma Folate and Total Homocysteine Concentrations." N. Engl. J. of Med. 340(19): 1449-54.

Kelly, P. J., Shih, V. E., Kistler, J. P., Barron, M., Lee, H., Mandell, R. & Furie, K. L. (2003). "Low Vitamin B6 but Not Homocyst(e)ine Is Associated With Increased Risk of Stroke and Transient Ischemic Attack in the Era of Folic Acid Grain Fortification." Stroke, May 8.

Lawrence, J. M., Petitti, D. B., Watkins, M. & Umekubo, M. A. (1999). "Trends in serum folate after food fortification." Lancet 354: 915-916.

Persad, V. L., Van den Hof, M. C., Dube, J. M. & Zimmer, P. (2002). Incidence of open neural tube defects in Nova Scotia after folic acid fortification. Cmaj 167(3): 241-5.

Quinlivan, E. P.,,McPartlin, J., McNulty, H., Ward, M., Strain, J. J., Weir, D. G. & Scott, J. M. (2002). "Importance of both folic acid and vitamin B12 in reduction of risk of vascular disease." Lancet 359(9302): 227-8.

Ranum, P. M. (1999). Report of the Micronutrient Assessment Program of Fortified PL480 Commodities. Washington DC, SUSTAIN.

Ranum, P. M. and F. Chome (1998). Results Report on the Vitamin C Pilot Program. Washington, DC, SUSTAIN.

Ray, J. G., Cole, D. E., Boss, S. C. (2000). "An Ontario-wide study of vitamin B12, serum folate, and red cell folate levels in relation to plasma homocysteine: is a preventable public health issue on the rise?" Clin Biochem 33(5): 337-43.

Ray, J. G., Meier, C., Vermeulen, M. J., Boss, S., Wyatt, P. R. & Cole, D. E. (2002). "Association of neural tube defects and folic acid food fortification in Canada." Lancet 360(9350): 2047-8.

Ray, J. G., Vermeulen, M. J., Boss, S. C.& Cole, D. E.. (2002). "Increased red cell folate concentrations in women of reproductive age after Canadian folic acid food fortification." Epidemiol 13(2): 238-40.

Solon, F. S., Klemm, R. D., Sanchez, L., Darnton-Hill, I., Craft, N. E., Christian, P.& West, K. P., Jr (2000). "Efficacy of a vitamin A-fortified wheat-flour bun on the vitamin A status of Filipino schoolchildren." Am J Clin Nutr 72(3): 738-44.

Yan, L., Prentice, A., Zhang, H., Wang, X., Stirling, D. M. & Golden, M. M. (2000). "Vitamin D status and parathyroid hormone concentrations in Chinese women and men from north-east of the People's Republic of China." Eur J Clin Nutr 54: 68-72.

Yellowlees, P. M. (1986). "Thiamin deficiency and prevention of the Wernicke-Korsakoff syndrome. A major public health problem." Med J Aust 145(5): 216-9.

Table 7.1 Micronutrient fortification levels and costs

Nutrient	Chemical Compound	Levels Added			Cost**
		As nutrient ppm	As compound gram/MT	As % of RDA per 100g	US$ per MT of fortified flour
Iron*	Elemental Reduced Iron	50	52	14% women	0.12
	Electrolytic Reduced Iron	50	51	31% men	0.16 –0.36
	Ferrous Sulfate	25	78	25% children	0.14
	Ferrous Fumarate	25	78		0.31
	Sodium Iron EDTA	15	111		0.96
Zinc	Zinc Oxide	15 – 25	19 - 31	15% - 25%	0.04 – 0.07
Calcium	Calcium Sulfate	1 – 2 g/kg	4400 – 8700	10% - 20%	0.57 – 1.14
	Calcium Carbonate		2500 – 5000		0.55 – 1.10
Selenium	Sodium Selenate	0.1 – 0.2	0.2 – 0.5	18% - 36%	0.01
Iodine	Calcium Iodate	0.2 – 0.4	0.3 – 0.7	13% - 27%	0.01
Folate	Folic acid	1.5 – 2.2	1.7 – 2.5	38% - 55%	0.06 – 0.08
Vitamin B1	Thiamin Mononitrate	2 – 5	2 – 5	17% - 42%	0.04 – 0.10
Vitamin B2	Riboflavin	2 – 4	2 – 4	15% - 31%	0.07 – 0.14
Niacin	Niacin (Nicotinic Acid)	20 – 50	20 – 50	13% - 33%	0.15 – 0.37
	Niacinamide				0.16 – 0.39
Vitamin B6	Pyridoxine Hydrochloride	3.2	3.8	25%	0.16
Vitamin B12	Cyanocobalamine, 1%	0.01	1.0	42%	0.17
Vitamin A	250SD Palmitate	5 – 10 IU/g		17% - 33%	0.78 – 1.54
Vitamin D	Vitamin D3, 100 SD	0.4 IU/g		20%	0.08
Vitamin C	Ascorbic Acid	15 – 50		(2% - 7%)	0.06 – 0.22

* Iron levels based on adding twice as much elemental reduced iron as ferrous salts in order to adjust for their having half the bioavailability.
** Cost estimate does not include shipping or duties.

Section 8 FORTIFICATION METHODS in LARGE MILLS

This section covers the properties of fortification premixes and how they are added to flour in large and medium size mills, typically those with milling capacities greater than 24 metric tons of wheat or maize per 24 hours or 1 MT/hr. It explains the different type of methods, equipment and designs used to produce flour that is correctly and uniformly fortified, and provides instructions on the installation, calibration and troubleshooting of fortification operations.

Fortification Premixes

A *premix* or *master mix* is a blend of micronutrients used to fortify flours. Seldom is only one nutrient added. The normal practice is to fortify flour with multiple micronutrients. It is more convenient to add them all at once as a single premix, the one exception to this is calcium, which is normally added separately due to its large bulk requirement

Advantages of vitamin/mineral premixes

1. *Nutrient Ratio:* If the premix has been properly designed, manufactured and mixed, the ratio between the different nutrients is constant. Because of this constant ratio, testing of only one of the nutrients in flour can verify that the delivery dose was correct for all. This assumes no destruction of vitamin activity or separation of the micronutrients after the premix was added. This single nutrient then acts as an *indicator* for all the other nutrients. Iron is often used as an indicator nutrient.
2. *Standardized Addition Rates:* The dilution of the premix can be adjusted through the use of a *carrier* to give a standardized addition rate that meets the needs of the production facility to produce a uniform level of fortification. Some mills are large enough to allow use of concentrated premixes while others are small and require a more dilute premix when the feeder cannot accurately handle the necessary low feed rate. Also, there is better dosage accuracy and mixing homogeneity with dilute premixes than with straight nutrients if the feeder is able to operate in the middle of its delivery range rather than at the lower end.
3. *Excipients:* The premix can have *excipients* and *free-flow agents* added to improve its flow or adhesion properties. Continuous flow flour mills require a free-flowing premix that does not clump or bridge in the feeder.
4. *Single Weighing:* A single premix requires only one weighing for batch systems or feed rate adjustment in continuous systems. This reduces labor requirements and greatly lessens the chance for error.
5. *Technical Support:* Often, but not always, the premix supplier will supply and service the feeder for adding the premix.

Design and composition of premixes

There are a number of considerations that are made in the design or formulation of a fortification premix. See Section 3 for a more detailed discussion of these.

1. *Fortification Standard:* A premix is designed to meet a **fortification standard** or add a set level of micronutrients. The former is the most common but adding set levels is becoming more commonplace.
2. *Natural Levels:* If designed to meet a fortification standard, the **natural levels** of the nutrients in the fortified product must be considered. The level added is to make up for the difference between the natural level and the standard.
3. *Overages:* An **overage** necessary to account for variation in that natural level, to make up for any processing or storage losses and to insure that the final level will be minimally achieved, must be included. An overage of 10% is often used when fortifying dry cereals. As an example, to fortify wheat flour, containing 12 ppm iron to the U.S. standard of 44 ppm, 35 ppm iron is typically added, which is the target less the natural level plus 10%.
4. *Nutrient Concentration:* The amount of each nutrient source must be adjusted on the basis of the **concentration** of the nutrient source used.

5. *Manufacturing Overage:* A small **manufacturing overage** (usually about 2%) is included to insure the premix meets label claims by assay.
6. *Addition Rate:* The desired **addition rate** of the premix then determines the final formulation. Ideally, the addition rate is set to be in whole units, such as 150 grams/ton.
7. *Free-flow Agents:* **Free-flow agents**, such as tricalcium phosphate or precipitated silica (silicon dioxide) may be added to keep the premix from clumping.
8. *Carrie/Excipient:* The **remainder** may be starch (wheat or corn), maltodextrin or an inexpensive mineral, such as calcium carbonate or calcium sulfate. Starch or maltodextrin is preferred as a carrier since it lowers the bulk density of the premix making it somewhat easier to handle and feed. Wheat or corn flour is not recommended for use as a carrier because of possible infestation and stability problems. Concentrated premixes with little or no carriers are desired when they are transported long distances or are subject to high shipping costs. However, small mills may have difficulty in accurately feeding these highly concentrated premixes.

Premix production and quality control

Micronutrient premixes are typically made by precision weighing of the ingredients and batch mixing in a ribbon or twin shell blender to produce a homogeneous mixture. Commercial premixes are customarily analyzed for all contained nutrients. This generates a certificate of analysis (COA) on each batch of product. This analysis, usually done by the premix supplier, is the most difficult and expensive aspect of commercial premix production. The COA is the guarantee from the manufacturer to the user that a particular batch of premix contains all of the desired nutrients in the correct amounts. In some countries (e.g. South Africa) premix suppliers must be certified by the government and required to submit product for periodic testing by a certified referee laboratory. Appendix E lists some of the larger, commercial premix suppliers.

Large mills or a group of mills in a particular area or country may choose to make the premix themselves rather than use a commercial product. This could be a cost-effective approach if only one or two micronutrients are involved. Since shipping distances would be reduced, it would allow production of a more dilute product better suited to small, local mills. For more complex premixes it may be preferable to rely on commercial producers because of their better access to raw material and analytical capabilities.

Packaging, storage and handling of premixes

Vitamin/mineral premixes should be packaged in air and watertight containers well protected from exposure to light. Typical packaging is a polyethylene bag inside a heavy, cardboard box. The package should be such that the bag can be easily resealed and the box closed after a portion of the product has been removed.

Premixes should be kept in their original containers in a cool dry place prior to use. Once opened exposure to the light and air should be minimized to prevent product degradation.

Handling of premixes at the mill

When handling the premixes the following precautions should be taken:

1. The operator should use a dust mask to prevent inadvertent inhalation of the active ingredients.
2. The operator should wash hands and skin areas exposed to the material during filling of the feeder hoppers.
3. The fortificant premix should be well identified to prevent accidental replacement with any other flour additive or premix (this can be achieved by using a color coded system which identifies the different additive feeders and additive boxes).
4. There may be some allergic skin reactions to flour fortificants such niacin. It is therefore recommended that the operator use gloves and long sleeve shirts when handling the product. A common occurrence is skin reddening caused by the vasodilatation effect of niacin. This effect is transitory and not dangerous but it can be annoying.

Upon receipt of the shipment, the production lot number(s) should be recorded and retained. It is recommended that a first-in, first-out (FIFO) system of stock rotation be employed since the vitamins in the fortificant premix have a limited shelf life in terms of their biological effectiveness and stability. This is particularly the case with vitamin A. Unopened packages of premixes containing vitamin A have an effective shelf life of six months in warm climates. The shelf life of premixes containing minerals and only

B vitamin is up to three years if unopened.   Once a premix box has been opened it should be used within a few weeks.

Mill preblends

Some mills may want to dilute concentrated premixes prior to use by mixing with flour and other flour improvers. This is usually done in a small mixer at the mill, making just enough preblend for a day's run. It is not advisable to make more than that since the flour-nutrient blend will not keep well. Flour improvers can be included in the blend, including enzymes, azodicarbonamide and ascorbic acid. Additives that should **never** be included in this blend for safety reasons are concentrated forms of potassium bromate and benzoyl peroxide (flour bleach).

Mill Fortification Methods

Mill fortification involves adding standard quantities of micronutrients to a set quantity of food and mixing them together so as to achieve a uniform product. There are three different types of methods that have been used to fortify flour or meal in large, continuous milling operations.

Batch mixing

Batch mixing is slower and more labor intensive than other methods but it has better accuracy and uniformity depending on the precision of the scale. It is usually done in smaller mills, as described in Section 9. There are now electronic digital scales accurate to a tenth of gram available for under $100. The problem with using such scales is that they are likely to disappear if not well secured.

Automatic systems of batch mixing in large-scale, continuous systems are now available. The flour and premix are metered into a mixer that rapidly blends it and discharges it into a bin or packout.   The alternate use of two mixers allows the system to operate continuously. This system is more expensive but does allow a somewhat greater level of accuracy and uniformity to be achieved.

Two-Stage mixing

A special two-stage mixing system was developed to fortify flour in the former Soviet Union. In this system the vitamins were first mixed with some of the flour to make a preblend, which was then mixed with a larger amount of flour as it flowed through the system. With the demise of the Soviet Union, flour fortification stopped and this equipment fell into disrepair. It reputably is still being manufactured and available through the Buhler Company. The supposed advantage of this method is that it allows for more accurate addition with variable flour flow, but the equipment is more expensive and not readily available in most countries.

Continuous metering

In this process the premix is continuously metered or *fed* into the flour flow using a precision microingredient powder feeder (dosifier). This is by far the most commonly used method because of its low cost and acceptable precision, and most of the following information relates to fortification by this technique.

For this method to be satisfactory a fairly constant flow of flour is required at the point of addition, or, in lieu of that, the use of a system by which the output of the feeder continuously adjusts to the flow rate of the flour. It also requires some degree of mixing after the point of addition. These two conditions are generally available in most mills, but if not, they can be achieved by installation of a conveying screw from a flour holding bin to another bin or to packout.

Flour Fortification in Continuous Systems

When micronutrients are added in continuous systems, they are added as a free-flowing, dry powder premix. The premix is continuously metered into the flour stream at a dosage rate dependent on the flour flow. It is important to ensure that the dosage is correct and that the flour flow and premix delivery rate is known and consistent. The premix can be added to flour at the end of the milling process, or it can be added after milling during packing or load-out on condition that adequate mixing is provided.

Equipment

The equipment needed to fortify flour in a continuous system includes:

1. A dry powder "feeder" or "dosifier" to meter out the fortificant or premix
2. A means to agitate the fortificant or premix so that it feeds smoothly and uniformly without bridging.
3. A mechanical or electronic means of adjusting the rate of flow of the premix
4. A means to deliver the premix from the feeder to the flour
5. Some level of mixing after the premix has been added to the flour.

Feeders (Dosifiers)

There are three general principles by which feeders control the amount of premix or fortificant added to flour.

- **Volumetric** addition is based on the principle that the **volume** of the material being added has a set weight when handled in a uniform manner. Volumetric addition is similar to using a cup or spoon to measure out ingredients in home baking. The same simple procedure can be used to fortify flour in small batch mixing processes. The most commonly used method to fortify flour at the mill is with volumetric feeders that dispense a set volume of a premix at a constant rate. The weight of the premix dispensed depends on its bulk density. Iron sources have higher bulk densities than vitamins and flour. The higher the density, the greater weight of material that is dispensed at the same feeder setting. The minimum error of measurement for volumetric addition is ±2%. Typical costs for this type of feeder are $1,000-$8,000.

- **Gravimetric** addition involves measuring the **weight** of material to be added on a continuous basis. There are weigh belt feeders for use in continuous systems that can give direct weightings of the material being dispensed, but they usually require a greater volume of material than used in most fortification operations. They could find application in calcium fortification. Typical costs for weigh belt feeders are $5,000-$10,000

- **Loss of weight** addition is based on taking continuous readings of the weight of the premix and feeding equipment. This is achieved by mounting the feeder on *load cells* that send out an electronic signal proportional to the total weight. The rate at which this weight drops with time indicates the true addition rate. This system is somewhat more complex and expensive than is required in most cereal milling operations. Typical costs for this type of feeder are $10,000-$20,000.

Types of Powder Feeders and Designs

There are many different types of powder feeders available that can be used to fortify flour. Appendix E lists companies providing such equipment. The different types of feeders range in degree of sophistication from complex to simple. They also range in cost from around $1000 to $25,000. Feeders also differ in how easy they are to clean, repair and maintain. There are three types of powder feeder mechanisms used in flour mills today: screw type, revolving disk, and drum type. These differ in the mechanism used to deliver a constant rate of powder (see diagrams). Most of the newer feeders manufactured today are of the screw type.

Screw feeder

The screw type feeder is powered by a variable speed DC electric motor, which is used to control the feed rate of the powder. The shape of the feed screw determines the feed rate capacity. Large capacity feeders may use a gearbox to increase and adjust the feed rate capacity. The premix is kept flowing by means of a large conditioning screw or flexible pulsating plates on the bottom of the hopper. Large hoppers may also use an intermittently run vibrator to prevent bridging. A low-level detector can be installed on the bottom of the hopper to indicate when the premix is close to running out. The on/off switch, speed control and low- level indicator light can be located near the feeder or at a remote location.

Advantages of this type of feeder are that it sustains a constant addition rate, has a wider range of delivery rates and hopper capacity, uses fewer mechanical parts and are less expensive to build. They can be more sanitary and easier to maintain than the other types of feeders. They are the main type of feeders available as new equipment.

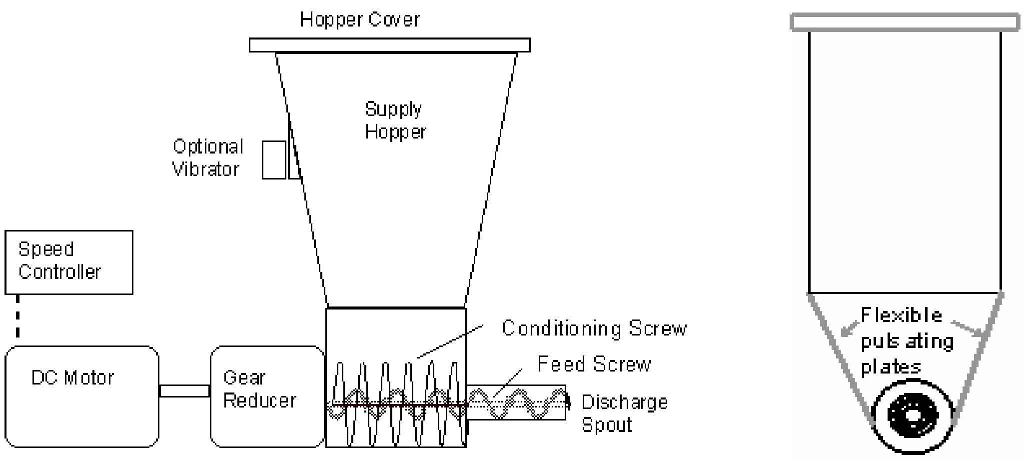
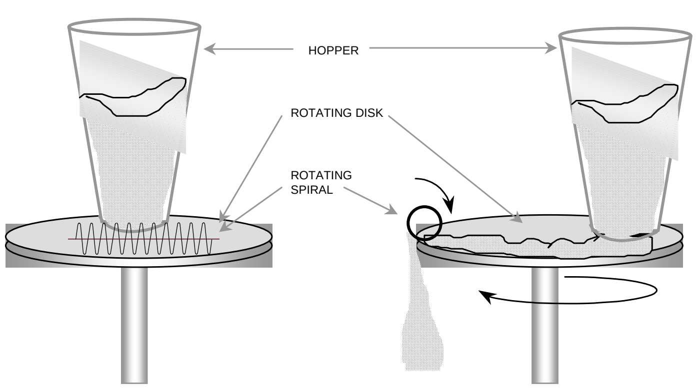


Figure 8.1 Screw type feeders

Revolving disk feeder

This older type of feeder uses a revolving disk equipped with a slide mechanism to control the rate of powder discharge. The disk revolves at a constant speed and can be run with either an AC or DC motor. The size of the hopper is usually smaller than the hoppers of other types of feeders requiring that it be refilled more frequently. This can be a disadvantage for larger flour mills since refilling takes up more of the miller's time. This type of feeder uses more mechanical components than the screw feeder.

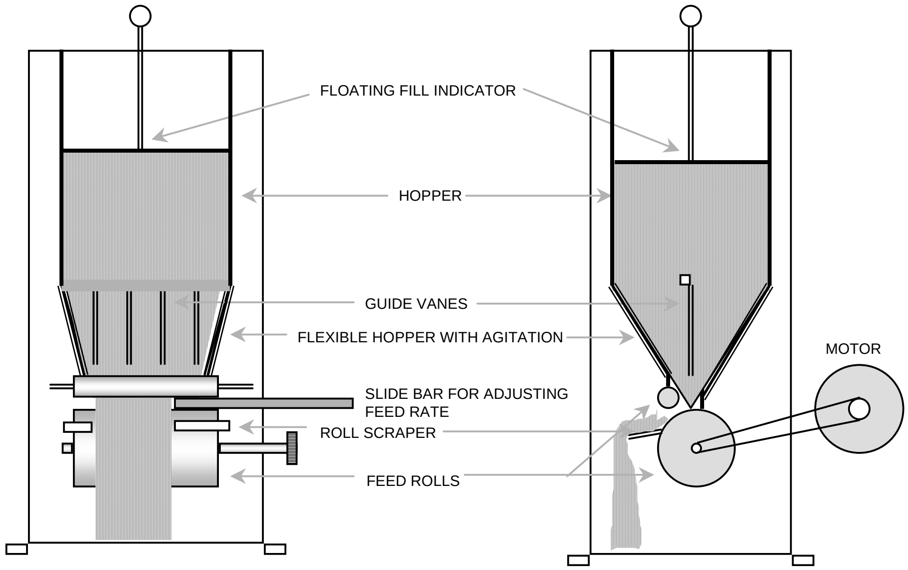
Figure 8.2 Mechanism used in Revolving Disk Feeders



Drum or roll type feeders

This type of feeder has been a workhorse for use in flour treatment, with many thousands of units still in use. It operates by allowing the powder to pass between two revolving cylinders, as shown in the diagram. Either a DC or AC motor can power the drum and a gearbox and a pulley system control the rotation speed. Pulleys and wheels of differing diameters can be used to make gross adjustments in the feed rate capacity. There is an adjustable gate, which is used to make fine adjustments in the rate of feed. This design requires more parts to operate and has higher maintenance requirements. Shear pins in the drive mechanism cause the feeder to stop working if large objects (bolts, plastic) get stuck between the rolls.

Figure 8.3 Drum Feeder



A new variation on drum feeders is to replace the motor with a variable speed DC drive motor allowing the addition rate to be adjusted electronically rather than mechanically. Variable speed AC drive motors are also available. It is advantageous to have capabilities for both mechanical and electronic controls since that affords better control over addition rates.

Fortification Systems for Different Size Mills

The number and size of the feeders needed to fortify flour at a mill depends on the capacity of the mill and the number of different products to be fortified. Generally, a mill needs only one feeder per milling unit, but larger milling units with multiple products may require additional feeders. It is advantageous to have at least one extra feeder as a spare in case of breakdown.

Feeder sizes

Powder feeders are available in different sizes. At the low end of the scale they can discharge amounts from 25 g per hour, and the largest can discharge up to 32 kg per hour. Generally, feeders used for flour fortification need to deliver only relatively small amounts of material. The size and number of feeders will be predicated on the capacity and hourly throughput of the mill or load-out system. There are different ways that the addition rate of a fortificant or premix can be adjusted to match the range of flour flow. One is the choice of the type and size of the feeder. Small volumetric feeders are best in smaller mills while gravimetric or loss of weight feeders may be needed when very high addition rates are required.

Feeding rate adjustments

Gross speed adjustments can be made by changing the size of gears or pulleys or the gear box itself. In screw type feeders the diameter of the feed screw determines the volume of the premix delivered at the same speed. Different size screws are available for most feeders. Fine speed adjustments are made through a controller for a variable speed motor, or a fine volume adjustment made using a mechanical gate at the outlet point. Finally, a more dilute premix can be obtained or made at the mill by batch mixing with flour.

When setting up a mill for fortification, the mill capacity determines the premix dilution and the type and capacity of the feeder required. Gross adjustments are then made by using different feed screws, pulleys or gears. Once installed and running, regular fine adjustments are made to match the flow of flour. Ideally, the feeder should be operating in the middle of its sized range rather than on either end.

Most of the feeders are volumetric, which deliver a known volume rather than weight of the material. The actual addition rate in weight per unit time for volumetric feeders depends on the bulk density of the fortificant or premix. This must be determined by running a test weight of the feeder output with time. A premix containing a large quantity of iron will have a higher density than a vitamin premix and will be fed at a higher addition rate at the same feeder setting.

Addition and Mixing Design Delivery Systems

Once the feeder has delivered its required quantity there are two ways that the material can be introduced into the flour stream.

Pneumatic conveying

The first is by pneumatic conveying. The premix drops into a venturi tube, which injects the premix into the air stream. The material is blown by positive pressure or sucked by a vacuum through a pipe into the flour collection conveyor. If that is not possible, some downstream location in the flour flow can be utilized allowing that some flour mixing is provided.

Gravity feed

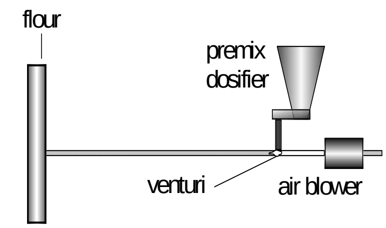
The second method of delivery is by gravity feed, where the feeder is placed above a conveyor. The premix is dropped directly into the flour as it flows through the conveyor, often the *flour collection conveyor*. This conveyor is designed to collect and blend the individual flour streams coming from the sifting equipment so that the final flour is uniform. The location where the flour fortificant is introduced to the flour conveyor is critical. It should be located in the front half of the collection conveyor above the blades of the mixing screw. If located too close to the discharge end the fortificant may not have enough time in the conveyor to be blended properly with the flour.

Location of Powder Feeders

Feeders should be placed in a dry location and away from sunlight. This will prevent the components from any potential interaction with sunlight. Vitamin A, riboflavin and folic acid are sensitive to light and atmospheric oxygen. Ideally, feeders should be placed in an area of the mill easily accessible to the operators and handy to the miller’s office or flour testing station. There should be room adjacent to the feeders for keeping a few boxes of the premix.

Pneumatic method

Pneumatic Method of Premix Delivery

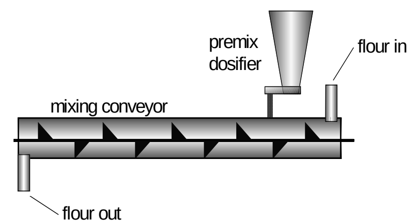


Pneumatic conveying of flour fortificants from the feeder to the flour collection conveyor has one key advantage. It permits the feeder to be located anywhere in the mill, allowing it to be conveniently located or added to a mill retroactively. However a pneumatic conveying system requires the investment of additional equipment such as blowers valve and piping. The pipes used to convey the material should have a minimum number of sharp bends and twists to prevent the possibility of clumping and blocking of the pipes by the flour fortificant. The venturi tube should be checked occasionally to see if there is any build up of the premix, and cleaned if there is. Pneumatic conveying of flour does not provide much mixing of the premix into the flour, so it should lead to a mixing conveyor or sieve rather than directly into a flour holding bin.

Gravity feed system

The gravity feed system requires the feeders to be located on top or above the flour collection conveyor. The material falls directly from the discharge end of the feeder into the flour stream. New mills are ideally suited to the installation of gravity feed locations, whereas older mills may be configured in a way that prevents the installation of this type of system. The equipment requirements of a gravity system are less than those for pneumatic conveying systems.

Gravity Feed Method of Premix Delivery



The feeder can sit either directly on top of a flour collection conveyor or on a platform or on the floor directly above it with the discharge spout feeding into a mostly vertically tube dropping down to the collection conveyor. The premix must enter the flour stream at least 3 meters from discharge end of the flour collection conveyor to ensure adequate blending. The collection conveyor may be well above the floor. This requires construction of a platform from which the hopper can be filled. If necessary because of space restrictions, feeders can be installed on the floor above and the premix dropped to the conveyor through a shoot.

Alternatively, the feeder can be connected to the flour discharge spout of a plansifter. The sifter flour spout must have a significant amount of flour entering into the flour collection conveyor on the floor below. The sifter flour spout must enter the flour stream at least 3 meters from discharge end of the flour collection conveyor to ensure adequate blending.

The 3 meter distance requirement may be lifted in some mills where the flour is pneumatically blown from the collection conveyor to either a packing bin or flour storage bin, or where the flour collection conveyor discharges into another conveyor and the total length of the mixing distance after the premix is added is at least 3 meters.

Electrical interlock system

It is highly recommended that an electrical interlock system be installed between the feeder motor and the motor driving the flour collection conveyor. An interlock causes the feeder to stop if the flour collection conveyor stops. This will prevent the inadvertent over-treatment of the flour, if there is a mechanical breakdown in the mill. In pneumatic delivery systems an interlock should be made between the feeder and the blower to insure that the feeder cannot be turned on without the blower operating. This will prevent buildup of the premix in the pneumatic lines followed by over-treatment of flour once the blower is turned on. An alternative approach is to have an automatic shut off switch on the feeder that is hooked up to a flour flow indicator or a pressure indicator in a pneumatic system.

Continuous monitoring system

Most mills are designed to have a constant flow of flour, but "*chokes*" or blockages may interrupt flow. Over-treatment of a portion of flour can occur if the flour stops or slows down but the feeders keep operating at the same rate. This can be corrected automatically by having the speed of the feeder slaved to the flour flow through the use of an electronic controller. This requires equipment that continuously monitors the rate of flour flow and generates an electronic signal proportional to that flow rate. This signal is then used to control the motor speed of the feeder.

Additional mixing conveyor

Most mills do not have automated control capabilities and must adjust the feeders manually as described in the following section. Some of the small, older mills do not have a point in the system with a known, constant flow of flour, which makes continuous fortification difficult to achieve. One solution to this is to install a mixing conveyor running from a flour holding bin to the packout bin. The feeder would drop or blow the premix into the start of the special conveyor.

Instructions for Fortification Operations

The following step-by-step procedures should be followed in setting up and calibrating feeders.

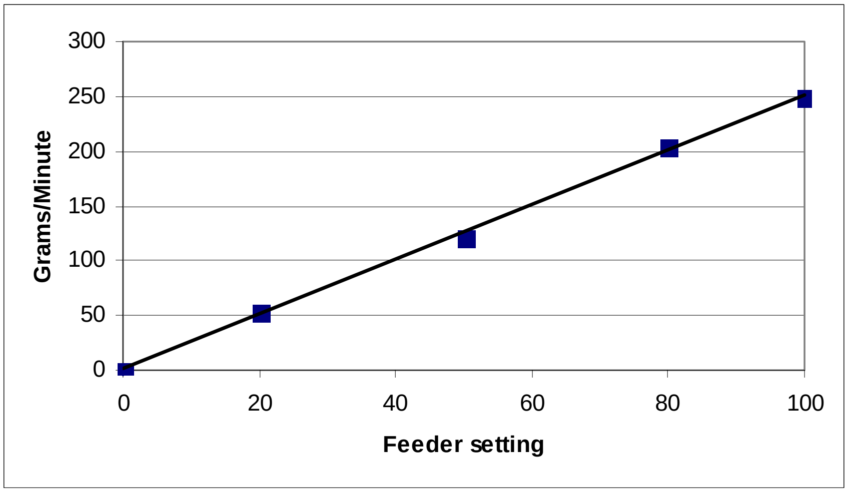
Feeder set up

1. Locate and install the feeder based on optimal mill equipment configuration. Note position requirement on flour collection conveyors for gravity feeder set up. Insure there is adequate mixing of flour after point of premix addition.
2. Install voltage regulator if there is a large variation in electrical voltage (over ± 20%).
3. Install the electrical interlock system either directly to flour collection conveyor motor or mill control panel.
4. Check to see that light indicating low premix level in hopper is operating.

Feeder calibration

1. Fill hopper about half full with premix to be added
2. Set feeder to maximum discharge.
3. Run feeder for 2 minutes.
4. Weigh premix.
5. Calculate maximum discharge per minute.
6. Optional: repeat above with different speed or percent settings.
7. Using graph paper or spreadsheet program, prepare a chart (see following figure for example) showing feed discharge rate per minute for different feeder speed settings from 0 to 100% of maximum discharge.

Figure 4 Example of feeder calibration curve



Flour Production Rate Determination

Use following procedure to determine actual flour production (not rated capacity) in kg or tons per minute.

1. While the mill is running count number of bags packed per 20 minutes or use on-line flour scale (if one is installed).
2. Calculate the flour production rate using the following formula. This is the actual production rate per minute.

Weight of bags in kg x number of bags per 20 minutes divided by 20 = kg flour per minute.

Premix Feed Rate Calibration

Use this is to determine the feed rate of premix in grams per minute required to fortify the flour at the recommended level.

1. Determine the recommended addition rate of premix (from supplier specifications)
2. Calculate premix feed rate per minute using formula:

Premix Weight in grams per tonne divided by 1000 = grams per kg flour

Premix Weight per kg multiplied by production rate per minute in kg = Premix Weight required per minute.

3. Adjust the control/dial on feeder to deliver weight of premix per minute.

Fortification Operation

1. Start mill up and let run for at least 15 minutes to reach normal production rate.
2. Start feeder at required setting.
3. Ensure feeder hopper contains premix.
4. Conduct check weighing (see following procedure) at start of mill production run and every 2 hours to verify correct addition. Adjust if addition rate is above or below target. Recheck addition rate using check weigh procedure.
5. Visually check feeder during production run to ensure sufficient premix in hopper and that feeder is operating properly.
6. At end of production run turn off feeder before shutting down mill.
7. Maintain production records showing:
    a. Lot number of premix used
    b. Check weights

c. Time of check weighing
d. Times of production run start and finish

Quality Control in Mill

Quality assurance and quality control for fortification is covered in Section 10. The following steps should be taken to ensure on-line quality control of the fortification process:

1. *Check Weighing:* The weight of premix discharged over a specific time (1 or 2 minutes) is measured and compared to the target weight for the premix.
2. *Iron Spot Test:* The iron spot test is carried out on the flour every 4 hours and at the start and end of each production run. (see Section 10 for method)
3. *Production Records:* Production records of the amount of fortified flour produced should be maintained.
4. *Inventory Records:* Premix inventory and usage records need to be kept.
5. *Premix Usage Reconciliation:* Reconciliation of actual usage versus target need to be kept.

Troubleshooting

Following are some of the problems that can occur with fortification and how to prevent them.

Compaction and erratic flow of premix

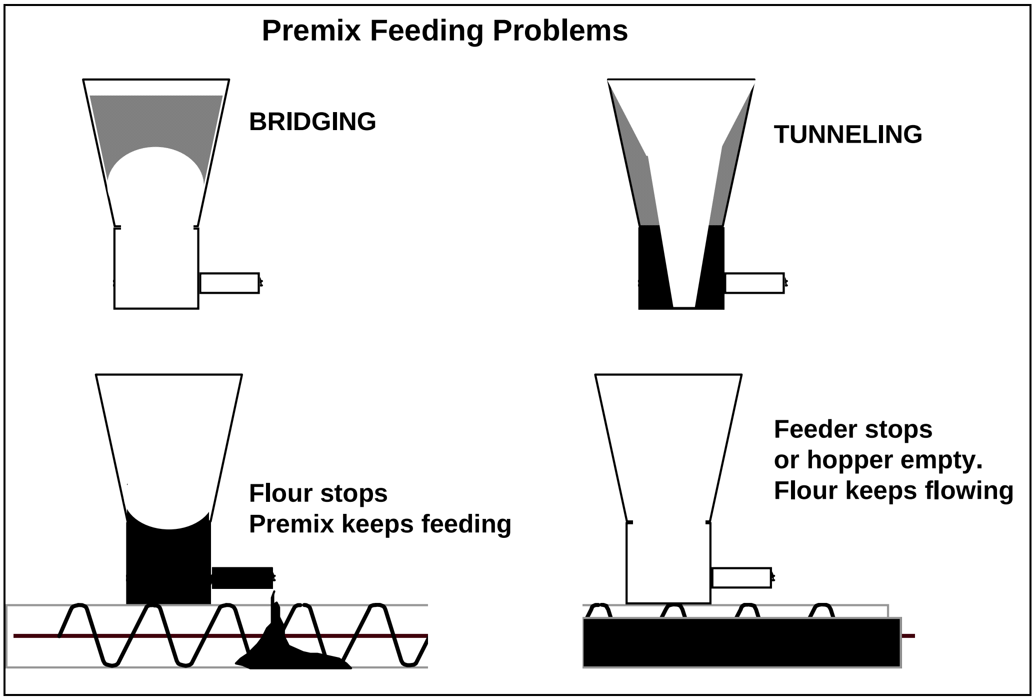
Compaction and stickiness contribute to the material balling-up, bridging or tunneling in the feeder. This can result in variability in the feed rate since a loose material will feed slower in weight per unit time than the compacted material.

Actions:

1. Have premix supplier change type or levels of free-flow agents used or type of diluent used.
2. Install mechanical agitation in premix feeder hoppers.
3. Dilute premix with starch or semolina.

Feeder Problems.

The following diagram shows the potential problems that can be encountered with premix feeders:



Actions:

1. Make frequent visual inspections of feeders.
2. Use a feeder with a different design or mechanical action.
3. Install mechanical agitation device on feeder.
4. Install electrical interlock between mill and feeder controls.
5. Install low-level alarm on feeder hopper.
6. Empty feeders if left unused for any length of time.

Electrical power supply variations

Feeders and controllers must operate in a consistent, uniform manner to ensure adequate fortification of the flour as it is milled. Electrical voltage power fluctuations do occur in many mills due to national grid supply problems and local generator variability.

Actions:

1. Use voltage regulators when single voltage feeder motors have been installed.
2. Use 3 phase motors.

Magnetic separation of iron

Elemental iron powders are attracted to magnets while iron salts (ferrous sulphate and ferrous fumarate) are unaffected. Magnets - made from iron, ceramic and rare earth elements - are used to remove tramp iron from flour. Only rare earth magnets are strong enough to actually pull elemental iron powders out of flour as it passes by the magnet. The magnet quickly becomes saturated with the iron powder and a state of equilibrium is reached causing no additional iron to be removed. Extensive experience and one study have shown it is possible to have magnets in the flour line with no removal or segregation of iron while still retaining the ability of the magnets to remove tramp iron. Tramp iron is thousands of times larger than the added iron powder and will be much more strongly attracted to the magnet. However, if a problem is suspected, first verify this by checking the surface of magnets to see if they hold large amounts of iron powder. If the magnet has a manual cleaning system, as do most of the new tube magnets, check the amount of iron powder that is removed on cleaning. If there is a problem, consider the following actions.

Actions:

1. Install magnets so that the flour stream acts as a continuous cleaning mechanism as it passes over the magnet.
2. If the iron powder bridges between the magnet tubes, use a magnet system with a larger distance separating the tubes.
3. Place magnets prior to the addition of the premix and rely on sieves to remove tramp iron after that point.
4. Use a non-magnetic iron source.

Segregation and loss of micronutrients

Some of the added vitamins and minerals can be destroyed, segregated or removed from the flour or meal due to pneumatic suction, sieving and other milling processes. This can show up as low values of vulnerable micronutrients (vitamin A, riboflavin) in the flour.

Actions:

1. Confirm that the premix formulation is correct for the type of flour being fortified.
2. Check the dust collector to see if it contains abnormally high levels of iron or vitamins. Riboflavin can give it obvious yellow color, but other micronutrients will have to be analytically tested. If this is the case, alter or remove the pneumatic suction applied to the flour after the point of addition, or fortify the flour at a later stage in the milling process.
3. Make sure the flour is not exposed to high heat (>40°C) or light during pneumatic conveying.
4. Do not run fortified products through purifiers.

Section 9 FORTIFICATION METHODS – SMALL SCALE FORTIFICATION

This section covers the methods for fortification of flour produced at small, neighborhood mills – typically those with a capacity of less than one ton per hour. For a comprehensive examination of small scale fortification please refer to the Small Scale Fortification Manual to be published by The Micronutrient Initiative, Ottawa, Canada.

Definition of Small Scale Fortification

In less developed areas of Africa, Asia, South America and Eastern Europe, only 20-50% of the population consumes pre-processed, packaged foods. A large segment of the population relies on the cultivation of various cereals that are then milled at home or locally. Milling on this small scale is usually done in 3 to 20 kg batches of grain, or the amount required by a household for 1 to 14 days. The very poor may resort to hand pounding of the grain to a coarse meal.

Small Scale Fortification (SSF) is defined as the addition of micronutrients to the milled cereal products in mills with a capacity of less than 1 metric ton per hour using a diluted premix or "preblend" format, with or without special dosing or blending equipment.

Household Fortification is the addition of micronutrients, in a sachet (e.g. *Sprinkles*) or crushable tablet form, to a food, usually cereal based, just prior to eating. This approach is usually directed at young children while SSF will benefit everyone in the household or extended family.

The Importance of Small Scale Fortification

Small scale fortification is important to be considered for the following reasons:

Lack of access to fortified flours from large processors

Large sections of the populations in many regions of the world do not have access to wheat and maize flours processed in large milling units. The following table illustrates the proportion of cereal flours produced at the small scale level in some countries and regions of the world.

Table 9.1 Estimated Proportion of Population in Various Countries Served by Small Scale Mills

Country/Region	**Small Mill Production Share**	**Population served by small mills**
India	80%	800 million
Pakistan	55%	91 million
Morocco	35%	10 million
Sub Saharan Africa	40-80%	225-270 million

Subsistence agricultural practices

The majority of the population is unable to grow or purchase sufficient quantities of nutritious food. Consequently the rural and peri-urban population suffers the effects of micronutrient malnutrition and subsequent health problems. Those living in rural areas, grow grains like maize, sorghum and millet on small plots of land, harvest it and dry it in the sun before grinding in a local hammer or disc mill. Maize meal is cooked into a porridge, and often eaten without meat or vegetables. In peri-urban areas, this segment of the population buys grain in the market and takes it to a local mill for processing.

Lack of dietary diversification

The food prepared from the products of small scale milling are very often the only food consumed. The continued consumption of only one staple food like wheat flour or maize meal results in micronutrient malnutrition as the unfortified staple food contains inadequate amounts of micronutrients.

Small Scale Milling compared to Large Scale Milling

The following table compares small scale fortification and large scale fortification. These factors need to be taken into consideration when planning and implementing small scale fortification.

Table 9.2 Comparison of Small and Large Scale Milling

Parameter	Large scale	Small Scale
1. Milling capacity (wheat or maize)	> 24 tons per day	< 24 tons per day or 1 ton per hour
2. Flour or meal production	> 50 tons per day	< 5 tons per day
3. Process	Usually continuous	Usually batch
4. Investment in equipment	Can be high	Low or none
5. Process control	Yes; industrial level	None or minimal; artisan or cottage industry level
6. Cost per unit of fortified product	Minimal, built into price of flour	Can be significant percentage of milling cost; recovery by toll
7. Quality assurance	Consistent, established but manageable practices	Inconsistent, not well established; requires constant attention
8. Human resources	Minimal but skilled	Moderate but largely unskilled
9. Consumer acceptance	High, because it is invisible	Uncertain because of high visibility and repeated consumer buying decision
10. Premix supply, packaging	Not of concern	Of concern: distribution and availability can be uncertain
11. Sustainability	High probability	Uncertain, depends on too many factors
12. Premix cost, shelf life	Not significant	Can be significant issues
13. Regulatory compliance	Easier to enforce	Difficult to enforce
14. Risk of contamination	Can be low	Can be high
15. Training	Minimal	Can be high
16. Promotion, IEC resources	Minimal	Usually significant
17. Feed material	Purchased by miller	Purchased or brought by customer
18. Product	May be segregated	May be sifted at mill or into fractions at home; may be sun-dried afterwards

Grains and Processing Steps

The following table shows the preparation, processing, post milling processing and quantities used in small scale milling practices. In all cases the extraction is 100%, meaning whole grain flour is produced.

Table 9.3 Small Scale Milling Practices

Grain	***Preparation***	***Process***	***Post milling Processing***	***Typical Quantity***
Maize	Dry clean De-germ, wet	Hammer Mill Stone Mill Plate Mill Hammer Mill Stone Mill Plate Mill	Converted into porridge Dried in sun before cooking	2.5 – 20 kg 2.5 – 5 kg
Wheat	Dry Clean	Hammer Mill Stone Mill Plate Mill	Converted into bread	2.5 – 20 kg
Millet	Decortication Wet	Hammer Mill Stone Mill Plate Mill	Sun Dried occasionally Converted into Porridge	2.5 –10 kg
Sorghum	Decortication Wet	Hammer Mill Stone Mill Plate Mill	Sun Dried occasionally Converted into porridge	2.5 – 10 kg
Beans	Dry Clean	Hammer Mill Stone Mill Plate Mill	Blended with other staples and cooked	2.5 – 5kg
Nuts	Dry clean	Plate mill	Separated into paste and oil	4 - 8 kg

Notes:
Grain: In some countries the raw materials may be blends of grains and legumes
Preparation: As all the materials are raw agricultural commodities they are usually cleaned by hand to remove foreign material. In the case of maize, millet and sorghum a wet decortication process may be carried out prior to milling.
Process/Extraction: The process describes the type of mill used.
Post Milling Processing: In some cases the milled grains may be damp and will be stored before processing into the final food. In this case the flour is dried in the sun.
Quantity: This is the typical range of the weight of grain brought to the village mill for processing. This amount may represent the food for several meals or the meal for the day of milling.

Description of Small Milling Practices

In a typical rural setting in Africa and parts of Asia, the customer is either woman or a child. The grain is brought in a sack, the amount depending on what one can carry, afford to mill, or is necessary for one's family for 1 to 14 days. Grain is volumetrically measured out using a "tin", usually a 20-25 litre metal container, typically holding about 17 kg of maize grain. The miller, almost always a male, discharges the contents of each tin into the mill's hopper tray, where he screens for pebbles (that could damage the mill) with his fingers, while the mill is operating. Meanwhile the customer ties her sack to the end of the discharge pipe to collect the meal. If the customer has brought more than one tin of grain, the miller fills the hopper as needed. The mill is run continuously as much as possible. The customer collects her sack of meal, pays the miller what he demands, the fee per tin being displayed on a chart. At no time does the customer query the miller about the milling fee he charges her, or the amount of meal she has collected, i.e., there is no measurement of the amount of meal collected in the sack.

If the customer wants a very white maize meal, free of bran, the grain is doused with water to wet the surface and fed to the dehuller, which has a typical extraction rate of about 60-65%. In some areas, the grain may be soaked for a few hours or even a day. The residue, about 35-40% of the weight of the grain, is often left on the mill floor, to be collected later by the miller and sold as chicken or animal feed. The miller does not share the resulting revenue with the customer. The dehulled product may be sifted on-site, and then dried in the sun for a few hours, before milling to flour, which may be sun-dried if it is damp.

In essence, the customer is very compliant, very trusting of the miller who is fully occupied with running the mill, feeding the grain, and collecting and recording the revenue. There is often a queue and customers can wait up to a couple of hours or longer. Conditions in the mill can be quite dusty, cluttered and bustling, with varying degrees of cleanliness. It is under these conditions that introduction of any SSF technologies for adding premix to the meal needs to be considered, on technical, economic and sociological grounds.

Fortification Methods

Stages of small scale fortification

(a) Preparing a diluted premix (preblend), and packaging it in bulk or pre-weighed sachets.
(b) Adding the appropriate amount of premix to the grain while it is being milled or adding premix to the product meal in a blender, after the grain is milled.

Challenges of small scale fortification

The challenge of SSF is to do it in a manner that is safe, timely and acceptable to the target population, at a cost marginally above that of milling. Moreover, the target population needs to demonstrate that they have the technical, human, logistical and economic resources to sustain SSF over the long term, independent of foreign donors or government subsidies. Local governments have the added responsibility of passing laws permitting food fortification and enforcing them through proper monitoring and testing.

Small scale fortification process

Cereal flours and foods can be fortified on a small scale by adding a micronutrient premix to the grain during milling or to the flour/meal after milling. This is achieved by using;

(a) A *calibrated scoop/spoon* to measure an amount proportional to the weight of grain, or
(b) A *sachet*, containing an amount appropriate for a set weight of grain, or
(c) A *small dosifier* that dispenses an amount proportional to the weight of grain flow

Fortification during milling - The first two dosing methods could be used whether the grain is milled by hand pounding or in a hammer or disc mill, whereas a dosifier may be used only in a hammer or disc mill. The milling action can adequately blend the premix and the cereal flour/meal. Where the milling operation permits, *direct addition* of the premix during milling can be an option.

Fortification after milling - When it is necessary to blend the premix with the maize meal after the milling process is complete, batch type blending may be necessary. This can be done using any one of: a large spoon/paddle, a mechanical blender attached to the mill drive, a stand-alone mechanical blender operated by hand, or a mixing pail. The following types of blenders have been used in Zambia and Zimbabwe:

1) A hand operated blender (HOB) which has a helical ribbon on a shaft that is rotated with a handle for mixing, with an outlet to discharge product. A stainless steel HOB originally designed in India for salt iodization programs was used for SSF of maize meal in Zambia. A less expensive cast iron model of the HOB was designed in Zimbabwe and is being used in Tanzania.

2) The second option is a rotating barrel with inside mixing flutes. The barrel could be rotated mechanically or by hand rolling across the floor, to mix in the fortification premix. While this method seems rudimentary, it is effective for small batches and has low labor costs. A 25 litre plastic pail called ODJOB, containing an integral baffle was identified to meet this purpose. The ODJOB is commonly used in North American homes for cement mixing by rolling the pail on the ground. To improve hygiene, efficiency, and human workload, the ODJOB can be mounted on a stand.

Premix and Preblend Considerations

The concentrated premixes made for large scale fortification can be used in small scale fortification once they are properly diluted to a preblend. But consideration to the following issues must be addressed when using these premixes in small scale fortification:

Fortification preblend production

The premixes used in large scale mills are too concentrated to be used at the small mill level. The concentrated premix must be diluted to a *preblend*, typically using the cereal being fortified as the diluent. The dilution factor will be determined by the weight of cereals typically processed for each customer at the small mill. This can range from 2 to 20 kg from one country to another and the traditional practices of the customer.

Once the dilution rate has been established, a centralized or district centre needs to be established to dilute and repackage premix. In Malawi a concentrated premix is diluted to 0.5% concentration using maize flour. The final dilute premix is then added to maize meal at the rate of 30 grams per kg. In Zimbabwe a premix sachet of 50 grams is added to 17kg of maize meal.

A fortification preblend has a much shorter shelf-life than the parent preblend – typically a few weeks rather than years – so it must be made in limited quantities close by to where it will be used.

Selection of iron form

Ferrous sulphate is not recommended for use in regions and countries producing high extraction flours and where weather conditions are tropical or extremely hot. Ferrous sulphate will interact with the oils that naturally occur in the grains and result in rancidity and “off” flavours.

Micronutrient interactions

Some micronutrients will react with each other in multiple fortificant premixes and in the target staple food. For example ferrous sulphate is not recommended for use in premixes containing Vitamin A because it will cause “off” flavour interactions and loss of potency. Vitamin C is not recommended for wheat flour since it is lost in baking but it can be used in some maize meal products.

Effect of further processing

In some cases the staple food that is brought to the mill may need further processing before being cooked in the home. For example, de-hulled maize is brought to the mill for milling but after the milling the maize meal is still moist. In this case the meal is spread out on mats in the sun to dry. Sun drying will effectively destroy most of added vitamin A, riboflavin and folic acid.

Monitoring and Quality Assurance for SSF

Following are monitoring and quality assurance requirements that can be applied to small scale fortification.

Equipment and preblend supplies

The milling industry or the project manager needs to be responsible for the purchase of blending equipment, weigh scales and premix supplies. This requires the project to have a good distribution system in place to be able to distribute premix during the year to remote locations.

Inspections

A third party inspection system or the project management should routinely inspect processing equipment to ensure that the blending method and system used is capable of consistent operation

Process validation of blending method

As part of the initiation of the SSF project, millers and assistants must validate the blending method used for consistency and ease of use. This would include a series of test runs (minimum of 10 runs with 3 samples from each lot of milled flour) using the same types of equipment found in the country or region using the blending method. Samples of unfortified material, fortified material and premix sample need to be analyzed quantitatively for iron content to determine blending efficiency.

Monitoring

Monitor flour ready for distribution through the use of an *iron spot test* method. This test will detect the presence of added iron to cereal flours. It can be used to compare the sample with a standard sample of a known level of added iron in the flour to give a rough index of the iron level. The iron spot test is described in Section 10 and given in Appendix I. In any program it is essential that adequate records of activities are kept. This allows for monitoring of the program and to facilitate auditing.

Quality Control at the Community Mill Level

Feeder/Dosifier calibration

In some countries such as CIS states, Moldova, Pakistan, and South Africa community mills may be large enough to use a feeder. In these cases it is appropriate to calibrate the feeder to the flour production rate. In addition the premix should be diluted because the flour production rate will be much lower than a typical commercial roller mill. A diluted premix or preblend, as used in other small scale or community based mills, is suitable.

The feeder or dosifier is normally equipped with a variable speed drive that allows for different discharge rates. The feeder should be calibrated so that at each speed setting from slowest to full speed the amount of material in grams delivered per minute can be calculated.

Preblend addition rates

The amount of preblend that can be added to the cereal flour in current SSF projects ranges from 10 to 30 grams per kilogram of cereal flour. Actual quantities of preblend added in these projects ranges from 10 grams to 200 grams depending upon the amount of cereal brought to the mill for fortification and the amount and number of micronutrients added to the flour. Current research has shown that preblend amounts added at the above rates does get uniformly dispersed into the cereal flour in community mills at the village level.

Process controls

The following process controls are recommended for use at the community mill level:

Check weighing

At the community mill the use of scales and volumetric measures for cereals and preblend is required to assure that the right amount of preblend is added to the cereal flour. In some cases the preblend is pre-weighed and packaged in sachets so that one sachet is added to a unit of cereal. In the case of cereals these can be either weighed using simple spring scales or a standard tin of known volume. When volumetric methods are followed it is recommended that the weight of grain in a known volume is measured and recorded. When mixed grains and legumes are brought for milling the use of a volumetric measure is not recommended as each component has a different bulk density and the proportion of cereals and legumes is not always the same.

Iron spot test

The iron spot test (given in Appendix I) is an essential tool to confirm the presence of *added i*ron in cereal flours. The test is relatively simple to use and can be used to test and compare actual production against a standard sample. The iron spot test can be used by millers, project team members and government inspectors as part of the quality control and quality assurance programme.

Sampling schedule

A sampling and inspection schedule needs to be in place to assure that fortification is being carried out consistently. The iron spot test can be carried out daily and inspections by project teams can be set up according to existing supervisor duties.

Section 10 QUALITY ASSURANCE AND CONTROL

This section provides information and guidelines on mill quality assurance (QA) and quality control (QC) related to the fortification of wheat flour and maize meal. It is not meant to be a comprehensive treatise on the subject, which has been well covered in other publications (FAO, 1995) (Nalubola and Nestel 2000; Nestel, Nalubola et al. 2002). Rather, it gives only the key aspects of a QA/QC programs related to cereal fortification, so as to help mills just starting to fortify flour, premix manufacturers and those applying for grants in cereal fortification better understand the nature and requirements of such programs.

There is a tendency in other materials to make this subject highly complex and involved, giving the mistaken impression that considerable cost and time will be needed for a QA/QC program on fortification. The goal here is provide a simplified version of the basic or minimal actions needed for a mill to assure conformance to fortification specifications and regulations. This, fortunately, does not require a large investment in equipment and personnel. The time and effort a mill need spend to effectively control fortification can be quite small.

While flour fortification does not have the same stringent requirements as the manufacture of pharmaceuticals or infant foods, it does require additional QA/QC measures over what the mill is accustomed to with regular, non fortified flour. Millers who fortify flour need to accept and practice these additional QA/QC requirements, recognizing that they will come under closer scrutiny by both their customers and government regulator to make sure that the product is properly fortified.

There are other aspects of fortification QC and testing that are covered in other sections of this manual. Appendix I provides analytical methods that can be used to test for micronutrients in flour. Section 11 on Regulations and Enforcement discusses government testing, monitoring and auditing of mills to see if they are properly fortifying. Special issues related to QA/QC in small mills are discussed in Section 9 while testing related to surveillance of nutrient intakes and effectiveness of fortification programs is covered in Section 14.

Definition of terms and abbreviations

QA/QC has a rich lexicon of special terms and abbreviations. Some of these are defined in the Appendix, but a few deserve special attention.

quality: The composite of material attributes, including performance features and characteristics of a production or service, needed to satisfy a customer's given need.

quality assurance (QA): A planned and systematic pattern of all actions necessary to provide confidence that adequate technical requirements are established, that products and services conform to established technical requirements, and that satisfactory performance is achieved.

quality control (QC): The overall system of technical activities whose purpose is to measure and control the quality of a product or service so that it adheres to specifications that meet the needs of its users.

total quality program: A program which is developed, planned, and managed to carry out, cost-effectively, all efforts to effect the quality of material and services from concept through validation, full-scale development, production, deployment, and disposal.

quality audit:. A systematic examination of the acts and decisions with respects to quality in order to independently verify or evaluate the operational requirements of the quality program or the conformance to specifications of a product .

total quality systems audit (TQSA): A comprehensive audit designed to ensure the quality of products provided in U.S. domestic and export food assistance programs.

Indicator nutrient: The use of a single nutrient in flour as an index of all the micronutrients added by a fortification premix. Given that the indicator nutrient is within specification, it follows that all the other micronutrients should be as well providing that the fortification premix is correct.

MSDS: Material Safety Data Sheets.

GMPs: A recognized set of *Good Manufacturing Practices*.
SOP: A S*tandard Operating Procedure* adopted for repetitive use when performing a specific action.
ISO 9000: An internationally recognized set of standards for qualification of global quality assurance and quality control standards. Adherence is accomplished through an application process for ISO 9000 certification in company standards for inspecting production processes, updating records, maintaining equipment, training employees and handling customer relations.
HACCP stands for *Hazard Analysis and Critical Control Point*, a method used to insure food safety by identifying potentially unsafe links in the food processing chain.
CV or COV: *Coefficient of Variation* is a statistical term used to describe the amount of variation within a set of measurements for a particular test. It is calculated by taking the variation of a set of data as a percentage of the mean.
control chart: A visual depiction of analytical results over time.
spot or grab samples: A sample taken at a single point in the process.

In simple terms, QA is the plan on how quality is to be continually achieved and QC is the testing or implementation of that plan.

Objectives of QA/QC

There are a number of reasons for having a QA/QC program on flour fortification:

1. To conform to government regulations or customer specifications.
2. To control costs. That is, not add more premix than needed to meet requirements.
3. To assure product safety.
4. To satisfy customer expectations.
5. To avoid regulatory action or bad publicity for non conformance.

To meet these objectives requires that the correct fortification premix is being uniformly added to the correct flour or meal in adequate amounts to meet the minimum standards and not in excessive amounts as to cause safety, product quality or cost problems. These objectives can be accomplished with standard production and inventory controls along with good manufacturing practices.

QA/QC in different size mills

The type and extent of QA/QC that a mill exerts over fortification will differ greatly depending on the size of the mill. Table 1.1 in the Introduction lists three categories of mills based on size: large (>3 MT/hr), medium (1-3 MT/hr) and small (<1 MT/hr).

Very large mills (> 8 MT/hr) and many large mills (8 to 3 MT/hr) have an active QA/QC program that includes the following:

- A QC manager or employee with QC responsibilities.
- A laboratory or designated space for testing wheat and flour.
- Flour and wheat testing equipment. These tests typically include protein or gluten, moisture or water content, ash content and/or color, amylase enzyme activity (falling number) and dough properties. Some labs also bake bread or prepare other baked products from the flour or meal as a part of their overall quality program.
- A written quality assurance plan that includes sampling procedures and a QC testing schedule.

The larger the mill, the more likely it is to have these capabilities. However, the smaller the mill, the less able it is to afford them or to have a need for them. For large mills the additional QA/QC required by fortification will fit in nicely to their current program, but for medium and small mills, it may necessitate adopting a new and unfamiliar set of procedures. In either case, mill personnel will have to be trained in this component of fortification since it involves unfamiliar procedures and tests.

Fortification premix control

Fortification is always accomplished at the flour mill by adding a *premix* of the vitamins and minerals required under the standards. The first step in quality assurance of flour fortification is to have a well-made premix. The formulation and dilution of the premix is normally not covered by regulations[16] but is agreed to by the mill and premix manufacturer so as to provide the most practical product that will meet the standards for each mill.

All reputable premix manufacturers provide Certificates of Analysis (COAs) for all micronutrients on each lot of premix (a lot or batch being the amount of premix that is blended together at one time). COAs should be kept filed at each mill and be made available to government or customer inspection if asked. The analytical methods used to test for the active ingredients in the premix should be documented. These methods are not those used to test for the levels of micronutrients in fortified flour. Distinct methods are required because of large differences in nutrient concentrations and presence or absence of interfering substances.

The mill should keep good inventory control of the fortification premix. This includes records on:

- Quantities, reception dates and lot numbers of all premix received in the plant.
- Regular (e.g. monthly) inventories of premix on hand.
- Quantity of fortified flour produced during the same period.

Premix suppliers should provide technical information or fact sheets on each type of premix. These sheets should include information on premix composition, application rate, packaging, handling and storage. Many countries will also want an MSDS on the premix. Current information and MSDS sheets from all premix suppliers used should be on file at the mill.

Each container of premix should have a label indicating the type of product (code number or name), lot number, kosher or halal certification if required and application rate.

Premix manufactures should inform the mills what the acceptable shelf life is of their product. Most fortification premixes are good for a couple years when stored in sealed, unopened containers. Premixes containing vitamin A, however, have a shorter shelf life. If there is a question whether a premix is still good after extended storage, a mill should contact the manufacturer providing them the lot number and storage conditions. Mills should not use premix that has sat around in an opened container for months, or exposed to fire or water, nor should they expect the supplier to take back or give credit for such materials. Once a premix has been received in good condition at the mill, the miller is responsible for storing it. A *first in, first out* (FIFO) inventory control should be employed.

All these records should be available to inspectors. One of the simplest ways to see whether a mill has been fortifying flour is to compare the quantities of premix used to the amount of fortified flour produced. This is not a surefire method, since mills can keep false records, but it has proven a very effective means of ensuring compliance when combined with other checks discussed below.

Feeder calibration and maintenance

Most flour mills fortify flour continuously using an ingredient feeder or dosifier to meter the premix into the flour as it flows through the mill. The alternative is to use a batch mixing system where a set quantity of the premix is weighed out and blended into a set quantity of flour. In continuous systems the fortification feeder must be adjusted to add the correct amount of premix based on the *flour flow rate* and the addition rate specified for that premix. Mills have to know, or determine, the flour flow rate, typically in Metric Tons per hour, kg/min or some similar unit, in order to make this calculation.

The premix addition rate should be checked daily by running a "check feed rate" on the feeder, and changed according to any change in the flour flow rate. This is done simply by putting a plate or cup under the discharge spout of the feeder for 60 seconds and weighting the amount of premix collected to an accuracy of 0.1 grams. A standard lab balance or electronic scale can be employed for this purpose.

[16] An exception to this is South Africa, where regulations cover the premix as well.

Mill QA/QC

The standard procedures utilized by large flour mills to insure that flour is properly fortified includes:

- Use of a quality feeder whose delivery of premix can be tied into the flow rate of the flour and which will stop when the flour flow stops.
- Regular checking of feed rates on feeder. Once per 8 hr. shift is recommended.
- Regular iron spot tests on flour. Once per 8 hr. shift is recommended
- Checking of premix usage against production of flour that should have been fortified. This typically should be done on a monthly basis.
- Optional quantitative testing of iron on a weekly or monthly basis. An outside lab is recommended.
- Optional quantitative testing of all fortification components in a composite sample on a monthly or quarterly basis. This must be done by an outside lab. Some premix companies offer this service to their customers at no charge.

Record keeping

Good record keeping is the key to mill QA/QC in flour fortification. Each mill should have a written plan of what records they want kept, how the data is to be entered, who should collect them and where they are to be kept. Records of all of the above activities should be kept by the mill and made available to government or flour customer inspection or audits when requested.

Analytical testing

Flour samples should be tested to verify that it has been properly fortified. Three types of testing are possible:

- **Qualitative tests** show simply the presence or absence of an added micronutrient. An example is the black light (uv light) test for riboflavin. This type of test is used to see if a flour has been fortified or not.
- **Semiquantitative tests** give a rough indication of the level of an added nutrient. Examples are the iron spot test and a color test recently developed for vitamin A in flour. This type of test tells whether the level added is low, normal or high.
- **Quantitative tests** give an actual value for the level of micronutrient in the sample. Unlike the other two types, which respond to added micronutrients, quantitative tests generally measure total content or both the natural and added levels, but some test methods have been developed to show only the added.

Sampling

The way flour samples are obtained and handled is an important component of the analytical procedure, particularly when submitted for quantitative testing, and should be well documented in the QA plan. The best place to sample is at or directly prior to packout, since this represents the final mill product. Composite samples are preferred to spot samples for quantitative testing, but spot samples are acceptable for the iron spot test.

A good composite samples would have 7 spot samples taken over an 8 hour period. One example is to place 50 gram spot samples into an opaque container holding at least 700 grams of flour. When all 7 samples are collected the composite is mixed by inverting and shaking the container. A single sample is then taken for testing. It is always good practice to retain all or part of the original sample in case something goes wrong with the other sample. A minimum composite sample consists of 3 spot samples taken over a hour period.

Samples of fortified flour or meal must not be exposed to direct sunlight or strong indoor lighting. They should be well labeled with the name of the mill, date, type of flour and whether or not they are fortified (unfortified samples are often collected to establish base line natural levels). If regular samples are to be collected, it is also a good idea to number them consecutively. This sample data should be entered into a mill sample record book. There is no point in taking and testing samples unless they are well documented.

When a mill first starts fortification they may want to have a number of spot samples of fortified protect tested along with some unfortified samples to gain a better picture of their capacity to fortify correctly along with the level and variation they can expect. For example, they may take a spot sample every 6 hours over a 3 day period and have them tested for iron as the indicator nutrient. They may also want to take a couple composite samples and have those tested for all the added micronutrients.

But once fortification has commenced, it is not practical to run that many samples on a regular basis. Medium and small size mills may wish to send in a composite sample a couple times a year, large mills may want to do it every month or quarter, while very large mills may wish to do that more often. Such samples are generally tested for all added micronutrients but testing one or two indicator nutrients may be sufficient.

When an official inspector is collecting samples it is recommenced that the sample taken be separated into three separate samples, one for the test; one for the mill and a third one in case the other two do not agree to their results.

Iron spot test

The iron spot test (AACC method 40-40, Iron- Qualitative Method) (see Appendix I) is universally used by millers to check whether flour has been properly fortified. This is a simple, inexpensive, semiquantitative procedure that should be run on fortified flour on a regular basis, typically every 2 or 4 hours for a large mill. It should be run at least once every eight hour shift at a minimum for flour sampled during production.. It may also be run on flour sampled in the warehouse to verify it has been properly labeled as being fortified.

The test will indicate whether the flour is under-fortified or over-fortified to a sufficient degree for the mill to take corrective action. The iron spot test can also indicate whether the iron being added is reduced, elemental iron, or a salt (ferrous sulfate or ferrous fumarate), in case the mill is adding both types. While this test is only for iron, it can be assumed that if the iron is correct, the other added micronutrients (i.e. folic acid) will be correct as well since they are added as a single premix.

Quantitative testing

While much can be accomplished through simple record keeping, feed rate checks and iron spot tests, there are times when it will be useful if not necessary to do quantitative testing of the micronutrients in flour. There are quantitative analytical procedures available for all the micronutrients that might be added to flour, many of which are provided in Appendix I. These differ greatly in complexity, analytical error (CV), type of equipment and skill needed to run them, as well as cost.

It is strongly recommended that quantitative tests on flour not be run by the flour mill. Rather they should be run by an outside laboratory that is familiar with the methodology and can run the test on a regular basis. The reasons for this are:

- The cost of the equipment and trained personnel needed to run many of these tests are beyond the resources of most milling companies.
- These tests need to be run on a regular basis if accurate results are to be obtained. Mills would only run these on an occasional basis and would never become very skilled in them.
- Mills would not save money by running these tests themselves over sending them out to an outside lab, particularly if they can have it done at no charge by their premix supplier.
- Flour mills in countries that have been fortifying flour for many decades, such as those in the United States and Canada, do not run these tests themselves, which shows that internal testing is not necessary for a successful QA/QC program.
- Quantitative results are normally used to show to a customer or government inspector that a product has been correctly fortified. Results from an outside laboratory have greater credibility than those produced internally.
- Suitable outside testing facilities are generally available for most countries.

There are some rare instances where a mill still believes they need a quantitative testing capacity, despite all the above reasons not to. In that case the spectrophotmetric test (AACC 40-41B) for iron, given in

Appendix I, should be the first method considered. This is a fairly inexpensive method and iron is the most likely nutrient to be used as an *indicator* of adequate fortification in government control. This method requires an ashing oven (generally available in most large mills) and a spectrophotometer or colorimeter. There are a couple of different methods depending on the iron color reagent employed. The wavelength at which the absorption is read depends on the particular reagent employed.

The most difficult part of this procedure is the extraction of the iron from the flour ash. This is prone to contamination and requires boiling acids that must be conducted under a good laboratory exhaust hood. This is a very corrosive process best done with non metal hoods and equipment. Natural iron content in flour can vary greatly and is harder to extract than added iron. Once the extract has been prepared it is a fairly simple procedure to read the iron content with a spectrophotometer.

Since folic acid is now routinely included in flour fortification, some mills may wish to test it along with iron. Others may wish to test for vitamin A when that is being added. Unfortunately, tests for both of these vitamins are quite difficult and are best left to outside labs.

Lab flour standards

It is recommended that the mill make and maintain a set of fortified flour standards for use in both the iron spot tests and any quantitative analyses. A separate set of standards is needed for each type of flour or meal being fortified. These should be kept in a sealed container preferably in a refrigerator and made fresh every couple months or when they are used up.

As an example, if a particular type of flour is being fortified with a premix added at 150 grams/metric ton (0.15 grams/kg), make up the following 4 samples:

1. Unfortified flour A
2. A + 0.10 grams/kg premix
3. A + 0.15 grams/kg premix
4. A + 0.20 grams/kg premix

It is recommended that these standard flours be run along with an unknown sample. Sample #3 with the correct fortification addition should be run every time a quantitative test is performed. The results on this known set of flour samples will help the mill correctly read the iron spot test, particularly during the first couple months that it is performed. In quantitative testing repeated assays on a single known sample will help verify its accuracy of the method, quantify its precision (CV) and help correct for any bias that may exist.

Utilizing nutrient assay data

One of the more misunderstood and contentious aspects of flour fortification is how to interpret and utilize analytical results from quantitative tests, whether performed by the mill or by an outside laboratory. Ideally, one would like to see all the results fall just above the minimum fortification standards or within the minimum-maximum range if there is one. Obviously, that does not always happen.

Some millers even question the value of quantitative testing of micronutrient content considering their high analytical error. They feel that the variability of premix addition in a well run mill is far less than the variability or assay error of the analytical testing. However, government regulatory control and some large flour customers require quantitative testing to confirm compliance to standards. Because there is pressure on the mills to consistently show good results, some mills or laboratories may want to “fudge” or “dry lab” the micronutrient testing. One indication that this might be happening is a CV under 8%, which is not generally achievable. Having a suspect laboratory run blind samples that are known to out of specification is one way to check whether they are reporting actual assay values.

Both mills and government regulators need to understand that there will be considerable variation in the results of quantitative tests of added micronutrients, with CVs of 10 to 20% common, depending on the type of nutrient being tested. A CV higher than 20% indicates a possible problem, either in the production or analytical testing, which needs to be corrected.

Very little can be achieved by having just one or two test results on spot samples. Rather, one needs a collection of results over time, preferably on composite samples, in order to really assess how well a mill is doing in fortifying flour. A mill is doing a good job in fortification if the average of 7 or more tests is above the minimum standard and the CV is less than 16%. It may be that one or two of the results are very low or very high. It the mill can assign a cause to those occurrences, such as a flour choke or the feeder to stop working knowing to have occurred, those results can be excluded from the average.

Following are some possible situations where corrective action is indicated:

Situation	***Possible causes***	***Possible actions***
A single indicator nutrient or all of the nutrients tested are consistently 80% or less of the minimum standard or greater than 140% of minimum.	Wrong feed rate, Wrong premix, High analytical error	Increase feed rate Change premix, Check for bias on test method
One nutrient is consistently high or low but the others are okay.	Wrong premix formulation, High analytical bias on the problem nutrient.	Reformulate premix, Check accuracy of method
High variability (CV) of all nutrients but their relationship to each other stays relatively constant.	This is indicative of high process variability rather than analytical error.	Check for causes of process variability such as erratic flour flow rate, high frequency of chokes and pneumatic separation.
Low thiamin but other nutrients okay.	Thiaminase in maize meal, High pH (>8)	Use different source of maize.
Low riboflavin or folic acid but other nutrients okay.	Exposure to ultraviolet light.	Protect flour and samples from light exposure.
Low iron but other nutrients okay	Low natural iron levels, Magnetic separation, Pneumatic separation	Reformulate premix, Use non magnetic iron, Add premix at different point.
Low vitamin A but other nutrients okay.	Vitamin A separation, Poor quality vitamin A.	Reconfigure point of addition, Use premix with better quality vitamin A.

.

References

Fortification Basics, Principles of Assay Procedures., OMNI.

Anon. Food Fortification: Technology and Quality Control. Rome, Italy, FAO.

Nalubola, R. and P. Nestel (2000). Manual for Wheat Flour Fortification with Iron
Part 3, Analytical methods for monitoring wheat flour fortification with iron. Washington, DC, the MOST project.

Nestel, P., R. Nalubola, et al. (2002). Quality Assurance as Applied to Micronutrient Fortification. Washington, DC, ILSI.

AACC. Approved Methods of the American Association of Cereal Chemists. 10th ed. The Association, St. Paul, MN, 2000.

Appendix C GUIDELINES ON DESIGN AND PROCUREMENT OF PREMIXES FOR THE FORTIFICATION OF MILLED CEREALS

This section provides practical advise on the type and source of fortification premix to add to milled cereal staples (primarily wheat flour and maize meal) in different countries or regions. It is arranged in a series of steps that depend on a country's food laws and regulations. Additional information on this topic is provided the Fortification Manual, particularly section 5 (Types and Levels of Micronutrients to Add).

There are three situations discussed:

1. Meeting a country's existing cereal fortification standards.
2. Voluntary flour and maize fortification.
3. Working to establish cereal fortification standards.

In Countries with Existing Fortification Standards

Currently, nearly 50 countries have cereal fortification standards or customary fortification practices (see Figure 1 below and Tables 5.2A for wheat flour and 5.2B for maize meal in Section 5 of the Fortification Manual for actual fortification standards in different countries.)

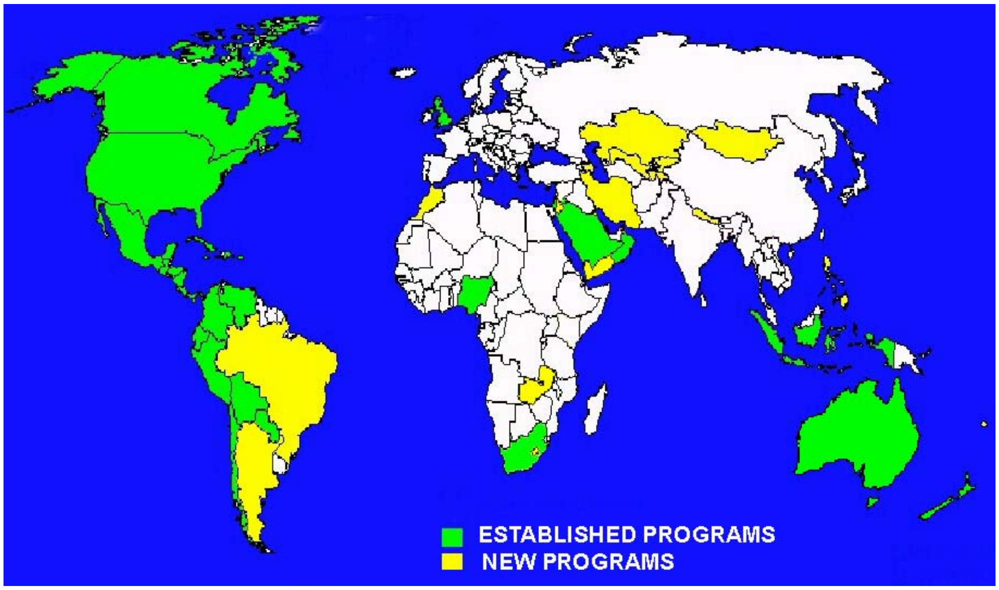


Figure 1. Countries with Fortification Programs

If a country does have fortification standards there are likely to be appropriate fortification premixes available from one of the many premix suppliers. While any of these premixes could be used, they may differ in the following aspects.

1. *The source of the micronutrients used.* For example, one supplier may use niacinamine as the source of niacin (vitamin B_3) while another may use nicotinic acid. Some countries specify the

type of micronutrients that can be added so one should check to make sure the premix is in compliance with those regulations.

2. *The levels of micronutrients added.* There is some leeway in the levels of micronutrients that can be added to meet a particular fortification standard. The premix supplier should provide evidence that they are adding sufficient but not excessive amounts of micronutrients to insure compliance with the appropriate standard. A few countries, such as South Africa, specify the amounts of micronutrients to add, but this is not the usual practice.

3. *The type of flour or meal the premix is designed to fortify*. Premixes can differ in the type and levels of micronutrients added based on the type of flour or meal it is designed to fortify. For example, lower levels of micronutrients may be needed for high ash, high extraction flour than for white refined flours. Also, the type of iron used on different types of flours can differ.

4. *The addition rate or premix dilution*. Premixes can be very concentrated to save on shipping and storage requirements, or dilute to better allow control of addition at the mill, particularly small mills. Higher addition rates require more dilution with an appropriate carrier such as starch or calcium salts. Mills should determine what addition rate they need and use a premix that meets that requirement. Alternatively, they can buy a concentrated premix and dilute it at the mill, usually with flour, to make its addition more manageable.

5. *Local availability*. Mills may prefer to procure premix from a local supplier. Most counties will not have premix manufacturing operations, so they will have to import it from a country that does. Shipping can add to the cost of the premix. Hopefully, the country will have minimal duties on imported premix. Some suppliers may maintain stocks of premix in country making it easier for mills to obtain it.

6. *Packaging.* Premix suppliers differ in how they package the premix. Containers can vary in size from 100 kg to 10 kg or less. The container itself can vary, from fiber or plastic drums to cardboard boxes or bags. The inner liners used may differ in their composition and how they are sealed.

7. *Quality, reliability and service.* While all premix suppliers promise these, differences may show up in actual practice. The supplier should provide Certificates of Analysis (COAs) for all the micronutrients present in each lot of premix. A good premix supplier should be able to advise the mill on what premix to use along with recommendations on how best to add it in the mill.

8. *C*ost. If everything else is equal, which is usually not the case, mills will want to buy from the supplier with the lowest cost. Costs can vary greatly for a variety of reasons, so mills should look around before purchasing.

There may be situations where a mill wants to add micronutrients that are not specified in the current standards. An example would be adding folic acid. Short of changing the standards, this could be possible depending on the regulation. For example, a country may require or specify fortification with certain micronutrients but allow fortification with others. Mills should consult with the government authorities prior to doing this.

In Countries without Fortification Standards

If a mill desires to fortify flour or meal in a country that does not currently have cereal fortification standards or regulations, the first step is to determine whether flour fortification is even allowed. A few countries have regulations that specifically prohibit adding most anything to flour. In that case the law must be changed or a special permit obtained before fortification can be practiced.

The next step is to find out if there are any regulations on the types and levels of vitamins and minerals that can be added to foods in general. Some countries have general regulations on food fortification that should be observed.

Assuming that the first two steps are completed and that fortification is possible, there are a couple of different approaches that can be taken in determining what type of fortification to use.

Comply to Regional Practices or Standards

For mills in countries that are located near countries with active cereal fortification programs and established standards, it may be sensible to fortify flour or meal in the same manner. This makes a lot of sense if the populations of the two countries are similar and there is active trade of cereal products between them. One big advantage in doing this is that the same premix can be used, which greatly improves its ability to be procured and helps lower the cost.

Examples of this situation include countries in Southern Africa using premixes designed to meet South African standards and countries in the South Pacific using the Indonesia premix. Countries may also wish to adopt regional standards, such as those in Central Asia, Central America and the Middle East, as shown in Table 1.

Table C1 Actual or Proposed Regional Flour Fortification Standards

(levels added)

	WHO/EMRO Middle East	*ADB/KAN Central Asia*	*ADB Proposed Southeast Asia*	*Southern Africa*
Iron (ppm)	30/60*	50**	30/60*	35**
Zinc (ppm)		22	30	15
Folic acid (ppm)	1.5	1.5	2	2
Thiamin (ppm)		2	2.5	1.94
Riboflavin (ppm)		3	4	1.78
Niacin (ppm)		10		23.68
Vitamin B_6 (ppm)				2.63
Vitamin A (IU/kg)				5951
Cost ($/MT)[17]	$0.32	$0.69	$0.74	$1.99

*30 ppm iron if ferrous sulfate, 60 ppm if elemental iron powder.
**As electrolytic reduced iron

Table C2 PL-480 Food Aid Fortification Standards

(final level)

	Wheat	*Maize*
Iron (ppm)	44	28.7
Folic acid (ppm)	1.54	1.54
Thiamin (ppm)	6.4	4.4
Riboflavin (ppm)	4.0	2.65
Niacin (ppm)	52.9	35
Calcium (ppm)	1100	1100
Vitamin A (IU/kg)	22,050	22,050
Cost ($/MT)[1,18]	$4.30	$4.10

[17] The *Costs* shown are estimated, undelivered (FOB) costs of the premix at a major U.S. port given in the cost in U.S. $ needed to fortify one metric ton of flour. These costs are subject to change and are provided here only for comparison purposes. They are for the premix only and do not include equipment, quality control or other associated costs of fortification.

[18] This cost is for the premix that does not include calcium. The cost of calcium fortification is discussed in section 6 of the Fortification Manual (Minerals).

Comply to Food Aid Standards

A number of developing countries get large amounts of fortified wheat flour or maize meal in the form of food aid, usually from the United States under their *Food for Peace* (PL-480) program. This is fortified with iron, calcium, vitamin A and four B vitamins. Sometimes the World Food Program (WFP) and other donors obtain flour or meal locally. This could be fortified to PL-480 standards or other standards set by the WFP (as is the case for flour milled in Pakistan distended for Afghanistan). The PL-480 premixes are readily available but costly since they add a high level of vitamin A. WFP has yet to adopt a clear policy on what type of fortification they require for different regions.

Comply to a Milling Company Standard

Some international milling companies have routinely fortified flour to certain standards, often those used in the country in which the company is based. Examples of this are U.S. and Canadian milling companies fortifying to U.S/Canadian standards (now the same but once somewhat different in levels) and South African milling companies fortifying to RSA standards. An example of this is the long term, voluntary fortification of wheat flour in Haiti to U.S. standards, which does not have any requirement that the flour be fortified.

Use a Flour Enrichment Scheme

Enrichment is a type of fortification involving the replacement of micronutrients lost in the milling process. The lower the extraction, the lower the ash and the less micronutrients that are present in the final flour versus what was present in the original grain. A mill may chose to simply restore lost nutrients using a premix designed for that purpose. This approach has some major complications and limitations.

One first has to determine which of the possible ten micronutrients that are lost in milling are to be restored[19]. This approach precludes adding vitamin A, vitamin D, vitamin B_{12} (which are not naturally present in grains) or the high levels of folic acid that have been widely recommended and used. Another complication is the wide variation in natural levels of some micronutrients in whole grains, particularly iron, making it difficult to establish what the restoration level should be.

Despite these problems, it is possible to go with a simple enrichment scheme and obtain a premix that will accomplish that purpose, an example of which is shown in Table 3, the estimated cost of which is $0.80 per metric ton of fortified flour. The advantage of this approach is that it deflects criticism in countries not accustomed to flour fortification since you are simply adding back nutrients that were removed. One major disadvantage is that it may not meet the nutritional needs of the population that will consume the flour. Note that the percentage of the dietary requirements provided are comparatively low for riboflavin and folic acid.

Table C3 Possible Flour Enrichment to Whole Wheat Levels

Micronutrient	*Level added (ppm)*	*Final minimum level (ppm)*	*% RDA per 100g*
Thiamin	3.5	4.1	34
Riboflavin	1.1	1.1	8
Niacin	48	48	30
Folic Acid	0.23	0.4	10
Pyridoxine	3.2	3.4	26
Iron	47	54	30
Zinc	31	35	35

[19] There are other nutrients lost in the milling process but only ten have nutritional significance as discussed in Section 1.

Meet Customer Specifications

There can be cases where a major flour user, such as a national bakery or food aid donor, requests that flour be fortified to certain specifications that they provide. Such flour may be used locally or exported. Fortification premix companies can provide the appropriate premix. In rare cases the flour purchaser provides the premix.

Devise Customized Fortification

In lieu of any of the above situations, mills in countries without fortification standards may wish to develop their own fortification scheme and applicable premix that meets their particular nutritional, economic and technical requirements. Ideally a mill or milling industry should do this in collaboration with local health and government authorities, but there may be situations where that is not practical or would result in an extended delay in implementation. In that case the milling company may want to call on experts, such as are available through the Micronutrient Initiative or WHO, to devise a suitable premix.

Types of Fortification

An important distinction should be made on whether the planned fortification of flour falls under the category of *open market, mass or targeted* fortification, in the parlance of the WHO guidelines. If the former, the mill's primary objective is to increase sales and market share of a couple of their flour products, typically their premium, higher priced white flour. This may be accompanied by special labeling and promotion. Open market fortification is likely to be discontinued if the hoped for sales do not materialize.

Mass fortification, on the other hand, is generally applied to more flour products and its long term continuation is not dependent on sales. A milling company, or even the whole milling industry within a country, may decide on their own that fortification is a good thing and should be practiced as a general rule, even if there are no requirements to do so. This is not a common occurrence; nearly all mass programs result from government action.

Targeted fortification occurs when a major mill customer or potential customer, such as a food aid agency like CARE or WFP, requests a special, fortified flour for distribution in their food aid programs.

This distinction in the type of program impacts the nature and possible source of the fortification premix to use. An open market program would lean towards those micronutrients that have the greatest recognition and appeal to the intended market, usually the higher socioeconomic group that would purchase the premium products. Folic acid and calcium are current examples of such micronutrients. Premix cost is generally not an issue in this situation. Established fortification premix companies would be a good resource to use in devising premixes for this purpose.

Mass and targeted fortification programs would want to focus on those micronutrients that are in the greatest need by the general population, with iron and folic acid being good examples. Cost becomes a important factor in mass programs since milling companies are not as able to regain the cost of fortification in the case of low priced products as they can from the higher priced, premium ones. Premixes for use in mass programs should involve the collaboration of different stakeholders, particularly those in government and the milling industries. Premix or micronutrient manufactures, while a useful source of information, should not be directly involved in deciding the types and levels of micronutrients to add in a mass program.

Fortification for Different Types of Flours

Mills produce different types of wheat and maize products, as discussed in Section 4 of the Fortification Manual. These products can differ greatly in their micronutrient content, which is dependent on the extraction rate and the degree of refinement. A good measure of this is the flour *ash* content – the greater the ash level, the higher the micronutrient content. Whole-wheat flours contain all of the original micronutrient content in the wheat kernel, while micronutrients are concentrated in some mill products (eg. *second clears, red dogs*) causing them to have actual higher levels of some micronutrients than those present in the whole wheat.

Historically, only the refined, white flours with ash contents below 0.7% have been fortified since they contain the lowest level of micronutrients and are the primary mill products that go to direct human consumption. There are many countries, however, where a high extraction, high ash flour, such as *atta* flour, is common if not prevalent. Should such flours be considered for fortification, and if so, with what? This is one of the more difficult questions to answer.

These high ash, high extraction flours could act as a convenient carrier for certain deficit micronutrients, such as folic acid and vitamin A, which are not naturally present in wheat. They would be less than ideal for iron and zinc because of a high level of phytic acid they contain, which would decrease the absorption of these minerals, unless special forms such as iron-EDTA were used. Also, these types of flours are often made in smaller mills making fortification more difficult but not impossible.

Guidelines on Devising Mass Cereal Fortification Standards

Each country or region should establish their cereal fortification program based on the situation in their region taking into account the different considerations in this manual. The process of establishing standards and associated regulations is difficult and involved. It should always involve representatives from the medical community, the milling and baking industry, and the government, usually through the Ministry of Health. Consumer groups, educational/research institutions and interested NGOs may also be involved. This alliance must assess what is needed and what is feasible. All major stakeholders must "buy in" to the final regulations if acceptance and compliance are to be achieved.

While the cost of the fortification may not appear to be a major constraint at the beginning, experience shows us that it always will be one at the end. As a general rule of thumb, fortification programs for cereal staples that have an ingredient or premix cost in excess of $1.00 per metric ton of flour are difficult for the milling industry to accept. This restricts the types and levels of micronutrients to include, and makes it very difficult to require vitamin A and calcium. On the other hand, it makes little practical sense to go through all the trouble of adding just one or two micronutrients, when additional ones could be included for very little additional cost, providing the general population needs them.

Consideration of what vitamins and minerals to include in a mass fortification program can be broken down into the following hierarchy depending on the recognized level of deficiency and cost:

1. Extensive deficiency problems - Should be included in all fortification programs: Iron and folic acid.

2. Common deficiency problems, reasonable cost and well suited to flour fortification – should be considered for most fortification programs where their specific deficiency can be shown: Zinc, thiamin, riboflavin, niacin (in maize), pyridoxine

3. Common deficiencies but cost restraints – Consider inclusion if added cost is acceptable: vitamin A, niacin (in flour), calcium

4. Deficiencies less common but known to occur – Consider for inclusion only if a deficiency problem can be proven and there is no other reasonable intervention: selenium, vitamin B12, vitamin D

Following are some specific guidelines on cereal fortification on different types of flours.

<u>**White refined wheat flour** (with an ash content below 0.80%) and **refined, degermed maize meal**</u>

1. Most refined flours **should be enriched** with those micronutrients that are naturally present in the whole-grain and highly reduced in the milling process and that are known to be deficit in the general population consuming these products.
2. The level of micronutrients to add to refined flours and meals should be at least sufficient to achieve the natural levels in the whole grains (i.e. enrichment or replacement levels, see Tables 3.7 and 3.8.)
3. Appropriate adjustments should be made on the basis of bioavailability of the iron source used in the amount added. Current guidelines on iron fortification from SUSTAIN, PAHO and WHO should be considered.

4. Folic acid should be added to this type of flour at higher than replacement levels (e.g. 1.5 to 2.4 ppm) because of the now recognized need and benefits for this vitamin.
5. Higher than replacement levels for riboflavin (2 to 4 ppm) for this type of flour should be considered if the need exists.
6. Some non-staple refined flours, such as cake flour, may be exempted from enrichment in order to allow consumers to have a choice of an unfortified product.
7. All developing countries should, at a minimum, fortify this type of flour with iron and folic acid, and strongly consider including zinc and riboflavin.
8. If niacin and vitamin A are added, their levels should be kept low enough so that the cost of the full fortification is reasonable and acceptable. Vitamin A addition greater than 10,000 IU per kg is not recommended because of the high cost.

Medium extraction flours (ash content 0.8% to 1.0%) and maize meals

1. This type of flour should only be fortified if fortification of refined flours will not achieve the desired nutrition objectives or coverage of the target population.
2. Elemental reduced iron is acceptable but not ideal. Ferrous sulfate is not recommended but ferrous fumarate is possible. Iron-EDTA should be considered. Otherwise, the same types and levels of the other micronutrient used in refined flour can be applied. Vitamin A can be added if desired.
3. The level of added nutrients naturally present in cereals may be reduced due to the higher natural content in order to achieve the same final levels or standards.

High extraction flour (ash content >1.0%) and maize meal

1. Fortify this type of flour only if absolutely necessary to reach a target population.
2. If iron needs to be included, use iron-EDTA but at low levels (~15 ppm Fe).
3. The levels of vitamins added and the fortification standards for this type of flour should be based on meeting nutritional intake objectives and not on levels in whole grain.

Appendix C

MAJOR GLOBAL FORTIFICATION PREMIX SUPPLIERS

American Ingredients Co.
3947 Broadway
Kansas City, MO 64111
Ryan Simmons,
International Manager, Flour Service
Work: 800-669-4092, ext.
Fax: 816-561-0422
E-Mail: rsimmons@americaningredients.com

BASF Corp
28 Keepataw Ct
Lemont, IL 60439-4339
John R. Eterno
Phone: (630) 257-5568
Fax: (630) 257-9392
Email: eternoj@basf.com

Bogasari Flour Mills
Kunci Biru Bld.
Jalan Raya Cilincing No. 1
Tanjung Priok
Jakarta
Indonesia
Philip Purnama
Senior VP
Work: (021) 439-00-170
Fax: (021) 430 6956
E-Mail: ppurnama@mba1997.hbs.edu

DSM Nutritional Products
Av. Quilin 3750
Santiago
Chile
Hector Cori
Micronutrient Intervention, Projects Manager
Work: 562-441-3367
Fax: 562-221-6727
Email: hector.cori@dsm.com

Fortitech Co.
2105 Technology Dr.
Schenectady, NY 12308
Dr. Ram Chaudhari
VP, R & D
Work: 518-372-5155
Fax: 518-372-5599
Email: ramc@fortitech.com

Granotec
PO Box 3434 Correo Central
Santiago,
CHILE
Miguel Angel O. Gonzalez
President
Phone: 56 27400123
Fax: 56 27400176
Email: ma.gonzalez@granotec.com

Hexagon Chemicals Pvt. Ltd.
229 Oshiwara Industrial Center
Goregaon (W)
Mumbai 400 104
India
Kishore Shintre
Marketing Director
Work: 91-22-877 8529
Fax: 91-22-8529/30/31
E-Mail: knikhil@giasbma.vsnl.net.in

Research Products Co.
1835 E North St
PO Box 1460
Salina, KS 67401-1460
Steven Schorn
Technical Manager
Work: 913 825 2181
Fax: 913 825 8908
Email: steveschorn@researchprod.com

Watson Foods Co Inc
301 Heffernan Dr
West Haven, CT 06516-4151
Michael K. Weibel
VP, Technical
Phone: (203) 932-3000
Fax: (203) 932-8266
Email: Michael.Weibel@watsonfoods.com

The Wright Group
6428 Airport Rd
PO Box 821
Crowley, LA 70526
Salmon L. Wright
Phone: (337) 783-3096
Fax: (337) 783-3802
Email: sam@wenrich.com

Appendix I

ANALYTICAL METHODS USED IN FORTIFICATION

Spot Test for Iron in Flour

AACC Method 40-40
IRON-QUALITATIVE METHOD

Scope
Applicable to iron fortified wheat flour and iron fortified bread crumb.

Reagents
Dissolve 10 g KSCN in 100 ml water. Mix with equal vol 2N HCl just prior to use.
Hydrogen peroxide 3%.

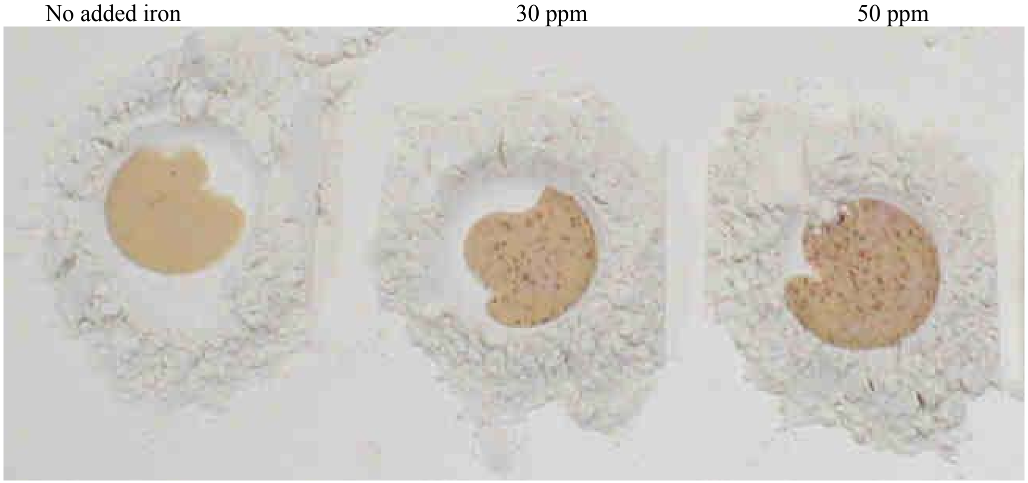
Procedure
Make a flat surface of the enriched flour or bread crumb by pressing down with a flour slick, spoon, the bottom of a small beaker or any suitable smooth surface. Drop a few mls of the freshly mixed HCl/thiocyanate reagent onto the surface followed by a few mls of the hydrogen peroxide sufficient to wet an area approx 1 inch in diameter. Let stand at least 10 min. under observation.

If added iron compounds are present they will show up as red spots on the surface. Reduced iron shows up as small dots that take time to appear. Ferrous sulfate shows up as larger spots that appear more quickly. The density of the spots provides an estimate of how much iron was added, which is best done by comparison to flours having known levels of added iron.

Reference
Schlesinger, H. I., and Van Valkenburgh, H. B. 1931. The structure of ferric thiocyanate and the thiocyanate test for iron. J. Am. Chem. Soc. 53:1212.

Example of iron spot test on flour with different levels of added iron.

No added iron 30 ppm 50 ppm



Determination of Folic Acid by HPLC Analysis

SCOPE

This method describes the procedure for the determination of folic acid in flour samples using the Dionex 500 Series HPLC.

This is not an official method.

CHEMICALS AND EQUIPMENT

CHEMICALS	**DESCRIPTION**
Acetonitrile	HPLC grade
Ascorbic acid	Analytical reagent grade
Deionized (DI) water	Nanopure, 18.2 megaohm
Flour	Unenriched
Folic acid	98% pure analytical reagent grade
Glacial acetic acid	Analytical reagent grade
Hexane HPLC grade	
Methanol	HPLC grade
pH buffers	4.00 and 7.00
Phosphoric acid	Analytical reagent grade
Potassium hydroxide	Analytical reagent grade
Potassium phosphate, dibasic	Analytical reagent grade
Reference flour	
Sodium acetate, anhydrous	Analytical reagent grade
Sodium chloride	Analytical reagent grade

EQUIPMENT	**DESCRIPTION**
Balance, analytical	Capable of weighing to 0.0001 gm
Balance, top-loading	Capable of weighing to 0.01 gm
Beakers 30 ml and 3,000 ml	
Column Phenomenex Bondclone, 150 x 3.9 mm,	10 µm, C18
Eppendorf pipet	5 ml adjustable

Flask	250 ml Nalgene with screw lids
Filter paper	Whatman #4, 12.5 cm
Food processor	
Funnels 60 mm powder	
HPLC system	Dionex 500 series with AD20 absorbance detector, GP50 pump, AS40 autosampler
Injection loop	100 µL
Pails	Plastic, one gallon, with lids
pH meter	
Shaker	Wrist action with timer
SPE tubes	Varian SAX quaternary amine ion exchange, 500 mg/10 ml
Syringes 20 ml disposable	
Syringe filters	Acrodisc, 0.22 µm
Test tubes	16 x 100 mm
Volumetric pipets	40 ml Class A
Weigh boats	
Weighing paper	8 x 8 cm

SOLUTION PREPARATION

Stock Standard

Weigh 453.5 ±0.1 gms of unenriched flour into the food processor bowl. Onto weighing paper, weigh out 0.1103 ± 0.0003 gms of folic acid. Tare the analytical balance, transfer the folic acid to the food processor bowl, and reweigh the weighing paper. Record the weight loss in the standards workbook. Close up the food processor and mix for 5 minutes. Transfer stock standard to a one gallon pail. After adjusting for the water content and purity, the stock standard will contain 100.0 mg/pound folic acid. Label with contents and date prepared.

Working Standards

Five working standards are made by diluting the stock standard. The weight of stock standard for the five working standards are: 1.14, 2.13, 3.18, 4.54, and 7.26 gms. The weight of unenriched flour for the five working standards are: 452.46, 451.47, 450.42, 449.06, and 446.34 respectively. Weigh the unenriched flour into the food processor bowl and add the corresponding amount of stock standard. Close up the food processor and mix for 5 minutes. Transfer working standards to a one gallon pail. The current bag of unenriched flour contains 0.156 mg/pound folic acid. The working standards will contain 0.407, 0.626, 0.857, 1.157, and 1.757 mg/pound folic acid, respectively. Label each container with contents and date prepared.

"A" Mobile Phase

Add 980 mls DI water to a 1,000 ml beaker. Add a magnetic stirring bar and place on a stir plate. Weigh out 8.20 ± 0.01 gms of sodium acetate. Transfer the sodium acetate to the beaker. Adjust the pH with acetic acid to a pH of 5.70 ± 0.05. Add 20 mls of acetonitrile. Pour into the mobile phase reservoir for delivery to HPLC system.

"B" Mobile Phase

Add 800 mls DI water to a 1,000 ml beaker. Add a magnetic stirring bar and place on a stir plate. Weigh out 8.20 ± 0.01 gms of sodium acetate. Transfer the sodium acetate to the beaker. Adjust the pH with acetic acid to a pH of 5.70 ± 0.05. Add 200 mls of acetonitrile. Pour into the mobile phase reservoir for delivery to HPLC system.

Extraction Solvent

Add 2,000 mls DI water to a 3,000 ml beaker. Add a magnetic stirring bar and place on a stir plate. Weigh out 34.83 ± 0.01 gms of potassium phosphate and transfer it to the beaker. Weigh out 1.00 ± 0.01 gms of ascorbic acid and transfer it to the beaker. Adjust the pH with phosphoric acid or potassium hydroxide to a pH of 8.50 ± 0.05.

Salt Eluent

To 250 ml of extraction solvent, add 25.00 ± 0.01 gms of sodium chloride. Stir until dissolved.

SAMPLE PREPARATION

NOTE: Normal analysis run consists of the 5 standards, 3 reference standards, and 24 samples.

Weigh out 4.00 ± 0.01 gms of sample. Transfer into a labeled screw capped flask using a funnel. Repeat for all samples and reference flours. Pipet 40 mls of extraction solvent into each flask, cap, and place on the wrist action shaker. When the shaker is full, shake the flask for 20 minutes. While the flask are shaking, prepare for filtration by placing funnels in 30 ml flask then fold a #4 filter paper into quarters. When the shaker has stopped, remove the flask, swirl, open, and pour into filter paper. Allow a minimum of 20 mls to filter before proceeding.

Place a syringe filter on the end of the 20 ml syringe. Remove the plunger, pour the filtrate into the syringe, replace the plunger, discard the first 1 ml of filtrate, and collect about 6 mls of filtrate in a test tube. Repeat for the other 15 flasks.

Place unmarked test tubes into the vacuum chamber in positions 1-12, 14, 17, 20, and 23. Place the top on the vacuum chamber. Check to make sure the pointer on the top is pointing to waste, change if necessary. Place a SPE cartridge into each stopcock. Turn on the vacuum and close the manifold. Fill the SPE cartridge with hexane. Open the stopcocks to allow the hexane to flow through until a thin film remains. Close the stopcock. *DO NOT LET THE CARTRIDGES GO DRY.* Repeat with methanol, then DI water. Pipet 5 ml of the first sample into the first SPE cartridge. Repeat for all 16 test tubes. Open the stopcocks to allow the sample to flow through until a thin film remains. Pipet 5 ml of DI water into each SPE. Open the stopcocks to allow the water to flow through until a thin film remains. Open the manifold. When the vacuum is at zero psi, turn the top so the pointer is pointing to collect. Pipet 5 mls of salt eluent into each SPE. Open the stopcocks to allow the salt eluent to flow through until a thin film remains. Open the manifold and turn off the vacuum. Remove the tubes, but keep them in the correct order.

Vortex the contents of each test tube. Pour contents into a labeled polyvial. Cap the polyvial.

EQUIPMENT PREPARATION

Column Switching

Open the door of the column holder. Locate the line going from the injector to the columns. If the line goes to the Dionex column, the line will need to be flushed. Disconnect the line from the Dionex column and place into a small beaker or flask. On the pump module, move the cursor so it is in front of "Remote", press the Select key so the display changes to "Local". Move the cursor up to in front of the "% A", type 100, and press "Enter". Move the cursor to in front of "mls/min", type 1, and press "Enter". Start the pump, run for 5 minutes, then stop the pump.

Connect the line to the inlet of the guard column. Use the blind in the guard column to seal the Dionex column. Connect the outlet of the column to the line going to the detector. Blind off the reaction tube outlet.

Sample Schedule

A sample schedule tells the computer what sample and type of sample is being analyzed. The easiest way to build a sample schedule is to open the last one, make changes, then save as a new file name. The next to last line is for cleaning the column and the last line shuts everything down.

ANALYSIS

Open the run window of PeakNet. Click on the second icon from the left to load a schedule. Chose the schedule that was developed in Step 6.3.2. After clicking on the last "OK", the pump will start. Let the system run for 30 minutes before continuing.

Load samples into AS40 automated sampler in appropriate order and press the Run button.

The Dionex software will automatically calculate mg/lb folic acid and print out a report for each standard and sample.

The following methods are summaries of official AACC methods giving The apparatus and regents.
For the full method see: *Approved methods of the American Association of Cereal Chemist, 10th Edition, The Association, St. Paul, MN 2000.*

Iron—Spectrophotometric Method

Inorganic Constituents AACC Method 40-41B

Final approval May 5, 1960; Reapproval November 3, 1999

This method determines iron content by reaction with orthophenanthroline and spectrophotometric measurement. It is applicable to cereals and cereal-based products.

Apparatus
1. Muffle furnace capable of maintaining 550°.
2. Platinum, silica, or porcelain crucible, approximately 60-mm diameter, 35-ml capacity. Porcelain evaporating dishes of about 25-ml capacity are satisfactory. Do not use flat-bottom dishes of greater diameter than 60 mm.
3. Spectrophotometer or colorimeter.

Reagents
1. Orthophenanthroline solution. Dissolve 0.1 g *o*-phenanthroline in about 80 ml water at 80°, cool, and dilute to 100 ml. Store in amber bottle in refrigerator for up to several weeks.)
2. Iron standard solution, 10 µg Fe/ml. a) Dissolve 0.1 g analytical grade Fe wire in 20 ml HCl and 50 ml water, and dilute to 1 liter. Dilute 100 ml of this solution to 1 liter; *or* b) dissolve 3.512 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in water, add 2 drops HCl, and dilute to 500 ml. Dilute 10 ml of this solution to 1 liter.
3. Hydroxylamine hydrochloride solution. Dissolve 10 g $NH_2OH \cdot HCl$ in water and dilute to 100 ml. Store in amber bottle in refrigerator. (This solution is stable for several weeks.)
4. Acetate buffer solution. Dissolve 8.3 g anhydrous sodium acetate (previously dried at 100°) in water, add 12 ml acetic acid, and dilute to 100 ml. (It may be necessary to redistill acetic acid and purify sodium acetate by recrystallization from water, depending on amount of Fe present.)
5. Prepare working standards as follows: Place aliquots of 10 µg/ml standard solution according to table below into 100-ml volumetric flasks, add 2 ml concentrated HCl to each, and dilute to volume.
Mix thoroughly by inverting flask 10–20 times. Using 10 ml of each of these standard solutions, continue under procedure beginning with step 8.

Aliquot of 10 µg/ml solution taken (ml)	*Final Fe Concentration (ppm)*	*Aliquot of 10 µg/ml solution taken (ml)*	*Final Fe Concentration (ppm)*
0	0	25	2.5
2	0.2	30	3.0
5	0.5	35	3.5
10	1.0	40	4.0
15	1.5	45	4.5
20	2.0	50	5.0

6. Ashing aid
a. Magnesium nitrate solution. Dissolve 50 g Mg(NO3)2·6H2O in water and dilute to 100 ml *or*
b. Redistilled HNO3.

Elements by Atomic Absorption Spectrophotometry

Inorganic Constituents AACC Method 40-70

Final approval October 16, 1991; Reapproval November 3, 1999

This method determines calcium, copper, iron, magnesium, manganese, and zinc in grains and cereal products.

Apparatus
1. Atomic absorption cereal products spectrophotometer. Several commercial models are available. Since each design is somewhat different, with varying requirements of light source, burner flow rate, and detector sensitivity, only gen-eral outline of operating parameters is given in Table I. Operator must become familiar with settings and procedures adapted to own apparatus and use table only as guide to concentration ranges and flame conditions. Single-slot burner may require that lanthanum be added to standard and sample solutions for all elements.
2. Ashing vessels, 150-ml beaker (Pyrex or Vycor) or 30-ml Vycor crucible.
3. Muffle furnace capable of operating at temperatures up to 525°.

Reagents
1. Water, distilled-deionized (greater than 10 megohm resistance). Use throughout procedure in all preparation and dilution of solutions.
2. Stock solutions. See Note 1.
a. Calcium, 25 µg Ca/ml. Dissolve 1.249 g CaCO3 in minimum amount *3N* HCl. Dilute to 1 liter. Dilute 50 ml to 1 liter.
b. Copper, 1000 µg Cu/ml. Dissolve 1.000 g pure Cu metal in minimum amount HNO3 and add 5 ml HCl. Evaporate almost to dryness and dilute to 1 liter with 0.*1N* HCl.
c. Iron, 1000 µg Fe/ml. Dissolve 1.000 g pure Fe wire in about 30 ml *6N* HCl with boiling. Dilute to 1 liter.
d. Magnesium, 1000 µg Mg/ml. Place 1.000 g pure Mg metal in 50 ml water and slowly add 10 ml concentrated HCl. Dilute to 1 liter.

TABLE I
Operating Parameters

Element	**Wavelength (Å)**	**Flame***	**Range (µg/ml)**	**Remarks**
Ca	4227	Rich Air-C_2H_2	2–20	1% La, 1% HCl
	4227	Rich N_2O-C_2H_2	2–20	Requires special burner
Cu	3427	Air-C_2H_2	2–20	
Fe	2483	Rich Air-C_2H_2	2–20	
Mg	2852	Rich Air-C_2H_2	0.2–2	May need La
Mn	2795	Air-C_2H_2	2–20	
Zn	2138	Air-C_2H_2	0.5–5	

*C_2H_2 = acetylene

e. Manganese, 1000 µg Mn/ml. Dissolve 1.582 g pure MnO_2 in about 30 ml
6N HCl. Boil to remove Cl and dilute to 1 liter.
f. Zinc, 1000 µg Zn/ml. Dissolve 1.000 g pure Zn metal in about 10 ml *6N* HCl. Dilute to 1 liter.
3. Lanthanum stock solution, 50 g La/liter ~5% HCl. Dissolve 58.65 g La_2O_3 (99.99%, low calcium content) in 250 ml concentrated HCl, adding acid slowly. Dilute to 1 liter.
4. Working standard solutions.
a. Calcium, 0, 5,10, 15, and 20 µg Ca/ml containing 1% La and ~1% HCl. To 25-ml volumetric flasks, add 0, 5, 10, 15, and 20 ml Ca stock solution (reagent 2a). Add 5 ml La solution and dilute to volume.
b. Other standard solutions. Dilute aliquots of solutions 2b, 2c, 2d, 2e, and 2f with 0.*5N* HCl to make at least four standard solutions of each element within range of determination.

References

1. AOAC International. 1995. Official Methods of Analysis of AOAC International, 16th ed. Method 965.09. The Association, Arlington, VA.
2. Gatehouse, B. M., and Willis, J. B. 1961. Performance of a simple atomic absorption spectrophotometer. Spectrochim. Acta 17:710.
3. Zook, E. G., Greene, F. E., and Morris, E. R. 1970. Nutrient composition of selected wheats and wheat products. VI. Distribution of manganese, copper, nickel, zinc, magnesium, lead, tin, cadmium, chromium, and selenium as determined by atomic absorption spectroscopy and colorimetry. Cereal Chem. 47:720.

Sources of Supply
40-70, Elements by Atomic Absorption Spectrophotometry
*Lanthanum (III) oxide, low calciu*m. Cat. no. 11266, **Alfa ÆSAR,** 30 Bond St., Ward Hill, MA 01835-8099, phone: (800) 343-0660, fax: (800) 322-4757, e-mail: info@alfa.com, website: www.alfa.com
Standard reference material. **National Institute of Standards and Technology (NIST),** Standard Reference Materials Program, 100 Bureau Dr., Stop 2322, Gaithersburg, MD 20899, phone: (301) 975-6776, fax: (301) 948-3730, e-mail: SRMINFO@nist.gov, website: www.ts.nist.gov (Use TS Services Page to find Std. Ref. Materials Program.)

Total Folate in Cereal Products

Microbiological Assay Using Trienzyme Extraction

Vitamins AACC Method 86-47
First approval November 8, 2000

This is a microbiological method employing the organism *Lactobacillus casei* subsp. *rhamnosus* (ATCC no. 7469) to determine the amount of folate present in foods and in vitamin concentrates. This method is semiautomated through the use of automated dilution and turbidity reading instruments or, optionally, the 96-well microtiter plate and reader system. See Note 1. Samples, with water added, are autoclaved to break up particles, gelatinize starch, and denature proteins to enhance enzymatic attack and make folate more extractable. Folate (pteroylglutamic acid in various forms) occurs naturally in foods bound to glutamic acid residues of varying chain lengths. Most naturally occurring folates cannot be utilized by the assay organism. Folic acid (pteroylglutamic acid) is extracted from the sample using a triple enzyme system. A protease and an amylase are used to digest the food matrix and aid in the release of folates. Desiccated chicken pancreas conjugase is used to hydrolyze folylpolyglutamates to folyldiglutamates, which, along with folic acid, can be utilized by the assay organism. The freed folates are extracted and diluted with basal medium containing all required growth nutrients except folate, and the turbidity of the *L. casei* subsp. *rhamnosus* growth response for the samples is compared quantitatively to that of known standard solutions. The method is applicable to cereal grains and cereal grain foods containing added folate (folic acid) or naturally occurring folates with levels from 5 µg/100 g to 100% folate.

Apparatus. See Note 2.
1. Centrifuge, clinical, to accommodate 20- × 150-mm test tubes.
2. Spectrophotometer, to read 20- ´× 150- (or 18- × 150-mm) test tubes.
3. Covered water bath, 37 ± 0.2°, with rotary shaker.
4. Disposable glass test tubes, borosilicate glass, 20- × 150-mm (or 18- × 150-mm).
5. pH meter, with long combination electrode.
6. Vortex mixer.
7. Volumetric flasks, class A, clear, low-actinic; 25, 50, 100, 250, 500, and 1000 ml.
8. Pipetter, Eppendorf repetitive, with 50-ml capacity.
9. Disposable tips, Eppendorf, 1.25 ml.
10. Adjustable digital pipet, Eppendorf, 100–1000 µl.
11. Pipetting machine(s), to deliver 1- and 5-ml aliquots. *Optional:* Pipetter, 12-channel, to use with 96-well microtiter plates.
12. Pipets, volumetric, class A; 1, 2, 3, 4, 5, 10, 20, 25, and 50 ml.
13. Autoclave, for sterilizing at 15 psi and 121–123°.
14. Refrigerator, set to 2–8°; freezer, set to –18°.
15. Centrifuge tubes, Oak Ridge type, 28.6 × 106.1 mm, polypropylene, reusable.
16. Centrifuge tubes, disposable.
17. Test tube racks, 4 × 12 in., to hold 20- × 150-mm test tubes.
18. Large rack, with cover, to hold four or more test tube racks.
19. Filter paper, Whatman no. 2V, 12.5 cm.
20. Balance, analytical, reading to at least four places.
21. Balance, top loading, three-place.
22. Hot plate stirrer.
23. Distilling apparatus for water. (Use distilled or double-distilled water throughout.)
24. Syringe, fitted with long needle, sterilizable, capable of delivering 50 µl.
25. Desiccator.
26. Inoculating loops and straight wire.
27. Bunsen burner
28. (*Optional for tube assay*) Sample changer, automated, assay-tube reading, modified with an air agitation system and connected to a spectrophotometer with a flow cell and either a printer or a computer, *or* a diluter and reader.
29. (*Optional microtiter plate system*) Microtiter plate reader, 96-well, and microtiter plates, 96-well. A reader with appropriate filter(s) for 570–630 nm and efficient software for calculation is suitable. See Note 3. Use of the microtiter plate systems requires filter sterilization of solutions, using 0.22-µm sterilization filter units (15-ml, 500-ml, and 1-liter sizes).

Reagents. See Note 4.
1. Acetic acid, glacial.
2. Adenine sulfate.
3. Agar, Bacto.
4. Agar, *Lactobacilus casei*.
5. □-Amylase.
6. *p*-Aminobenzoic acid.

7. Ammonium hydroxide.
8. Antifoam. Mix 1.5 ml antifoam with 100 ml water. Shake before using.
9. Ascorbic acid.
10. *L*-Asparagine monohydrate.
11. Biotin.
12. Calcium pantothenate.
13. Casein hydrolysate, vitamin-free, acid-hydrolyzed.
14. Celite (optional as filter aid).
15. Conjugase source, chicken pancreas, desiccated.
16. *L*-Cysteine-HCl.
17. Dextrose (glucose), anhydrous.
18. Ferric sulfate heptahydrate.
19. Folic acid reference standard.
20. Glutathione.
21. Guanine hydrochloride.
22. Liver.
23. Magnesium sulfate heptahydrate.
24. Manganese sulfate monohydrate.
25. Niacin.
26. Octanol.
27. Potassium phosphate, dibasic.
28. Potassium phosphate, monobasic.
29. Protease, pronase E.
30. Pyridoxine hydrochloride.
31. Riboflavin.
32. Sodium acetate, anhydrous, and sodium acetate-3H2O.
33. Sodium ascorbate.
34. Sodium chloride.
35. Sodium hydroxide.
36. Thiamin hydrochloride.
37. Toluene.
38. D,L-Tryptophan.
39. Uracil.
40. Xanthine.
41. Acetic acid 0.02*N*. Dissolve 1.2 ml of glacial acetic acid in ~500 ml water. Dilute to 1 liter with water.
42. Ammonium hydroxide (2+3), NH4OH (2+3). Carefully mix 2 volumes concentrated ammonium hydroxide with 3 volumes water.
43. Hydrochloric acid (1+1). Carefully mix equal volumes of concentrated HCl and water. See Note 4.
44. Phosphate buffer, pH 7.8 (extract buffer). Dissolve 1.42 g sodium phosphate dibasic and 1.0 g ascorbic acid in water and dilute to 100 ml. Adjust pH to 7.8 with 4*N* NaOH. Prepare fresh on day used. Assay requires approximately 35 ml of buffer for each sample and standard.
45. Phosphate buffer, pH 6.8 (assay buffer). Dissolve 1.42 g sodium phosphate dibasic and 1.0 g ascorbic acid in □85 ml water and dilute to 100 ml. Adjust pH to 6.8 ± 0.02 with 4*N* NaOH. Prepare fresh on day used. Assay requires 12–15 ml of buffer for each sample and standard tube for test tube assay and □2.5 ml of buffer for each sample and standard for microtiter plate assay option.
46. Phosphate buffer, 0.1*M*, pH 7.0 (buffer for making standard solutions). Dissolve 13.61 g potassium phosphate monobasic in water and dilute to 1 liter with same. Adjust pH to 7.0 with 4*N* potassium hydroxide.
47. Potassium hydroxide, 4*N*. Dissolve 224 g of potassium hydroxide in ~500 ml water. See Note 4. Cool; dilute to 1 liter with water.
48. Sodium hydroxide, 4*N*. Dissolve 160 g of sodium hydroxide in ~500 ml water. See Note 4. Cool, dilute to 1 liter with water.

49. Sodium hydroxide, 0.01*N*. Pipet 2.5 ml of 4*N* sodium hydroxide into a 1-liter volumetric flask. Dilute to volume with water.
50. Adenine-guanine-uracil solution. Dissolve 1.0 g each of adenine sulfate, guanine hydrochloride, and uracil in 50 ml warm HCl (1+1); cool; and dilute with water to 1 liter.
51. Xanthine solution. Suspend 1.0 g xanthine in 150–200 ml water. Heat to ~70°; add 30 ml NH4OH (2+3); and stir until solid dissolves. Cool, and dilute to 1 liter with water.
52. Asparagine solution. Dissolve 10 g L-asparagine monohydrate in water and dilute to 1 liter.
53. Vitamin solution for folate. Dissolve 10 mg *p*-aminobenzoic acid, 40 mg pyridoxine hydrochloride, 4 mg thiamin hydrochloride, 8 mg calcium pantothenate, 8 mg niacin, and 0.2 mg biotin in ~300 ml water. Add 10 mg riboflavin dissolved in ~200 ml 0.02*N* acetic acid. Add a solution containing 1.9 g anhydrous sodium acetate and 1.6 ml acetic acid in ~40 ml water. Dilute to 2 liters with water. (Not necessary to prepare if using commercial basal medium preparation in reagent 58.)
54. Saline, sterile. Dissolve 9 g NaCl in 1 liter water. Dispense 10-ml portions to 20- × 150-mm test tubes capped with plastic tops. Sterilize 15 min at 121–123° and store in refrigerator.
55. Salt solution B. Dissolve 20 g MgSO4-7H20, 1 g NaCl, 1 g FeSO4-7H2O, and 1 g MnSO4-H2O in water. Dilute to liter. Add 1 ml HCl.
56. PABA-vitamin B6 solution. Dissolve 50 mg *p*-aminobenzoic acid and 120 mg pyridoxine hydrochloride in 200 ml water. Add 0.95 g sodium acetate and 0.8 ml acetic acid to ~40 ml water. Combine the two solutions and dilute to 500 ml with water.
57. Agar maintenance medium. Into 1 liter hot water containing 10 ml of 100 ng/ml vitamin B12, dissolve 48 g *Lactobacilli* agar and 3 g Bacto agar. After agars dissolve, dispense 10-ml portions to 20- × 150-mm test tubes; plug with cotton; and cap. Cover tubes to prevent contamination; sterilize 15 min at 121–123°; and store in refrigerator.
58. Folic-acid-free, double-strength basal medium. Prepare as shown in Table I. (Alternatively, commercially available folic acid casei medium can be used. Prepare as directed, i.e., suspend 9.4 g casei medium and 50 mg ascorbic acid in 100 ml water; boil for 1–2 min; cool.)
59. Standard solutions. Use low actinic glassware.
a. Stock solution (100 µg/ml). Accurately weigh 50 mg USP folic acid that has been dried to constant weight and dissolve in 0.1*M*, pH 7.0 phosphate buffer in 500-ml volumetric flask. Dilute to volume with 0.1*M* phosphate buffer. Top with enough toluene to keep surface covered (usually 3–5 ml). Store in refrigerator. Check purity of standard, and verify concentration of stock solution by pipetting 10 ml stock solution to 100-ml volumetric flask and diluting to volume with 0.1*M*, pH 7.0 phosphate buffer.
Measure absorbance of solution at 282 and 346 nm, using 0.1*M*, pH 7 phosphate buffer as blank. Folic acid concentration (FA) in stock solution:

FA (µg/ml) = absorbance/absorptivity × DF × 1000 × MW
where absorptivity = 27,600 at 282 nm and 7200 at 346 nm, DF = dilution factor, and molecular weight (MW) = 441.4.

TABLE I
Preparation of Folic-Acid-Free, Double-Strength Basal Medium (reagent 58)

	Milliliters of Basal Medium to Prepare		
	250	**500**	**1000**
Add in order listed (ml)			
Adenine-quanine-uracil solution	**2.5**	**5**	**10**
Xanthine solution	**5**	**10**	**20**
Asparagine solution	**15**	**30**	**60**
Vitamin solution for folate	**50**	**100**	**200**
Salt solution B	**5**	**10**	**20**
PABA-vitamin B6 solution	**2.5**	**5**	**10**
Add ~100 ml water and the following solids (g)			
Vitamin-free casein, hydrolyzed	**2.5**	**5**	**10**
Dextrose	**10**	**20**	**40**
Potassium phosphate, dibasic	**0.25**	**0.5**	**1**
Potassium phosphate, monobasic	**0.25**	**0.5**	**1**
Sodium acetate-3H2O	**16.6**	**33.2**	**66.4**
Glutathione	**0.00125**	**0.0025**	**0.005**
Dissolve the following solids (g) in dilute HCl and add to above solution			
L-Cysteine-HCl	**0.125**	**0.25**	**0.5**
D,L-Tryptophan	**0.05**	**0.1**	**0.2**
Mix well, adjust to pH 6.8 with NaOH, and dilute to 1 liter with water.			

b. Working standard solution (1 µg/ml). Dilute 5 ml stock solution to □475 ml with water and adjust pH to □7.5 with HCl. Dilute to 500 ml with water. Prepare fresh on day of use. This will be diluted further when standards are run in parallel with samples.
c. Diluted stock solution (100 ng/ml) (for use in working inoculum). Dilute 10 ml intermediate stock solution to □90 ml with water and adjust pH to □7.5 with HCl. Dilute to 100 ml with water. Top with enough toluene to keep surface covered (usually 3–5 ml). Store in refrigerator. Prepare fresh; discard after use.
60. Liquid culture medium (50% basal medium with 0.4 ng/ml folic acid and 10 µg/ml solubilized liver). Suspend 0.1 g liver in 100 ml water. Hold mixture for 1 hr at 50° and filter. Top with enough toluene to keep surface covered (usually 3–5 ml). Store in refrigerator. Pipet 20 ml to a 1-liter volumetric flask. Add (via pipet) 8 ml of diluted folic stock solution (100 ng/ml). Dilute to volume with water. Mix equal volumes of solution with basal medium solution. Dispense 10-ml portions of diluted medium to 20- × 150-mm screw cap test tubes; autoclave 15 min at 121–123°; and cool tubes rapidly. Store in refrigerator. (A commercially available bacto micro inoculum broth has also been found satisfactory as liquid culture medium.)
61. Conjugase solution: chicken pancreas solution (5 mg/ml). Weigh 0.5 g chicken pancrease and add 100 ml pH 7.8 buffer. Stir vigorously for 10 min. Transfer to 20- × 150-mm test tubes and centrifuge for 10 min at ~2000 rpm. Decant supernatant through glass wool pledget into beaker; cover with parafilm; and store in refrigerator. Prepare fresh on day of use. Each sample and standard requires 4–5 ml of conjugase solution.
62. □-Amylase solution (20 mg/ml). Dissolve 0.5 g □-amylase in 25 ml water. Store in refrigerator. Prepare fresh on day of use. Each sample and standard requires 1 ml □-amylase solution.
63. Protease solution (2 mg/ml). Dissolve 0.05 g protease in 25 ml water. Filter

through glass wool pledget, if necessary, and store in refrigerator. Prepare fresh on day of use. Each sample and standard requires 1 ml protease solution.

Notes

1. Folates are light and oxygen sensitive. Use of yellow lighting and low actinic glassware is recommended. Preparation and storage of samples under subdued lighting is essential.
2. For quality assurance, glassware must be low-actinic and must be cleaned meticulously and heated 1–2 hr at 250° to destroy any folic acid residues present. Folic acid should be stored in a desiccator prior to preparation of stock standard solution. Potential sources of error are shown in Table II.
3. For some plate reader systems, blackwall microtiter plates may be necessary to assure against deviations in readings between edge-row wells and center-row wells.
4. ***Caution.*** Ammonium hydroxide, hydrochloric acid, and potassium or sodium hydroxide are extremely caustic and can cause severe burns. Protect skin and eyes. When using flammable liquids, perform operations behind a safety barrier when using steam or electric mantle heating. Use an effective fume removal device to remove flammable vapors as produced. Leave ample headroom in flasks, and add boiling chips before heating is begun. All controls, unless vapor sealed, should be located outside of vapor area. For toxic liquids, use an effective fume removal device to remove vapors as produced. Avoid contact with skin.
5. Filtered solutions can be set aside in the dark at 4° overnight.
6. Use of automatic pipetting machine (apparatus 11) is recommended.
7. To assure against contamination of sterile microtiter plates and solutions, assure that bench area is clean and not susceptible to airborne contaminants. Alternatively, use a biosafety hood, if available, as this will reduce the risk of contamination.
8. A minimum of 15 ml inoculated media (i.e., 15 ul inoculum in 15 ml medium) is needed per plate (i.e., one standard and five samples). Prepare amount needed plus □70 ml extra.

Reference

DeVries, J. W., Keagy, P. M., Hudson, C. A., and Rader, J. I. 2001. Collaborative study on determination of total folate in cereal products by microbiological assay using trienzyme extraction (AACC Method 86-47). Cereal Foods World 46:(in preparation).

TABLE II Potential Sources of Error

Cause	Type of Result[a]
Incomplete wetting of sample at extraction	L, random
Sample splattered during mixing	L, random
Incorrect preparation of extraction buffers	L, bias
Weighing error	H/L, random
Balance out of calibration	H/L,bias
Air bubbles in diluter	L, random
Diluter not in calibration	L, bias
Failing power board on spectrophotometer	H/L, random
pH electrode calibration error	L, bias
Sample spilled	L, random
Stress on assay organism	L, bias
Incorrect assay medium preparation	L, bias to *no* growth in assay tubes

[a] A random error can be high (H) or low (L) but of indeterminate magnitude. A high or low bias affects every individual analysis in the same way, at the same magnitude.

Possible Sources of supplies
Centrifuge, clinical, to accommodate 20- × 150-mm test tubes. Cat. no. C1450-1. **Baxter Travenol,** Lakewood, NJ 08701, phone: (732) 363-7519
Spectrophotometer to read 20- × 150- (or 18- × 150-mm) test tubes. Spectronic 20, **Milton-Roy Co.,** 104 Crandon Blvd., Key Biscayne, FL 33149, phone: (305) 361-0480
Covered water bath, 37 ± 0.2°, with rotary shaker. Blue M, **Baxter Travenol,** Lakewood, NJ 08701, phone: (732) 363-7519
Disposable glass test tubes, borosilicate glass, 20- × 150-mm (or 18- × 150-mm), cat. no. T1290-10A or T1290-9A; *pH meter*, with long combination electrode, Orion SA720, cat. no. 072000; *vortex mixer,* cat. no. S8223-1; *pipetor,* Eppendorf repetitive, with 50-ml capacity, cat. no. P5063-20; *disposable tips,* Eppendorf 1.25-ml, cat. no. P5063-28; **Baxter Travenol,** Lakewood, NJ 08701, phone: (732) 363-7519
Pipetting machine(s), to deliver 1- and 5-ml aliquots. Vial filler model DABEL, **National Instruments Co.,** 4119 Fordleigh Rd., Baltimore, MD 21215, phone: (410) 764-0900, fax: (410) 764-1675, website: www.filamatic.com
Autoclave, for sterilizing at 15 psi and 121-123°. No. N67CDS, **Allegiance Healthcare, Div. Of Cardinal Health,** 8855 McGaw Rd., Columbia, MD 21045, phone: (410) 290-8400, fax: (410) 290-7906
Centrifuge tubes, disposable. Cat. no. C3978-50, **Baxter Travenol,** Lakewood, NJ 08701, phone: (732) 363-7519; ***or*** no. 2070, **Falcon Plastics, Inc.,** 250 W. Wylie Ave., Washington, PA 15301, phone: (724) 222-2600, fax: (724) 222-4585, website: www.falconplastics.com
Balance, analytical, reading to at least four places. Cat. no. 01-909-409 Mettler AE260, **Fisher Scientific,** 4500 Turnberry Dr., Hanover Park, IL 60103, phone: (800) 766-7000, fax: (800) 926-1166, website: www.fishersci.com
Balance, top loading, three-place. Cat. no. 01-913-132 Mettler PE160, **Fisher Scientific,** 4500 Turnberry Dr., Hanover Park, IL 60103, phone: (800) 766-7000, fax: (800) 926-1166, website: www.fishersci.com
Distilling apparatus for water. No. A1212, **Barnstead International,** 2555 Kerper Blvd., Dubuque, IA 52001, phone: (800) 446-6060, fax: (563) 589-0516, e-mail: mkt@barnstaedthermolyne.com, website: www.barnsteadthermolyne.com
Sample changer, automated, assay-tube reading, modified with an air agitation system and connected to a spectrophotometer with a flow cell and either a printer or a computer. Escargot fractionator, model SC-30 or model 222, **Gilson Co.,** P.O. Box 200, Lewis Center, OH 43035-0200, phone: (740) 548-7298 or (800) 444-1508, fax: (740) 548-5314, e-mail: sales@gilsonco.com, website: www.gilsonco.com; ***or*** *diluter and reader,* Autoturb II, **Mitchum-Schaefer Inc.,** 4251 N. Shadeland Ave., Indianapolis, IN 46226, phone: (317) 546-4081, fax: (317) 546-4195, website: Mitchum-Schaefer.com
Sterilization filter units, 0.22-µm (15-ml, 500-ml, and 1-liter size). Nalge disposable filterware, **Nalge Nunc International Corp.,** 75 Panorama Creek Dr., P.O. Box 20365, Rochester, NY 14602-0365, phone: (800) 625-4327, fax: (800) 625-4363, e-mail: orders@nalgenunc.com, website: www.nalgenunc.com; ***or*** disposable filterware, **Corning Glass Works,** U.S. 11 S., Martinsburg, WV 25401, phone: (304) 267-1200
Agar. Bacto, cat. no. 0140-01, **Difco Laboratories,** P.O. Box 331058, Detroit, MI 48232-7058, phone: (800) 521-0851, fax: (313) 462-8517
Agar, pure culture of *Lactobacilli casei.* Cat. no. 7469, **American Type Culture Collection [ATCC],** 10801 University Blvd., Manassas, VA 20110-2209, phone: (703) 365-2700, fax: (703) 365-2750, e-mail:

sales@atcc.org, website: www.atcc.org; ***or*** cat. no. 0900-15, **Difco Laboratories,** P.O. Box 331058, Detroit, MI 48232-7058, phone: (800) 521-0851, fax: (313) 462-8517

-*Amylase*. No A-6211, **Sigma Chemical Co.,** P.O. Box 14508, St. Louis, MO 63178 or 3050 Spruce Tree, St. Louis, MO 63103, phone: (800) 325-3010 or (314) 771-5765, fax: (314) 771-5757, website: www.sigmaaldrich.com or www.sigmaaldrich.com/Europe

Antifoam. Antifoam AF or B, **Dow Corning Corp.,** P.O. Box 994, Midland, MI 48686, phone: (989) 496-4400, fax: (989) 496-6731, website: www.dowcorning.com

Casein hydrolysate, vitamin-free acid-hydrolyzed. Hy case amino, ICN cat. no. 104864, **Kraftco Corp. Humko Sheffield,** 1099 Wall St. W., Lyndhurst, NJ 07071, phone (201) 935-9105

Conjugase source, chicken pancreas, desiccated. Difco 0459-12, **Baxter Travenol,** Lakewood, NJ 08701, phone: (732) 363-7519. No substitutions for source.

Folic acid reference standard. Cat. no. 28600, **U.S. Pharmacopeia Convention, Inc.,** Reference Standards, 12601 Twinbrook Pwky., Rockville, MD 20852, phone: (301) 881-0666 or (800) 227-8772, fax: (301) 816-8148, e-mail: custsvc@usp.org, website: www.usp.org

Liver. No. 0133-01, **Difco Laboratories,** P.O. Box 331058, Detroit, MI 48232-7058, phone: (800) 521-0851, fax: (313) 462-8517

Potassium phosphate, monobasic. Cat. no. 7100-2.5*NY, **Baxter Travenol,** Lakewood, NJ 08701, phone: (732) 363-7519

Protease, pronase E. Cat. no. P-5147, **Sigma Chemical Co.,** P.O. Box 14508, St. Louis, MO 63178 or 3050 Spruce Tree, St. Louis, MO 63103, phone: (800) 325-3010 or (314) 771-5765, fax: (314) 771-5757, website: www.sigmaaldrich.com or www.sigmaaldrich.com/Europe

Folic acid casei medium. Cat. no. 0822-15, **Difco Laboratories,** P.O. Box 331058, Detroit, MI 48232-7058, phone: (800) 521-0851, fax: (313) 462-8517

Bacto micro inoculum broth. Cat. no. 0320-02, **Difco Laboratories,** P.O. Box 331058, Detroit, MI 48232-7058, phone: (800) 521-0851, fax: (313) 462-8517

Data anlysis software for 96-well microplate method. Microplate Manager, **Bio-Rad Laboratories,** 2000 Alfred Nobel Dr., Hercules, CA 94547, phone: (800) 424-6723 or (510) 741-1000, fax: (800) 879-2289, website: www.bio-rad.com

Preparation of Sample AACC Method 62-05

Preparation of Sample: Bread
Final approval April 13, 1961; Reapproval November 3, 1999

Objective

This method prepares a bread sample for various analyses. This is accomplished by drying the sample at room temperature, determining the mois-ture loss, and further processing the bread by reducing it to a fine 20-mesh grind. The sample may then be subjected to chemical analysis.

Procedure

1. Select representative loaf of bread and weigh to ±0.2 g. When determining whether bread conforms to USDA standards, weigh loaf no sooner than 1 hr after removal from oven. Place loaf on large sheet of smooth paper and cut into slices 2–3 mm thick, taking precautions not to lose any crumbs. Let cut slices dry on paper at room temperature until they are in approximate equilibrium with mois-ture of air; usually 15–20 hr will suffice.
2. Weigh dried slices and crumbs, and compute percent moisture lost on air-drying.
3. Grind air-dry bread to pass a 20-mesh sieve in a mill that produces mini-mum heating of sample, mix thoroughly, and store in an airtight container for chemical analysis.

Notes

1. If sample is to be employed for iron determination, do not grind dry material but reduce to suitable fineness with a wooden rolling pin.
2. If sample contains raisins or other fruit, proceed as directed above, except grind by passing twice through a food chopper instead of grinding.

Vitamins AACC Method 86-90

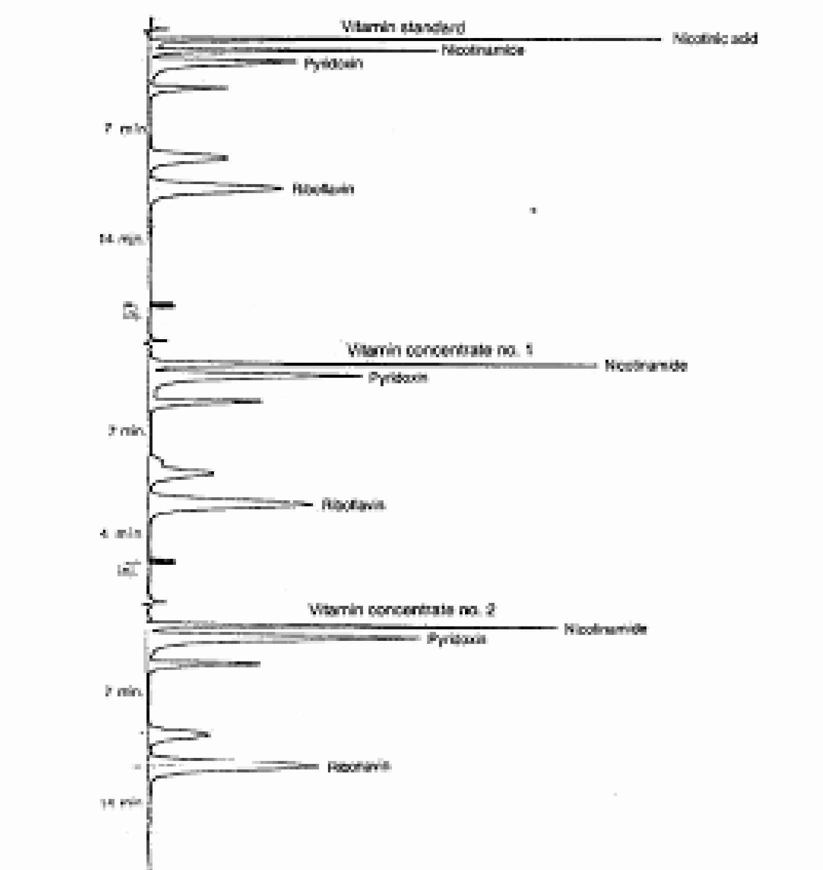
B-Vitamins in Vitamin Concentrates by HPLC
Final approval October 15, 1997; Reapproval November 3, 1999

B-vitamins are dissolved in ionic-strength-adjusted aqueous acetic acid. They are separated by paired-ion high-performance liquid chromatography (HPLC) and quantitated by comparing their absorbance with absorbance of standard solutions at 285 nm. This method measures the presence and quantity of niacin, niacinamide, pyridoxine (vitamin B6), and riboflavin (vitamin B2) in vitamin concentrates containing a minimum of 0.1% of any one of the analytes.

Apparatus
1. High-pressure liquid chromatography system, including
a. Absorbance detector.
b. Pump, for metering solvents, capable of precise delivery of 1.0 ml/min.
c. Injector, with 20-µl sample loop.
d. Column, 4.6 mm × 25 cm, 10 µm. See Note 1.
2. Volumetric flasks, 2-liter
3. Volumetric flasks, low-actinic, 100-ml.
4. Ultrasonic bath, heated.

Reagents
1. Mobile phase. See Note 1.
a. Part A: in 2-liter volumetric flask containing 1000 ml water, dissolve 2.0 g pentanesulfonic acid, sodium salt, and 20 ml acetic acid. Dilute to volume with water.
b. Part B: in 2-liter volumetric flask containing 1000 ml methanol, dissolve 2.0 g pentanesulfonic acid, sodium salt, and 20 ml acetic acid. Dilute to volume with methanol.
2. Standard solutions. Prepare fresh monthly and store in low-actinic (amber) glassware.
a. Niacin stock solution (1.0 mg/ml). Weigh 100 mg niacin; dissolve, and dilute to 100 ml with part A of mobile phase.
b. Niacinamide stock solution (1.0 mg/ml). Weigh 100 mg niacinamide; dissolve, and dilute to 100 ml with part A of mobile phase.
c. Pyridoxine hydrochloride stock solution (1.0 mg/ml). Weigh 100 mg pyridoxine hydrochloride; dissolve, and dilute to 100 ml with part A of mobile phase.
d. Pyridoxine HCl working stock solution (100 µg/ml). Pipet 10 ml stock solution into 100-ml volumetric flask and dilute to volume with part A of mobile phase.
e. Riboflavin stock solution (100 µg/ml). Weigh 25 mg riboflavin; dissolve and dilute to 250 ml with part A of mobile phase (be sure all riboflavin is dissolved. Sonicating in 50° water bath for about ½ hr will help dissolve it).
f. B-vitamin working standard. Pipet 10 ml each of niacin, niacinamide, and riboflavin stock solutions and 10 ml pyridoxine working stock solution into 100-ml volumetric flask that contains 1.0 g $CaCO_3$. Add approxi-mately 25 ml part A mobile phase, mix well, and place in sonicator for 30 min at 45–50°. Remove and cool to room temperature in water bath. Dilute to volume with part A mobile phase and mix well. Final solution will contain: niacin, 100 µg/ml; niacinamide, 100 µg/ml; pyridoxine HCl, 10 µg/ml; and riboflavin, 10 µg/ml. See Note 2.
3. Calcium carbonate, HPLC grade.

Fig. 1. Sample chromatogram, showing standard and two vitamin concentrations.



Notes

1. The following method modifications also achieved successful separations:
Column: C-18 Bondapak, 10 µm, 3.9 mm × 30 cm.
Mobile phase: Instead of 2.0 g of pentanesulfonic acid, use 1.0 g pentanesulfonic acid and 1.0 g hexane sulfonic acid in both parts A and B. Use 75% A and 25% B.
2. All standard solutions should be stored at or below 4°. Riboflavin stock solution will be more stable if stored in the dark with a thin layer of toluene above solution.
3. Order of elution is niacin, niacinamide, pyridoxine hydrochloride, and riboflavin. (See Fig. 1.)

Sources of Supply

86-90, B-Vitamins in Vitamin Concentrations by HPLC

*Detecto*r. Waters model 486 tunable UV/visible absorbance detector (also, replacement parts for model 440 absorbance detector), **Waters Associates,** 34 Maple St., Millford, MA 01757, phone: (508) 478-2000 or (800) 252-4752, fax: (508) 872-1990, e-mail: info@waters.com, website: www.waters.com; ***or*** Varichrom variable-wavelength absorbance detector (model 9050). **Varian Inc. Scientific Instruments,** 3120 Hansen Way, Palo

Alto, CA 94304-1030, phone: (650) 213-8000, e-mail: customer.service@varianinc.com, website: www.varianinc.com
Pump. Beckman model 110B or 118, **Beckman Instruments, Inc.,** 7624 W. 101 St., Bloomington, MN 55438, phone and fax: (952) 941-5126
Column. Lichrosorb RP-8, 10 µm, 250- × 4.6-mm, cat. no. 8843, **Alltech Associates, Inc.,** 2051 Waukegan Rd., Deerfield, IL 60015-1899, phone: (847) 948-8600 or (800) 255-8324, e-mail: alltechemail@alltechmail.com, website: www.alltechweb.com; ***or*** µ-Bondapak-C18, **Waters Associates,** 34 Maple St., Millford, MA 01757, phone: (508) 478-2000 or (800) 252-4752, fax: (508) 872-1990, e-mail: info@waters.com, website: www.waters.com