

Qualitative Spot Test for Zinc in Fortified Maize Flour and Rice using Dithizone

Standard Operating Procedure

Purpose

The purpose of this test is to quickly and inexpensively assess the presence of added zinc in fortified cereal grains, namely maize flour (white and yellow) and rice (white and brown).

Scope

Use this method to test for the presence of added zinc in fortified foods, specifically yellow and white maize flour and white or brown rice using extruded or coated kernels.¹

Synopsis

Zinc in fortified maize flour and fortified white or brown rice (using extruded or coated kernels) reacts with a solution of dithizone to form pink-colored spots.

Time Required²

10–15 minutes

Safety

Refer to pages 7–8 for reagent safety information before preparing reagents and conducting the assay.

Equipment

Prepare the reagent in a laboratory setting so you can take appropriate safety precautions if needed. The completed dithizone reagent may be used in both laboratory and field environments.

¹ We also applied this assay to fortified refined wheat flour during the validation process. See Annex 3 for application in refined wheat flour results.

² This refers to time of sample preparation to time of result interpretation and recording; it does not include time required for reagent solution preparation.

Table 1. Equipment Needed

Laboratory Equipment	Qty	Non-Laboratory Alternative	Qty
Analytical balance capable of weighing to at least 0.1 milligram (mg)—Ohaus Explorer Precision or equivalent	1	not applicable (N/A)	
A balance capable of weighing ~25 grams (g)	1	N/A	
Weigh boats—250 milliliter (mL), VWR 10803-170 or equivalent	1	~20 centimeter ² sheet clean white paper	1
General purpose disposable polyethylene ³ transfer pipettes—VWR 16001-174 or equivalent	1	N/A	
Amber volumetric flask—100 mL, 1 liter (L)	1	N/A	
Beakers—50 mL, 250mL	1	N/A	
Polyethylene reagent bottles ⁴ —100 mL, 1.0 L	1	Any plastic or polyethylene capable of containing 1.0 L.	1
Vortex mixer—VWR 58816-121 or equivalent	1	Shake the solution (if the bottle lid is tight) or mix manually (e.g., with a plastic spoon) ⁴	
Disposable gloves (as required)	-	N/A	

Reagents

- 2.0 x 10⁻³ molarity (M) dithizone solution⁵

Procedure

The use of disposable gloves is recommended. The test does not require a fume hood. Use clean materials to avoid contaminating the sample.

Fortified Maize Flour Samples

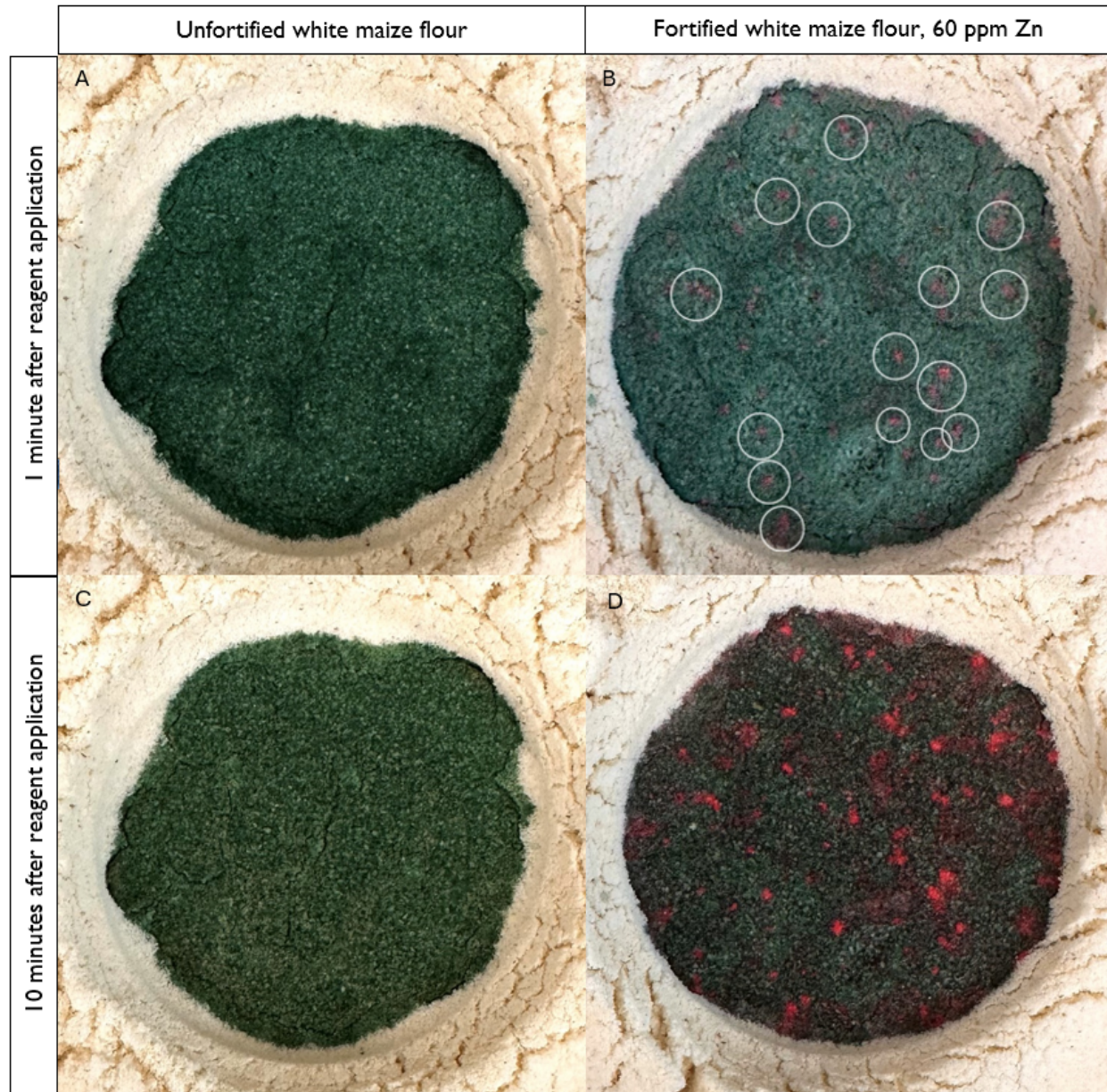
1. Weigh approximately 25 g (~55 mL) sample into a 250 mL weigh boat, or by volume in a 100-250 ml graduated beaker, and transfer it to a flat surface, a petri dish, or a white tile.
2. Spread the top of the flour pile to create a well in the sample, using the bottom of a small, clean 50 mL beaker or spatula.
3. Using a polyethylene transfer pipette, carefully add 10–15 drops of the dithizone solution to wet the flattened surface area.
4. Set aside for 10 minutes. If pink-colored spots appear, added zinc is present.

³ Should not be glass.

⁴ Plastic is ideal for minimizing metal contaminants.

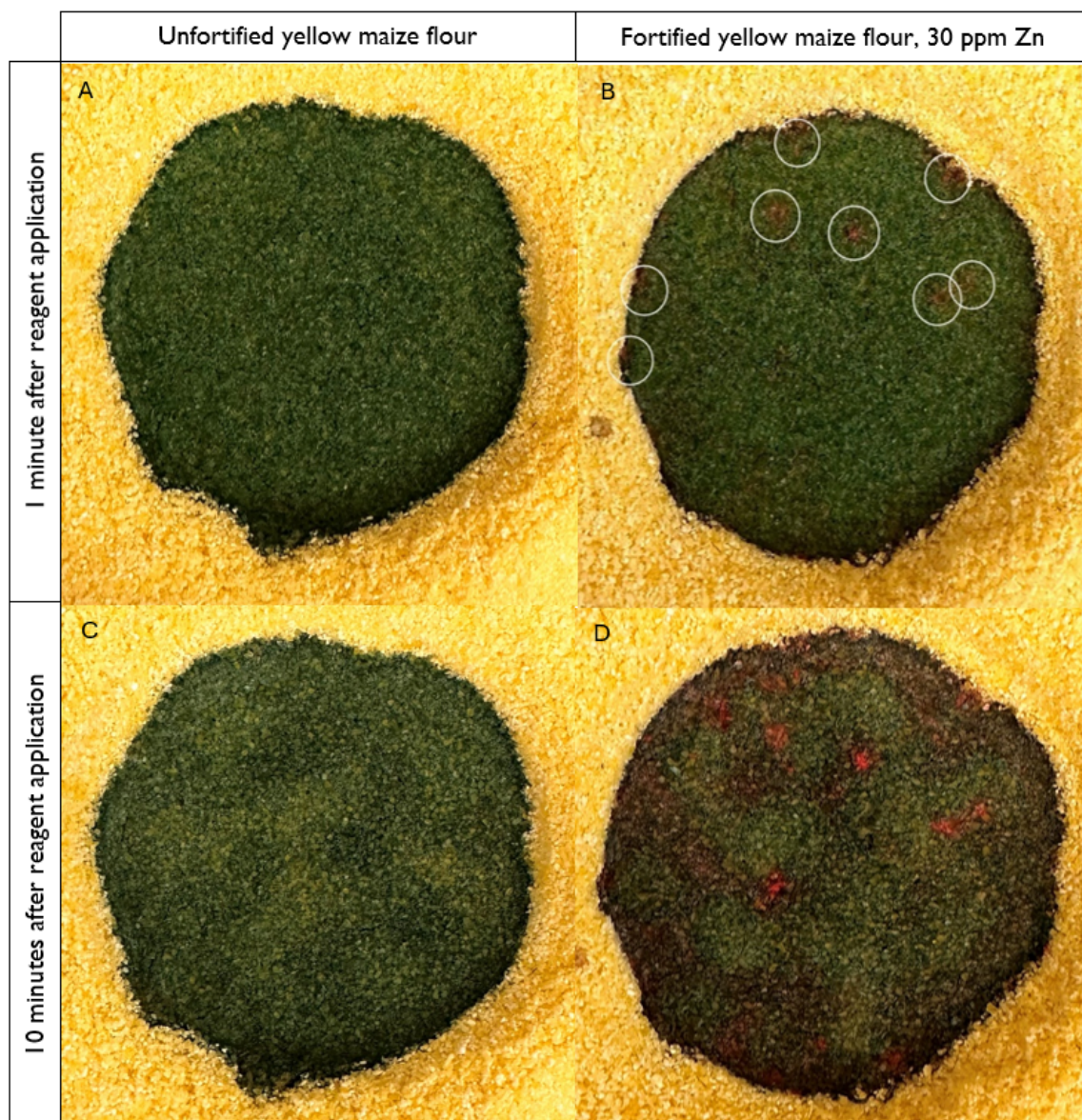
⁵ See Annex 1 for reagent preparation instructions.

Figure 1. Zinc Spot Test in White Maize Flour Samples



Bright pink spots indicate the presence of added zinc in white maize flour (B, D, on the right) compared against a negative control (A, C, on the left).

Figure 2. Zinc Spot Test in Yellow Maize Flour Samples

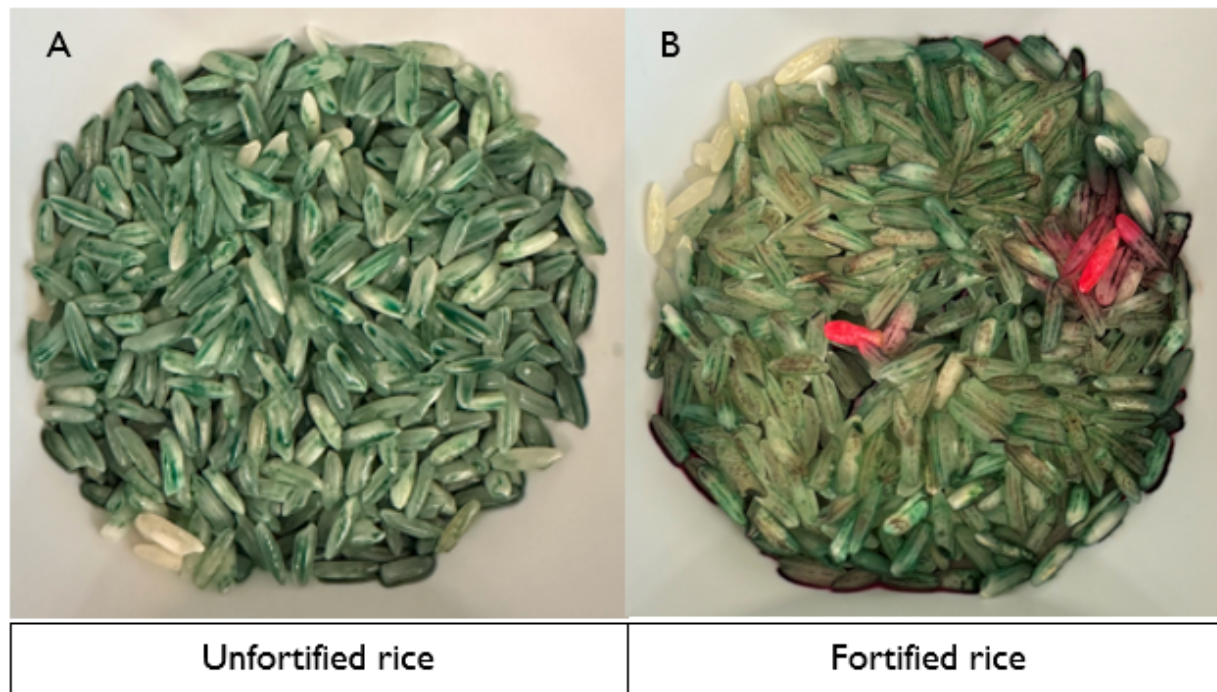


Bright pink spots indicate the presence of added zinc in yellow maize flour (B, D, on the right) compared against a negative control (A, C, on the left).

Fortified Rice Samples

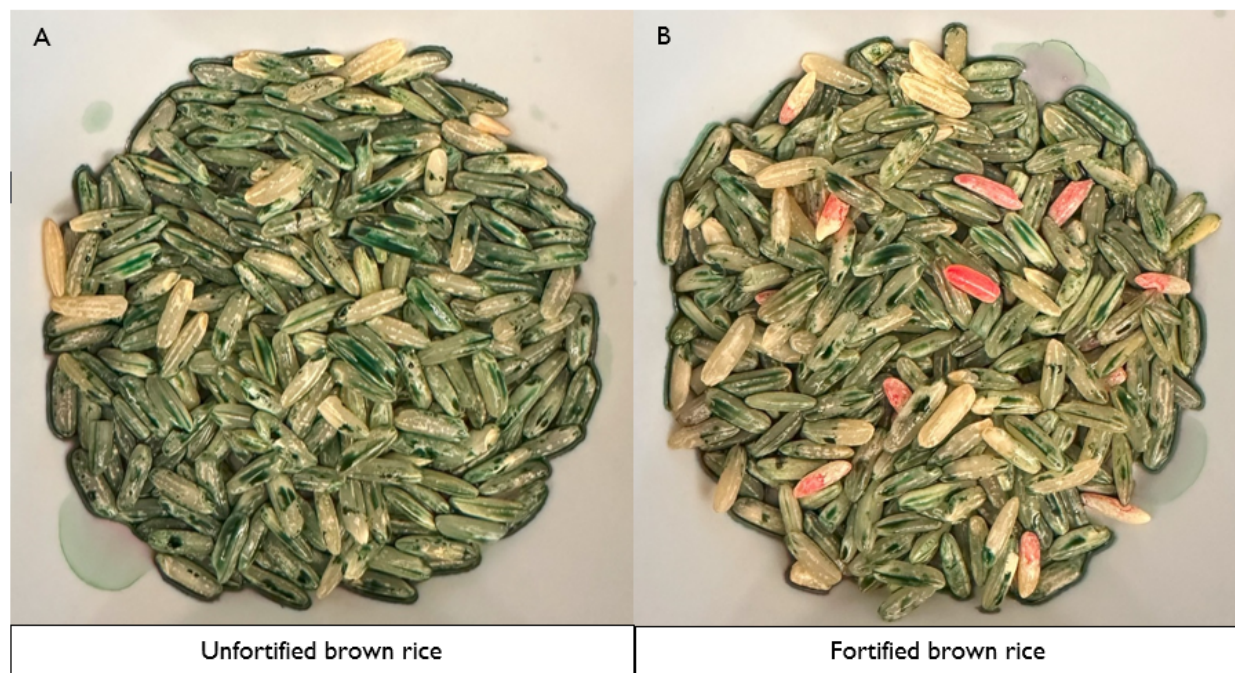
1. Weigh approximately 50 g (~35 mL) sample into a 250 mL weigh boat, or by volume in a 100-250 mL graduated beaker, and place it on a flat surface, a petri dish, or a white tile.
2. Spread the rice to make a flat thin layer of the sample.
3. Using a polyethylene transfer pipette, carefully add 10–15 drops of the dithizone solution to wet the flattened surface area.
4. Set aside for 10 minutes; if pink-colored spots appear, added zinc is present.

Figure 3. Zinc Spot Test in White Rice Samples



Bright pink spots indicate the presence of added zinc-fortified extruded kernels in white rice (B, on the right), compared against a negative control (A, on the left).

Figure 4. Zinc Spot Test in Brown Rice Samples



Bright pink spots indicate the presence of added zinc-fortified coated kernels in brown rice (B, on the right), compared against a negative control (A, on the left).

Safety of Reagents

Dithizone

According to the Materials Safety Data Sheet (MSDS), dithizone may cause mild skin and eye irritation when in contact. Use gloves when handling and avoid contact with skin or eyes.

First aid measures:

- If inhaled, move to an area with fresh air.
- In the event of skin contact, take all contaminated clothing off immediately. Rinse skin with water/in a shower.
- In the event of eye contact, rinse out with plenty of water. Remove contact lenses.
- If swallowed, drink up to 16 ounces of water.

Acetone

Acetone is considered an eye, skin, and lung irritant, according to its MSDS. As a volatile organic fluid, acetone is highly flammable in liquid and vapor form and may cause eye irritation and drowsiness or dizziness in prolonged exposure to fumes. Take appropriate safety measures during its use.

First-aid measures:

- General advice: If symptoms persist, call a physician.
- In the event of inhalation, remove to fresh air. If breathing is difficult, provide oxygen if available and trained to do so. If the person is not breathing, perform cardiopulmonary resuscitation if trained. Seek medical attention.
- In the event of eye contact, flush the eyes with water immediately and continue rinsing for at least 15–20 minutes. Use an eyewash station if available. Get medical attention.
- In case of skin contact: Wash off immediately with plenty of water for at least 15 minutes. If skin irritation persists, call a physician.
- In the event of ingestion, rinse your mouth with water and drink plenty of water afterward.
- Most important symptoms and effects: Symptoms of overexposure may be a headache, dizziness, tiredness, nausea, and vomiting. It may also cause pulmonary edema indicated by breathing difficulties (e.g., shortness of breath), coughing, or chest pain.

Disposal of Samples After Testing

- Containment and labeling: place tested samples in a sealable, clearly labeled container, such as a sealable plastic bag. The label should include the contents and any relevant hazard information.
- Check your local, state, or national regulations regarding the disposal of chemically contaminated materials.
- If in a laboratory or institutional setting, consult with your environmental health and safety officer or waste management team for specific instructions.

Acknowledgments

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Annex I. Reagents and Preparation of Solutions

Reagents

1. Dithizone—ThermoFisher CAS 60-10-6 (256.33 g/mole [mol]), analytical grade
2. Acetone—ThermoFisher CAS 67-64-1 (58.08 g/mol), analytical grade

Preparation of Solutions

This solution should be prepared in an area with adequate ventilation to prevent lung irritation. Avoid inhalation of reagent fumes.

2.0 x 10⁻³ M Dithizone Solution

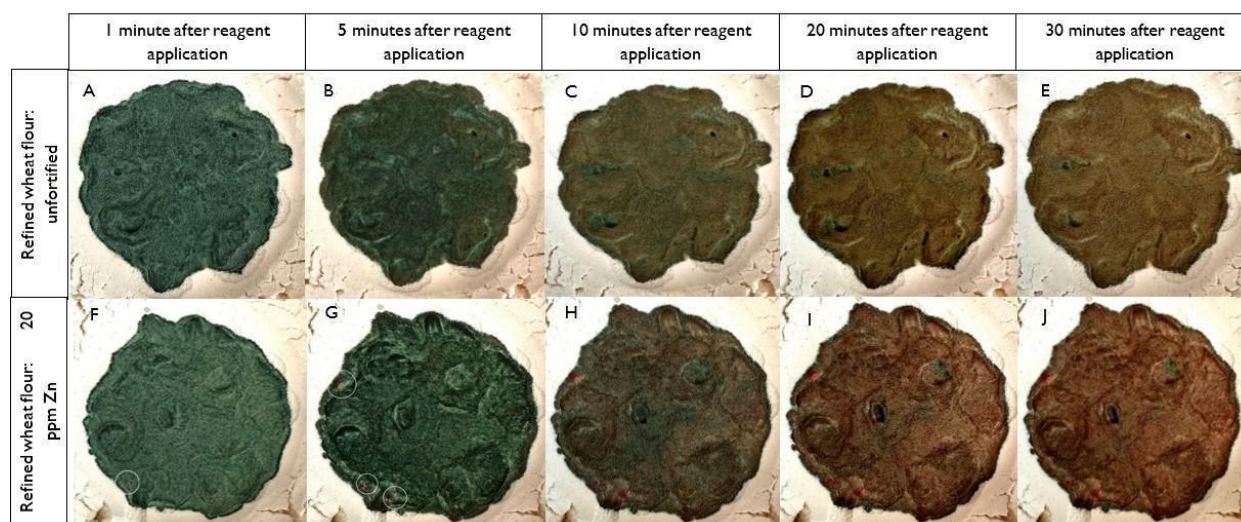
1. Weigh out 50 mg dithizone and add it to ~60 mL acetone in a 100 mL amber volumetric flask.
2. Mix by inversion several times, and open the flask to release vapors if formed.
3. Bring to volume with acetone. Mix by inversion.
4. The solution may be used for up to 3 months; keep refrigerated and in the dark.

Note: Dithizone oxidizes quickly when exposed to light and warm temperatures. Avoid light during reagent preparation and keep refrigerated in an amber volumetric flask/media bottle immediately after preparation and between uses.

Annex 2. Zinc Spot Test Applied to Zinc-Fortified Refined Wheat Flour

The dithizone reagent was also applied to refined wheat flour during the validation process. As shown below in Figure 5, the dithizone reagent identifies the presence of zinc in zinc-fortified refined wheat flour (C & D, bottom) when compared to unfortified refined wheat flour (A & B, top) with pink spotting. For qualitative testing of fortified refined wheat flour samples, an assay using xylenol orange⁶ is recommended.

Figure 5. Zinc Spot Test in Wheat Flour



Bright pink spots indicate the presence of added zinc in refined wheat flour (F through J, on the bottom), compared against an unfortified control (A through E, on the top), examined over 30 minutes.

⁶ USAID Advancing Food Fortification Opportunities to Reinforce Diets. 2024. *Qualitative Spot Test for Zinc in Fortified Wheat Flour and Rice using Xylenol Orange: Standard Operating Procedure*. Arlington, VA: USAID AFFORD.



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