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PAKISTAN STANDARD SPECIFICATION FOR FORTIFIED WHEAT FLOUR (AATA) (1ST REVISION)

PAKISTAN STANDARDS & QUALITY CONTROL AUTHORITY
Standards Development Centre, PSQCA Complex, ST-7/A, Block-3
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PAKISTAN STANDARDS & QUALITY CONTROL AUTHORITY

(STANDARDS DEVELOPMENT CENTRE)

(AGRICULTURE & FOOD DIVISION)

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27.	Director (Technical)	Continental Biscuits (Pvt) Ltd., 6th Floor, PIDC, House **Karachi.**
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37.	Mr. Zuzzer A. Shamsuddin, Consultant,	Hamilton Courts, Clifton Road, **Karachi.**
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39.	Director,	Quality Control Centre, PSQCA, **Karachi.**

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2.	Mr. Lal Muhammed Rajar, Examiner (Agri. & Food)	Standards Development Centre, PSQCA, **Karachi.**

PAKISTAN STANDARD SPECIFICATION
F O R
FORTIFIED WHEAT FLOUR (AATA) (1ST REVISION)

0. FOREWORD

0.1 This Pakistan Standard was adopted by the Pakistan Standards & Quality Control Authority, Standards Development Centre, on **09-03-2017** after the draft finalized by the Cereal Pulses and their Products Technical Committee had been approved by the National Standard Committee for Agriculture and Food Products.

0.2 While formulating this Standard the Technical Committee is responsible for the preparation of this Pakistan Standard.

0.3 In an effort at nutritional upgrading, Fortified Wheat Flour (Aata) to which vitamins and minerals have been added is at present being prepared and marketed in the country. This standard is expected to help in exercising proper quality control in the manufacture of fortified wheat Atta of good quality under hygienic conditions.

0.4 Wheat Aata is prepared in Pakistan by grinding whole wheat grains either in small stone mills operated by animals or human labour, in large mills using mechanical power or in large roller flour mills or other means.

0.5 Pakistan Standard for Wheat Flour (Aata) PS:380 for Whole Meal Wheat Aata has already been published. Separate standard on Fortified Wheat Atta is also being brought out simultaneously.

0.6 For the purpose of deciding whether a particular requirements of this standard is complied with final value observed or calculated expressing the result of test or analysis shall be rounded off in accordance with PS: 103-1991 (1st Rev). Method of Rounding off Numerical Values the number in significant places retained in the rounded value shall be same as that of the specified value in the standard.

0.7 All the ingredients and processes shall be in accordance with PS:3733 for Halaal Food Management System Requirements for any organization in the Food Chain.

1. SCOPE

1.1 This standard prescribes the requirements and the methods of sampling and test for fortified wheat Atta, hereafter termed Fortified Wheat Flour (Aata).

2. REQUIREMENTS

2.1 The Fortified Wheat Flour (Aata) shall be prepared by thoroughly and uniformly admixing recommended suitable level of iron, folic acid, zinc and Vitamin-B_{12} with Wheat Aata of good quality (see PS:380 for Whole Meal Wheat Aata). It shall be in the form of powder having characteristics taste and flavour. The product shall also be free from rancidity and insect, rodent or fungus infestation. It shall also be free from fermented, musty or other objectionable odour. It shall neither have any ingredient other than those specified nor any extraneous matter.

NOTE—The appearance, taste and odour shall be determined by sensory evaluation tests.

2.1.1 The fortified wheat flour should be prepared by adding NaFeEDTA as an inert carrier. The vitamins and the minerals shall be of pharmaceutical or food grade as per CAC Standard.

The product shall also conform to the requirements given in Table-1.

TABLE 1 - REQUIREMENT FOR FORTIFIED WHEAT FLOUR (AATA) (Clause 2.4)

SL No	Characteristics	Requirements	Method of Test Reference to PS
1	Moisture, percent by mass, max.	14	*Appendix B of PS:380
2	Total ash (on dry basis), percent by mass, Max	2.0	*Appendix C of PS:380
3	Acid insoluble ash (on dry basis), percent by mass, Max	0.1	*Appendix D of PS:380
4	Gluten (on dry basis), percent by mass, Min	8.0	*Appendix E of PS:380
5	Total protein (N x 6.25) (on dry basis), percent by mass, Min	8.0	
6	Crude fibre (on dry basis), percent by mass, Min	2.0	*Appendix F of PS:380
7.	Acidity percent expressed as Sulphuric Acid max.	0.115	Determined by the alcoholic extraction process
8.	Total Aflatoxin in Wheat Flour shall not be exceed	20 ppb	-

- Pakistan Standard Specification for Whole Meal Wheat Aata

TABLE 2 LEVEL OF MINERALS AND VITAMINS

1	Folic acid	Not less than 1.0 ppm	Appendix B of this standard
2	Iron (in the form of NaFeEDTA)	Not less than 15 ppm	Appendix C of this standard
3	Zinc (in the form of Zinc oxide)	Not less than 30 ppm	
4	Vitamin B_{12}	Not less than 0.008 ppm	

2.3 HYGIENIC CONDITION

The Fortified Wheat Flour (Aata) shall be prepared manufactured, packed, stored and distributed under hygienic conditions (PS:3111) for Code for Hygienic Condition for Food Processing Units.

3. PACKING AND MARKING

3.1 PACKING

3.1.1 Aata packing shall be made in 5,10, 20, 40 Kg or any other suitable size acceptable to the purchaser and vendor.

3.1.2 The packing material shall be a food grade cotton, jute, polypropeline or any other suitable material agreed between the purchaser and vendor.

3.1.3 The packing will be sealed or stitched properly to avoid any contamination, loss of Aata from the packing or any infestation.

3.2 MARKING

Each Atta bag shall be clearly marked in such a manner that the dye or ink does not penetrate into the material. Each bag shall be suitably marked so as to give the following information.

a) Name of the product i.e. Fortified Wheat Flour (Aata)
b) Name and address of the Miller / Manufacturer.
c) List of ingredients/ additives
d) Trade Mark
e) Average net weight
f) Batch and Code Number
g) Date of manufacture and expiry
h) PS: Mark and License Number
i) Logo of Fortified Wheat Flour (Aata).

3.2.1 The product shall be labeled as prescribed in PS:1485 for labeling of Pre-packaged foods.

4 SAMPLING

4.1 The method of drawing representative samples of the material and the criteria for conformity shall be as prescribed in appendix-A.

5 TEST

5.1 Tests shall be carried out as prescribed under 2.1 and in the appropriate Pakistan Standards specified in column 4 of Table-1.

5.2 QUALITY OF REAGENTS

Unless specified otherwise, pure chemicals shall be employed in tests and distilled water (PS:593) for Water for Analytical Laboratories shall be used where the use of water as a reagent is intended.

APPENDIX – A

Sampling Techniques and Management of Sample

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

Composite Sample

Flour samples of approximately 250 g, each taken with plastic or metal utensils from 5-7 different bags, are thoroughly mixed together in sample mixer for 10 minutes. Composite samples are divided into three fractions, sealed and labeled separately. One sample is retained in mill as reference sample, while two others are given to relevant authorized person who sends one sample to the laboratory for analysis and holds the third one. If the mill owners are not satisfied with the analysis results of the authorized laboratory they can use the reference sample held at mill for duplicate analysis. The following important points should be taken into account for composite sampling:

1. The samples should be labeled for their identification. The labels should comprise the information about name of mill, sampling date and time and type of flour whether fortified or unfortified.
2. Unfortified flour samples should also be taken for comparing the results with fortified one.
3. Avoid keeping the fortified flour or food samples in direct sun light or in front of sharp light.
4. The storage of samples in a sequence should be ensured for easy tracking.

When an official inspector is collecting samples it should 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 and would stay with Inspector.

Control Sample

As the wheat flour contains natural iron contents in varied degree and it is difficult to determine narrow range for natural Iron contents in the flour, so it is important to collect a control sample from the same batch of flour which being fortified and sampled for quantitative testing. The comparison between total iron contents from the Fortified Wheat Aata and non fortified Wheat Aata will determine the value for added Iron contents.

APPENDIX - B

DETERMINATION OF FOLIC ACID BY HPLC ANALYSIS

1. CHEMICALS AND EQUIPMENT

1.1 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 grad
Reference flour	
Sodium acetate, anhydrous	Analytical reagent grade
Sodium chloride	Analytical reagent grade

1.2	**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

2 SAFETY

3 OPERATION

3.1 SOLUTION PREPARATION

3.1.1 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.

3.1.2 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 is: 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.

3.1.3 "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.

3.1.4 "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.

3.1.5 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.

3.1.6 Salt Eluent

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

3.2 SAMPLE PREPARATION

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

3.2.1 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.

3.2.2 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.

3.2.3 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.

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

3.3 **EQUIPMENT PREPARATION**

3.3.1 Column Switching

3.3.1.1 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.

3.3.1.2 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.

3.3.2 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.

3.4 **ANALYSIS**

3.4.1 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.

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

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

APPENDIX - C

QUANTITATIVE TESTING OF IRON IN FORTIFIED WHEAT FLOUR (AATA) THROUGH SPECTROPHOTOMETRIC METHOD (ADOPTED FROM AACC METHOD 40-41B)

This method is approved by AACC for quantitative determination of iron in cereals and cereal based food products.

1.1 Principle

Organic constituents in a food sample are broken down by dry or wet ashing at high temperature (500-550 ºC) and the inorganic constituents are dissolved in slightly acidic solution. Solubilized ferrous iron is then reacted with a chromogenic reagent, orthophenanthroline, in the presence of a reducing agent (such as hydroxylamine hydrochloride), resulting in a pink-colored complex. The concentration of iron is determined by its spectrophotometric absorbance at 510 nm.

1.2 Materials

1. Volumetric flask 25 mL, 100 mL, 250 mL and 1000 mL
2. Beakers, 250 mL
3. Manual volumetric pipettes (200-1000 mL)
4. Porcelain crucibles
5. Watch glasses
6. Pipette tips
7. Graduated tubes
8. Tips for ‘blue’ pipettes
9. Test tubes, 10 mL

1.3 Equipment

1. Vortex mixer
2. Analytical balance
3. Spectrophotometer (521, 535, or 562 nm)
4. Hot plate
5. Muffle furnace
6. Eppendorf pipette (100 and 500 mL)

1.4 **Reagents**

1. Sodium acetate (CH3COONa.3H2O).
2. Hydrochloric acid (HCl)
3. α-α-dipyridyl (2,2' bipyridine) (C10H8N2)
4. Bathophenanthroline, 4,7-diphenyl-1,10-phenanthroline-disulfonic acid (C24H16N2O6S2)
5. Hydroxylamine hydrochloride (NH2OH.HCl).
6. Iron standards should be chosen from the following: - Electrolytic iron - Ferrous ammonium sulfate, (NH4)2Fe(SO4)2.6H2O - Iron standard

1.5 **Procedure**

1.6 **Preparation of Solutions**

a. Hydrochloric acid (HCl, 6M): Add 200 mL deionized water to a 500 mL beaker. Then gradually add 250mL concentrated HCl. Let it cool before transferring to a 500 mL volumetric flask and make up the volume with deionized water. Finally, transfer this solution, considered to be stable for indefinite period of time, to glass flask and close with a glass stopper.

b. Hydrochloric acid (HCl, 0.96M): Transfer168 mL deionized water into a 500 mL beaker and then gradually add 32 mL 6M HCl. The solution thus prepared is considered to remain stable indefinitely which is later on transferred to a glass flask closed with a glass stopper.

c. Hydroxylamine hydrochloride (10%): To a 500 mL beaker add 50 g hydroxylamine hydrochloride and then 400 mL deionized water. After complete dissolution, through stirring with a glass rod, transfer this solution to a 500 mL volumetric flask and make up the volume with deionized water which can be retained for indefinite time in glass flask having glass stopper.

d. Bathophenanthroline (0.025%) in sodium acetate (2M) (Bathophenanthroline0.025%/CH3COONa-2M): Sodium acetate trihydrate (108.8 g) and bathophenanthroline (0.10 g) are mixed with deionized water (400 mL) in 500 mL beaker with a glass rod using moderate heat, if necessary, for complete mixing/dissolution. Make sure that bathophenanthroline is fully dissolved because it is feebly soluble at room temperature. Store this solution, considered to be stable for 3 to 4 months, in a glass or plastic flask and is discarded if pink color develops which is an indication of its contamination with iron.

1.7 **Preparation of Iron Standards**

a. Standard (1000 ppm): Dilute reagent DILUT-IT, according to manufacturer's instructions, in a 1 L flask using deionized water. Alternatively, dissolve 3.512 g Fe(NH4)2(SO4)2.6H2O in distilled water, add 2 drops of concentrated HCl and dilute to 500 mL.

b. Standard (10 ppm): To a 100 mL volumetric flask, add 1.0 mL (measured with a volumetric pipette) of 1000 ppm iron standard. Then add 16 mL of 6M HCl and adjust the volume with deionized water.
c. Preparation of standards: Prepare standards in 100 mL volumetric flasks. To make concentrations of 0.0, 0.3, 0.6, 1.2, 2.4, and 4.0 ppm, which are equivalent to 0, 7.5, 15, 30, 60, and 100 ppm (mg/kg) of iron in flour, add 8 mL 6M HCl to each flask, then add a corresponding quantity of 10 ppm iron standard (see table below). Adjust to volume with deionized water. Store in dark glass flasks and cover with glass stoppers. These standards are stable for 2 to 4 weeks in a temperature controlled environment.

1.8 (a) **Procedure Ashing the Sample**

a. Accurately weigh 2 g previously homogenized sample in duplicates and transfer to a porcelain crucible.
b. Ash the sample in muffle furnace at 550 °C for 4 hours. The sample is adequately ashed when a white or grey colored ash is obtained which is then cooled to room temperature.

1.8(b) **Solubilizing the Ash**

a. Add 5 mL of 6M HCl to the porcelain crucible allowing the acid to wash the walls of the crucible and evaporate until dry on the hot plate, taking care that the sample does not splash outside the crucible at any time.
b. Dissolve the residue in 5 mL 6M HCl and place on hot plate for 5 minutes.
c. Filter into a 50 mL Volumetric flask using a Pasteur pipette. Wash the crucible with distilled water and quantitatively transfer the contents of the crucible into filter paper.
d. Add 5 mL of 10% hydroxylamine solution to the flask and mix by smoothly rotating the flask and adjust the volume with deionized water.

1.9 **Iron Determination**

1. Label, in duplicate, 10 mL test tubes for standards (0.0, 0.3, 0.6, 1.2, 2.4, 4.0, and up to 10 ppm), control, and samples.
2. To each corresponding tube, add 5 ml of standard, control, or sample.
3. Add 0.5 mL 10% hydroxylamine solution. Vortex.
4. Add 4 mL of dipyridyl-0.025%/sodium acetate-2M or bathophenanthroline0.025%/sodium acetate-2M solution. Vortex and leave for 20 minutes.
5. Read the absorbance of the solution in each tube in a spectrophotometer at 521 nm for dipyridyl or 535 nm for bathophenanthroline. Adjust to zero using distilled water.

1.10 **Interpretation/Calculations**

1. Plot concentration of iron in ppm (y) versus absorbance (x).
2. The concentration of iron can be calculated directly using a regression equation. To report the concentration of iron in mg of iron per kg of food, multiply the results obtained in ppm (mg/L) as:

   Iron (mg/kg) = conc. of iron (mg/L) x [(50 x 10-3 L) / (sample in kg)]

   Iron (mg/kg) = conc. (ppm) / weight (g) x 50

1.11 **Notes**

1. Make sure that all glasswares used are appropriate for mineral analysis. Reagents should be of analytical grade with as low concentration of iron as possible.

2. Distilled and deionized water should only be utilized with a conductivity less than 2 mSi/cm or 10-6 (ohm.cm)-1.

3. When using the dipyridyl chromogen, it is critical to maintain the pH of the solution between 5 and 6. If necessary, add sodium acetate buffer solution.