

AS 476:2014

Afghanistan National Standard

**FORTIFIED WHEAT FLOUR SPECIFICATION
ANNEX 1: FORTIFICATION, SAMPLING AND TESTING**

Afghanistan National Standards Authority

0. FOREWORD

0.1 Fortification of food is a public health measure aimed at improving and maintaining the health of individuals in the population through provision of an adequate level of nutrient intake by the addition of nutrients to processed foods or food products.

0.2 The roller milling of wheat flour to produce a refined white flour (low extraction rate) with long shelflife (before the onset of unpalatability due to fat rancidity) results in the loss of up to 80% of the naturally present vitamins, minerals, and fat. These nutrients are contained in the bran and germ fractions and are removed by sieving the flour after grinding of the wheat grain. Fortification is designed to replace these micronutrient losses and supply additional vitamins and minerals to rectify dietary deficiencies in the consumers.

0.3 In contrast, flours milled to include all the germ and bran, or a high percentage of these components (high extraction rate flours), have short shelf lives due to their high fat content, but retain the natural micronutrients within the flour. Wholegrain flours also retain phytates in the bran fraction which bind (chelate) essential minerals, particularly iron and zinc and inhibit their bioavailability. The minerals used for the fortification of high extraction rate flours must resist this chelation by phytate if they are to be available for digestion and absorption into the body.

0.4 Wheat flours are considered to be one of the most cost effective, technically feasible and widely used vehicles for B group vitamins, folic acid, Vitamin A and minerals through centralized milling,

Wheat flour products, especially bread and biscuits are also consumed by most sectors of the population.

0.5 This Afghanistan Standard is drafted in order to assist in alleviating of problem of deficiency in B group vitamins, folic acid, Vitamin A and minerals and to ensure that the safety and quality of fortified products are complied with, and the respective vitamins are supplied in the right amount and form.

0.6 The WHO recommended micronutrients for addition to low and high extraction flours are given in Table 1.

Table 1 WHO recommendation on addition of nutrients to flour

Nutrient	Flour extraction rate	Compound	Level of nutrient to be added in parts per million (ppm) by estimated average per capita wheat flour availability (g/day)			
			<75g/day	75-149g/day	150-300g/day	>300g/day
Iron	Low	NaFeEDTA	40	40	20	15
		Ferrous sulphate	60	60	30	20
		Ferrous fumarate	60	60	30	20
		Electrolytic iron	NR	NR	60	40
	High	NaFeEDTA	40	40	20	15
Folic acid	Low or high	Folic acid	5.0	2.6	1.3	1.0

Vitamin B12	Low or high	Cyanocobalamin	0.04	0.02	0.01	0.008
Vitamin A	Low or high	Vit A palmitate	5.9	3	1.5	1
Zinc	Low	Zinc oxide	95	55	40	30
	High	Zinc oxide	100	100	80	70
NR – Not Recommended						

1. SCOPE AND FIELD OF APPLICATION

This Afghanistan standard prescribes requirements, methods of sampling and tests for fortified wheat flours intended for human consumption. The standard is applicable only where there exists no other specific prescribed Afghanistan standard for a particular grade of fortified wheat flour.

2. Terminologies

For the purpose of this Afghanistan standard the following definitions shall apply:

2.1 Wheat flour - Derived from the milling of clean wheat to produce a fine flour for the production of bread, biscuits, noodles or other wheat-based products. Flours may vary in degree of bran and germ removal as specified in the standard.

2.2 Extraction rate -

$$\text{weight of clean wheat} - \text{weight of bran and germ removed}) \times 100 / \text{weight of clean wheat}$$

2.3 Fortificant – A micronutrient compound to be added to food vehicle.

2.4 Fortification - The addition of one or more micronutrients to food for purpose of preventing or correcting a demonstrated deficiency of one or more micronutrients in the population or specific population group.

2.5 Food vehicle - A food stuff identified to be fortified with the prescribed micronutrient (s) as prescribed in this standard.

2.6 Micronutrient - A natural or synthesized vitamin, mineral or trace element that is essential for normal growth, development and maintenance of life; and of which a deficit will cause characteristic biochemical or physiological changes.

3 REQUIREMENTS

3.1 Description

Fortified wheat flours shall be a food stuff composed of the fine endosperm of wheat including varying proportions of wheat germ and bran according to the extraction rate of the flour, to which B group vitamins, folic acid and minerals have been added.

3.2 Physical and chemical requirements

Wheat flour shall be milled from wheat of good quality, free from foreign materials, substances hazardous to health, excessive moisture, insect damage and fungal contamination and conform to this Afghanistan Standard (Ref No) based on Codex Standard 152-1985 (Rev 1-1995 Wheat Flour).

4 FORTIFICATION

4.1 The level of micronutrients to be added in wheat flour will be in compliance with the WHO recommendations (Table 1).

Based on Afghanistan Statistical annual book's data (2011/12) and decision of ANSA/TC3/SC1 members it is to be agreed that the daily adult intake of wheat flour in Afghanistan is around 430gr/day.

4.2 Micronutrients should be added as a prepared premix of stated inclusion rate from a recognized micronutrient premix supplier.

It is not permissible for the premix to be prepared by the miller. The premix must be supplied with a certificate of analysis.

Table 2: Micronutrients to be added to each kg of wheat flour

Micronutrient	Target Content (mg/kg)	Acceptable Range (mg/kg)	Chemical form	Method of reference test
Folic acid	1.0		Folic acid	
Vitamin B12	0.008		Cyanocobalamin	
Iron	15	12-18	NaFeEDTA	AOAC 944.02* (see Appendix 1)
Zinc	50		Zinc oxide	

Note: Ranges are given only for micronutrients that can be measured as added in the finished flour.

4.3 Permitted fortificants

For the purpose of vitamin fortification of wheat flour, the fortificants must conform to the United State Pharmacopoeia, British Pharmacopoeia, Food Chemical Codex, the FAO/WHO General Principles for the use of Food Additives of the Codex Alimentarius, Volume 1 or the European Pharmacopoeia.

5. METHOD OF FORTIFICATION

The method of fortification must ensure uniform distribution of the added fortificants within the flour such that the all samples provide levels for vitamin A and iron within the minimum and maximum limits set in Table 3. Batch or continuous fortification processes may be used, although most roller mills operate a continuous milling and fortification process. The most common process of continuous fortification is illustrated below (Fig 1), though other technologically competent process may be used.

The fortification stage typically requires the uniform distribution of approximately 150g – 250g of micronutrient premix in each ton of wheat flour. Actual addition levels will be advised by the micronutrient premix supplier. Premixes may vary in density depending on formulation and carrier.

The fortification system should comprise the following or their equivalent.

1. A flour flow from the milling and sifting section which is known and constant.
2. A 'feeder, or 'dosifier' for the metering of dry micronutrient premix.
3. A means to agitate the premix so that it feeds smoothly and uniformly without bridging into the feeder .
4. A mechanical or electronic means of adjusting the rate of output flow of premix.
5. A conveying system to deliver the premix from the feeder to the flour.
6. A mixing capability after the premix is added to the flour.

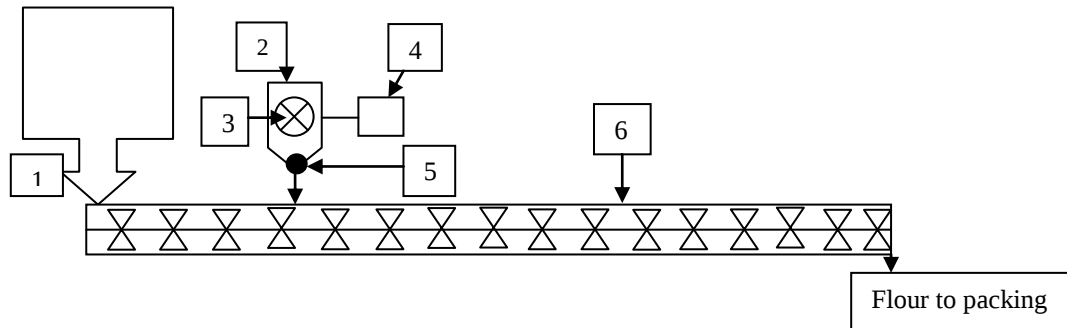
Within the concepts of Good Manufacturing Practices, the mill must ensure routine, effective and calibrated control of :

-
- flour flow into the fortification line,
- premix from the feeder into the mixer
- dwell time in the mixing system

The capacity of the micronutrient feeder must be consistent with the production rate of flour coming from the mill and where the feeder is operating within 30-70% of its maximum delivery rate.

If necessary, the premix may be diluted with wheat flour to a uniform blend before use.

Fig 1. Example of Continuous Fortification of Wheat Flour



6. RECORDS OF PERFORMANCE

It is a requirement within this Standard that all mills retain for inspection records relating to the performance of fortification. These are:

1. Certificate of license to prepare fortified wheat flour
2. Certificate of analysis and inclusion level of premix from the supplier
3. Date of premix delivery/batch number and date of usage.
4. Daily record of flow rate of flour through the mill
5. Daily record of quantity of premix used
6. Daily record of calibration of premix feeders
7. Daily record of confirmatory tests that flour has been fortified using the rapid NaFeEDTA (حديد گريدہ) assay procedure (Appendix B1/2)
8. Written Standard Operating Procedures (SOP) related to the calibration of all components of the flour fortification system.

7. HYGIENE AND QUALITY MANAGEMENT

Fortified wheat flour shall be produce in accordance with the requirements given in Afghanistan Standard ABC based on the Codex Recommended General Principles of Food Hygiene CAC/RCP 1-1969 Rev 4-2003 including the adoption of the principles of Hazard Analysis Critical Control Point (HACCP).

8 SAMPLING AND TESTING

- 8.1** The method of drawing representative samples from a warehouse shall be in accordance with Codex Sampling Plans For Prepackaged Foods AQL 6.5 (XOT13), (or Afghanistan Standard equivalent)
- 8.2** Routine sampling on line at the mill will conform to the maintenance of Good Manufacturing Practice through Standard Operating Procedures and in relation to the production output of the mill.
- 8.3** Testing shall be done in accordance with the respective prescribed methods provided in Tables 2 and 3 and Appendix 1. The method in Tables 2 and 3 shall be used as the reference method for the determination of NaFeEDTA. The rapid photometric method (Appendix 2) shall be used as the routine method for determination NaFeEDTA.

NOTE: “Pure chemicals” shall mean chemicals that do not contain impurities which affect the results of analysis (chemicals of analytical grade).

8.4 In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated is to be rounded off, it shall be done in accordance with Afghanistan Standard XYZ

9. PACKAGING, MARKING AND LABELING

- 9.1** Packing – Fortified wheat flour shall be packed in food grade, non-absorbent material which has no adverse influence upon the composition of the product, its properties and appearance. The package shall be sunlight resistant and sealed to safeguard the product in terms of the following.
- a) Safety and hygiene, that is, prevent contamination of the product
 - b) Nutrition value; that is minimizes loss of nutrients.
 - c) Technological qualities of the products; and
 - d) Organoleptic qualities of the product and the food to which is to be Applied

9.2 Marking and labeling

Containers or bags of fortified wheat flour shall be legibly and indelibly marked with the following information

- a) Name of the product
The name of the product shall be fortified wheat flour
- b) Date marking.
The date of minimum durability (preceded by the words “Date of manufacture and expiry date”) shall be declared by the day, month

and year in uncoded numerical sequence except that the products with a shelf life of more than three months; the month and year shall suffice.

- c) List of ingredients
- d) The statement “store in cool and dry place away from sunlight” shall be included in close proximity to the date marking.
- e) The fortified vitamin nutrient and the average quantity in mg per 100 g of the product.
- f) The name, postal and physical address of the manufacturer of the product.
- g) Country of origin
- h) Net content by mass in SI units
- i) Lot/Batch identification number in code or in clear
- j) Manufacturers registered trade mark; if any.

9.3 Certification mark

- In the case of voluntary implementation, each container of fortified wheat flour may also be marked with the recognized mark of competent certification body.
- After the implementation has become compulsory, Each container shall be marked with the logo of supervising body..

10 CAVEAT

Separation: If any part, section or provision of this Act shall be declared invalid or unconstitutional, other provisions or parts thereof which are not affected thereby shall remain in full force or effect.

Appendix 1 RECOMMENDED REFERENCE METHOD FOR THE MEASUREMENT OF IRON ADDED AS NaFeEDTA IN FORTIFIED WHEAT FLOUR

Cunnif, D (Ed). Official Methods of Analysis of AOAC International. 1997. 16a ed. AOAC International, Gaithersburg. No.944.02. See: Quantitative spectrophotometric method for determining total iron in wheat flour pp 16-20 in “Manual of methods for determining micronutrients in fortified foods. 2010 USAID” is explained as follow:

Cunnif, D (Ed). Official Methods of Analysis of AOAC International. 1997. 16a ed. AOAC International, Gaithersburg. No.944.02. See:

Cunnif, D (Ed). Official Methods of Analysis of AOAC International. 1997. 16a ed. AOAC International, Gaithersburg. No.944.02. See:

***Note:** Method measures total iron in flour (intrinsic + fortificant). To determine the level of added iron as fortificant, it is necessary to measure the total iron in the untreated flour and deduct this from the total in fortified flour.

Method outline:

1. Dry ash 1.0g fortified flour at 550°C for 6 hours.
2. Oxidize all iron in ash to ferric iron with concentrated nitric acid.
3. Evaporate nitric acid and solubilize iron in concentrated sulphuric acid.
4. Reduce all iron to ferrous iron using hydroxylamine hydrochloride.
5. React ferrous iron with 1, 10 phenylanthroline to develop red/orange colour.
6. Measure colour absorbance at 510nm in UV spectrophotometer.
7. Calculate iron concentration using a calibration curve prepared using standard iron solutions.
8. Repeat using unfortified flour to determine intrinsic iron content.
9. Added iron = Total iron – intrinsic iron

C. Quantitative spectrophotometric method for determination of total iron in wheat flour

I. References

Cunnif, D (Ed). Official Methods of Analysis of AOAC International. 1997. 16a ed. AOAC International, Gaithersburg. No.944.02.
AOAC. Official Methods 944.02

II. Principle

The determination of total iron in foods usually includes the total combustion of organic materials leaving only the ash, which contains the mineral part of foods. This process transforms all iron present to the oxidized ferric form (Fe³⁺). A solution of the ash is prepared using hydrochloric acid and the iron (III) is reduced to iron (II) using hydroxylamine hydrochloride. The ferrous ion (Fe²⁺) can be determined spectrophotometrically by forming colored complexes using several chromogens that interact with iron (Fe²⁺) such as 1,10-phenanthroline.H₂O; bathophenanthroline, (a disulphonic salt of 4,7- diphenyl – 1,10 phenanthrolyne); α,α - dipyridile (2,2' bipyridine); or ferrozyne (acid[3–(2-pyridyle)- 5,6 –bis-(4- phenylsulphonic) –1,2,4- triazine). The color reaction has

to be performed under pH-controlled conditions suitable for the chromogen. In order to reduce the competition by hydronium ions (H_3O^+) for the ligand, a solution of 2 M sodium acetate is added.

III. Critical points

- Clean and wash all glassware following appropriate cleaning procedures for analysis of minerals.
- All reagents have to be analytical grade with the minimum possible content of iron. The water used has to be distilled and deionized, with less than $2\mu\text{Si/cm}$ conductivity, or 10^{-6} (ohm. cm) $^{-1}$.
- It is critical to maintain the pH of solutions between 5-6. If necessary, more sodium acetate can be added to increase the pH.

IV. Equipment and materials

- Analytical balance
- Cuvettes (1 or 3 mL capacity, 1 cm pathlength and suitable for reading in visible light)
- Furnace (Temperature > 500 °C)
- Funnels
- Graduated cylinders
- Porcelain crucibles
- Spectrophotometer UV/VIS
- Volumetric flasks (25, 100, 250 mL)
- Volumetric and graduated pipettes
- Vortex mixer

V. Reagents

- Hydrochloric acid (HCl), 37%, p.a., $d = 1.19\text{ g/mL}$, $\text{Fe} < 28\text{ }\mu\text{g/mL}$, mol wt 36.46.
- Nitric acid (HNO_3), p.a., 65 %, $d = 1.39\text{ g/mL}$, $\text{Fe} < 1\text{ }\mu\text{g/mL}$, mol wt 63.01.
- Sodium acetate trihydrated, ($\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$), p.a., 99% $\text{Fe} < 200\text{ }\mu\text{g/kg}$, mol wt 136.08.
- 1,10-phenanthroline-monohydrate, p.a., mol wt = 198.23.
- Hydroxylamine hydrochloride ($\text{NH}_2\text{OH}\cdot\text{HCl}$), p.a., mol wt = 69.49.
- Glacial acetic acid (CH_3COOH), p.a., mol wt. 60.05.
- Standards Solution for iron Ammoniacal Ferrous Sulfate, $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2\cdot 6\text{H}_2\text{O}$, mol wt 392.14

VI. Solutions

a. 1,10-phenanthroline. H_2O

Dissolve 0.1 g 1,10-phenanthroline. H_2O in ca 80 mL H_2O at 80° C, let it cool down, and dilute to 100 mL. Store in a dark bottle under refrigeration. The solution is stable for several weeks. Discard if the solution turns lightly pink, indicating that it has been contaminated with iron.

b. Acetate Buffer-2 M

In a 500 mL beaker add 68 g sodium acetate trihydrate, and dissolve in approximately 100 mL of deionized water. Add 60 mL of glacial acetic acid and dilute to 500 mL. Transfer the solution into a glass flask with hermetical cover. The solution is stable for indefinite time.

c. Hydroxylamine Hydrochloride –10 %

Add 10 g of hydroxylamine hydrochloride into a beaker, and dissolve with 100 mL of deionized water with the aid of a glass rod. Transfer the solution into a glass flask with hermetical cover. The solution is stable for indefinite time.

VII. Standard solutions

a. Primary Standard Solution of Iron – 1000 mg/L

Dissolve 3.512 g of $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ in distilled water, and add a few drops of concentrated HCl. Dilute to 500 mL in a volumetric flask. Transfer the solution to a plastic bottle. This solution is stable for indefinite time, unless a light pink color is observed indicating contamination.

b. Secondary Standard Solution of Iron-10 mg/L

Into a 500 mL volumetric flask pipette 5 mL of the Primary Standard Solution (1000 mg/L). Add 2 mL concentrated HCl. Fill with distilled water up to the 500 mL mark. Transfer the solution to a plastic bottle and store it in a cool dry place. This solution is stable for about 6 months.

c. Standard Solutions for the Calibration Curve

Solutions for the calibration curve will have iron levels from 0.0, 0.2, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 5.0 mg/L (ppm). Into 100 mL volumetric flasks, pipet the amounts of the Secondary Standards Solution (10 mg/L) that are specified in the table, and then make up to volume with deionized water.

Iron (mg/L,ppm)	Volume of the Secondary Solution (10 mg/L) to be added (mL)
0.0	0.0
0.2	2.0
0.5	5.0
1.0	10.0
1.5	15.0
2.0	20.0
3.0	30.0
4.0	40.0
5.0	50.0

Mix thoroughly by inverting the flask several times. Transfer the solutions into properly labeled plastic bottles. These standard solutions are stable for approximately six months.

VIII. Procedure

a. Dry digestion (ashing)

1. Clean the porcelain crucibles, and label using a high-temperature proof marker.
2. Dry crucibles in the oven at 110 °C and cool in a desiccators. Repeat until constant weight is attained.
3. Take about 100 g of the flour and grind in a mortar and pestle and mix well.
4. Weigh 1 g of the previously homogenized sample in duplicate. Weigh by difference directly into the crucibles using an analytical balance and record the weights accurately to 3 decimals (0.001 g).
5. Place the crucibles into the muffle furnace at 550 °C and heat for 6 hours.
6. Turn the oven off and wait until the temperature has decreased.
7. The ashing is complete when a white or grayish ash is obtained. If this is not the case, continue the ashing until white/grayish ash is obtained.
8. Let the crucibles cool down for 5 minutes and place in a desiccators for 1 hour until they reach room temperature.

b. Preparation of the ash solution

1. Add 5 mL of concentrated HNO₃ to the crucible, pouring the acid onto the inside walls of the crucible.
2. Evaporate the acid by heating the crucibles on top of a hot plate at low temperature, solution should not boil.
3. Dissolve the remaining residue by adding 2 mL of concentrated HCl, and heat for few minutes, taking care that the solution does not spill out the crucible.
4. Let the crucible cool down and transfer the solution quantitatively into a 25.0 mL volumetric flask. Wash crucible with distilled water and bring to volume with deionized water.

c. Determination of iron

1. Pipet 10.0 mL of the sample solution into 25.0 mL volumetric flask, then add 1.0 mL of hydroxylamine hydrochloride solution, mix well and let it stand for 5 minutes.
2. Pipet 10.0 mL of the standard solutions prepared in VII.c, into 25.0 mL volumetric flasks, and follow the same procedure as for the samples.
3. Add 5.0 mL acetate buffer and 4.0 mL of 1,10-phenanthroline to each flask. Mix well and color will start developing.
4. Let stand it for 30 min and then make up to volume (25 mL) using deionized water.
5. Turn on the spectrophotometer 15-20 minutes before using it to warm up.
6. Adjust the wavelength to 510 nm Set the mode to Absorbance.
7. Set the instrument to zero Absorbance using deionized water.
8. Read the absorbance of the 0.0-mg/L standard solution (blank) and record the absorbance.
9. Read the absorbance for the standard solutions and flour sample solutions.

10. If color intensity of the samples is too high, make appropriate dilution of the sample solutions and record the absorbance again.

Note:

1. This reducing step is very important because iron oxidizes to Fe (+3) during ashing and reaction with concentrated acids.
2. In the case of the other chromogenic agents (bathophenanthroline, a-dipyridyle, or ferrozine) use 2 mL of the solutions instead of 4 mL.
3. For the other chromogenic agents, the corresponding wavelengths are: bathophenanthroline: 535 nm; a-dipyridyle: 521 nm; and ferrozine: 562 nm.

IX. Calculation

1. Plot a graph of the absorbance values of the standard solutions (y-axis) against concentration (x-axis) and obtain the equation of the standard curve. The equation will be similar to the one obtained for soluble iron.
2. Calculate the concentration of soluble iron in the sample solution solving the standard curve equation for x.
3. Calculate the concentration of soluble iron in the flour sample using the equation below.

$$\text{Iron} \left(\frac{\text{mg}}{\text{kg}} \right) = \frac{[\text{Fe}] \times 25}{w}$$

Where w is around 1.0 g or the weight used in the ashing steps.

Appendix 2 RECOMMENDED ROUTINE METHOD FOR THE MEASUREMENT OF NaFeEDTA IN FORTIFIED WHEAT FLOUR

The method is a modern rapid kit assay based on the solubilisation of iron compounds in water at different temperatures and times followed by the reaction of the solubilized iron with a colour complexing reagent.

The kit is available from www.bioanalyt.com using extraction reagent vials iEx IRON and photometer iCheck IRON.

Method outline:

1. Dilute 100g of flour fortified with NaFeEDTA in 1000ml of water and shake vigorously to prepare a homogeneous suspension.
2. Syringe 0.4ml of suspension into an activated iEx reaction vial and shake vigorously.
3. Incubate vial for 3 hours at 50°C to develop colour reaction. Shake vigorously every 15 minutes.
4. Centrifuge vial at 300rpm (rounds per minute) for 1 minute to separate a clear upper phase.
5. Place vial in iCheck photometer to measure colour absorbance and calculate total iron concentration in mg/kg flour.
6. To obtain the level of intrinsic iron, repeat the assay using unfortified flour, but incubate for 60 minutes at 20°C (step 3).

7. Added iron = Total iron – intrinsic iron

Reference:

- Fortified wheat flour version 4.0 2011. wfp251105.pdf
- “Manual of methods for determining micronutrients in fortified foods. 2010 USAID”
- Central Statistic Organization of Afghanistan (Year book 1390)