# CHAPTER III

# MATERIALS AND METHODS

## Materials

1. Rice, glutinous rice, millet and corn, 30 samples of each, were randomly sampling from markets in Bangkok.

2. Chemicals

   2.1 Standard solution

      2.1.1 Cupric nitrate standard solution for AAS (1.000 mg/ml)(British Drug House)

      2.1.2 Zinc nitrate standard solution for AAS (1.000 mg/ml)(British Drug House)

   2.2 Hydrochloric acid, A.R.(British Drug House)

   2.3 Nitric acid, A.R.(British Drug House)

   2.4 Perchloric acid, A.R.(British Drug House)

   2.5 Triton X-100 (British Drug House)

3. Instruments

   3.1 Analytical balance (Mettler H 311)

   3.2 Atomic absorption spectrophotometer (Varian AAS-775)

   3.3 Blender (Janke & Kunkel, Ika-Werk)

3.4 Carbon rod atomizer (Varian CRA-90)

3.5 Hot air oven (Termaks)

Methods

Sample containers and all glasswares (Pyrex) were scrupulously cleaned with non-ionic detergent (Triton X-100) 0.1% solution and thoroughly rinsed with deionized water.

1. Determination of zinc

1.1 Preparation of sample

Each sample was ground in blender. The ground sample of approximately 0.5 g was accurately weighed in 100 ml beaker. Digestion of the sample was carried out essentially according to the method described by AOAC (50). Nitric acid (5 ml) was added to each ground sample and left for a few minutes until the sample was soaked thoroughly. Then, perchloric acid (60%; 1.5 ml) was added and the mixture was gently heated on a hot plate until frothing ceased. The mixture was kept on heating until nitric acid was almost evaporated and continuously heating to give the white fume of perchloric acid, then cooled at room temperature, and hydrochloric acid (6 N; 5 ml) was added. The mixture was transferred to a 25 ml volumetric flask and adjusted to volume with deionized water. The digested solution was kept in an air-tight

polyethylene bottle prior to the measurement. The amount of zinc was determined by a flame atomic absorption spectrophotometer (51).

### 1.2 Preparation of standard solution

#### 1.2.1 Preparation of standard solution for rice and glutinous rice

Working standard solutions were prepared from a 1000 ppm Zn stock standard at the concentrations of 2,4,6 and 8 ppm Zn in deionized water. The final standard solutions were prepared according to standard additions method. Digested sample solution (1.8 ml) was added to each standard tube. Aliquots. (0.2 ml) of working standard solutions at various concentrations, or deionized water, were then added. The final volume of each tube was 2.0 ml and the final concentrations of standard Zn were 0, 0.200, 0.400, 0.600, 0.800 ppm as shown in Table 1. The volume ratio of digested solution to working standard solution was, therefore, 9:1. For the measurement of unknown samples, the digested solutions were also diluted with deionized water by the ratio of 9:1.

Table 1 Preparation of standard Zn solutions for the determination of rice and glutinous rice

| Tube No. | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Sample (ml) | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 |
| Working standard(ppm) | - | 2 | 4 | 6 | 8 |
| Working standard(ml) | - | 0.2 | 0.2 | 0.2 | 0.2 |
| Deionized water(ml) | 0.2 | - | - | - | - |
| Total volume(ml) | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Final concentration(ppm) | 0 | 0.200 | 0.400 | 0.600 | 0.800 |

1.2.2 Preparation of standard solution for millet and corn

Working standard solutions were prepared from a 1000 ppm Zn stock standard at the concentrations of 0.4, 0.8, 1.2 and 1.6 ppm Zn in deionized water. The final standard solutions were prepared according to standard additions method (see 1.2.1 for more detail) as shown in Table 2. The volume ratio of digested solution to working standard solution was, therefore, 1:1. For the measurement of unknown samples, the digested solutions were also diluted with deionized water by the ratio of 1:1.

Table 2 Preparation of standard Zn solutions for the determination of millet and corn

| Tube No. | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Sample (ml) | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Working standard (ppm) | - | 0.4 | 0.8 | 1.2 | 1.6 |
| Working standard (ml) | - | 1.0 | 1.0 | 1.0 | 1.0 |
| Deionized water (ml) | 1.0 | - | - | - | - |
| Total volume (ml) | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Final concentration (ppm) | 0 | 0.200 | 0.400 | 0.600 | 0.800 |

1.3 Analysis

The absorbance values of standard solutions and digested solutions were determined by flame mode (51), using the instrumental settings and conditions as followed:-

| | |
|---|---|
| Lamp current | 5 mA |
| Wave length | 213.9 nm |
| Spectral band pass | 1.0 nm |
| Integration time | 3 sec |
| Flame | air-acetylene |

Background correction mode was used

1.4 Calculation

Standard curve (Figure 1) was prepared by plotting concentration against absorbance. Concentrations of zinc in the digested solutions were obtained from the

standard curve by extrapolating the absorbance against the concentration. Then, the concentrations of zinc in the samples were calculated from:-

$$\text{Zn } (\mu g/g) = \text{concentration (from standard curve)} \times \text{dilution factor} \times \frac{25}{0.5}$$

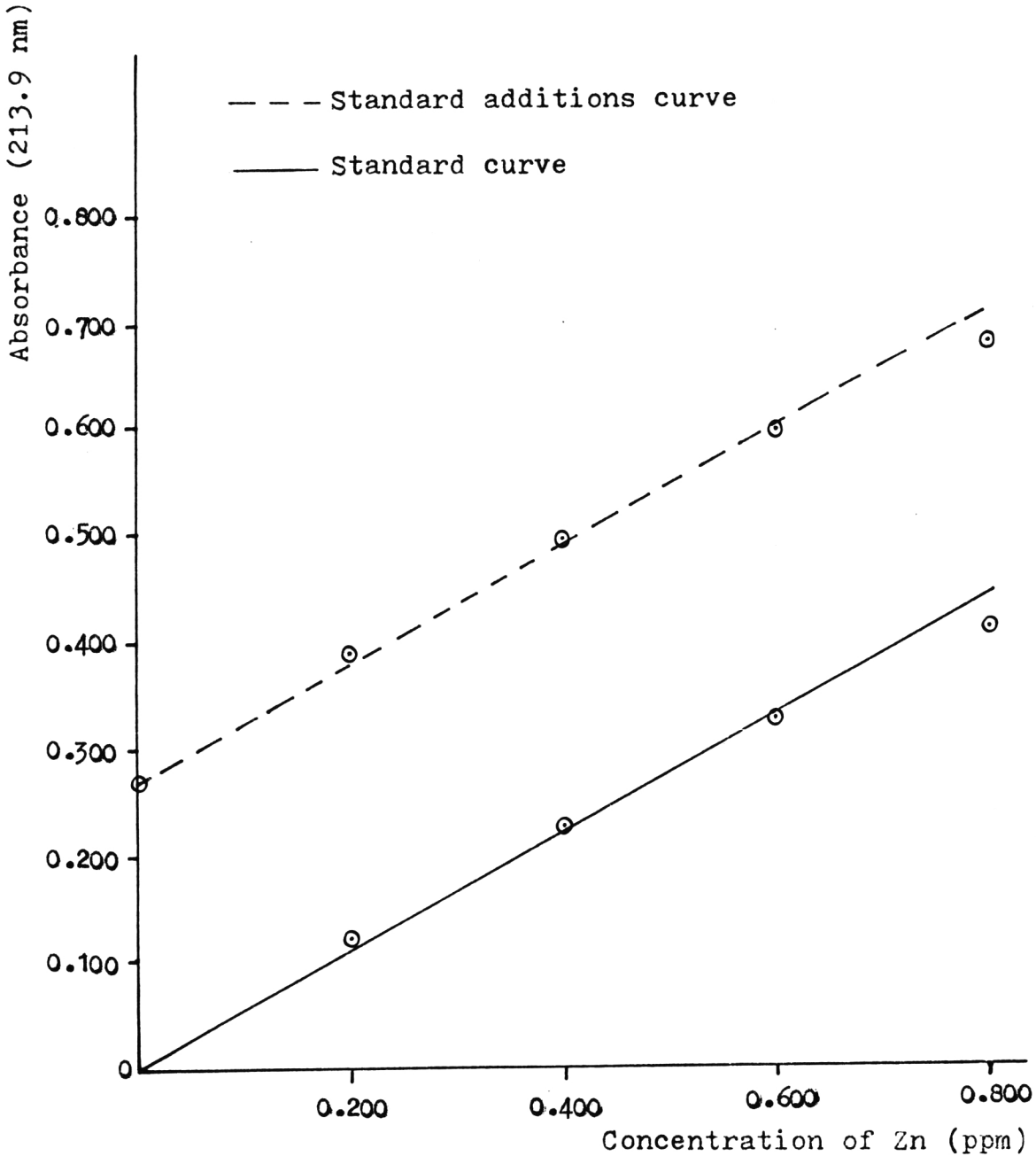


Figure 1 Standard curve for Zn

2. Determination of copper

2.1 Preparation of sample

Ground sample of approximately 2 g was accurately weighed in a 125 ml beaker. Nitric acid (5N; 20 ml) was added to each sample and the mixture was gently boiled for 30 minutes, cooled at room temperature, filtered through filter paper (Whatman # 41) into a 25 ml volumetric flask and adjusted to volume with deionized water. The digested solution was kept in an air-tight polyethylene bottle. The amount of copper was determined by a flameless atomic absorption spectrophotometer (52).

2.2 Preparation of standard solution

2.2.1 Preparation of standard solution for rice, glutinous rice and corn

Working standard solutions were prepared from a 1000 ppm Cu stock standard at the concentrations of 1.25, 2.50 and 5.00 ppm Cu in 2% $HNO_3$, and final standard solutions were prepared according to standard additions method. To the series of standard tubes, 1.8 ml of digested solution was added. Aliquots (0.2 ml) of working standard solutions at various concentrations, or deionized water, were then added and mixed. The final concentrations of added standard Cu were 0, 0.125, 0.250 and 0.500 ppm as shown in Table 3. The volume ratio of digested solution to working standard solution was, therefore, 9:1. For the measurement of unknown samples, the digested solutions were also diluted with deionized water by the ratio of 9:1.

Table 3 Preparation of standard Cu solutions for the determination of rice, glutinous rice and corn

| Tube No. | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Sample (ml) | 1.8 | 1.8 | 1.8 | 1.8 |
| Working standard (ppm) | - | 1.25 | 2.50 | 5.00 |
| Working standard (ml) | - | 0.2 | 0.2 | 0.2 |
| Deionized water (ml) | 0.2 | - | - | - |
| Total volume (ml) | 2.0 | 2.0 | 2.0 | 2.0 |
| Final concentration (ppm) | 0 | 0.125 | 0.250 | 0.500 |

2.2.2 Preparation of standard solution for millet

Working standard solutions were prepared from a 1000 ppm Cu stock standard at the concentrations of 0.375, 0.750 and 1.500 ppm Cu in 2% $HNO_3$, and final standard solutions were prepared according to standard additions method. To the series of standard tubes, 1 ml of digested solution and 1 ml of deionized water were added. Aliquots (1 ml) of working standard solutions at various concentrations, or deionized water, were then added and mixed. The final concentrations of added standard Cu were 0, 0.125, 0.250 and 0.500 ppm as shown in Table 4. The volume ratio of digested solution to working standard solution and deionized water was, therefore, 1:2. For the measurement of unknown samples, the digested solutions were also diluted

with deionized water by the ratio of 1:2.

Table 4 Preparation of standard Cu solutions for the determination of millet

| Tube No. | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Sample (ml) | 1.0 | 1.0 | 1.0 | 1.0 |
| Working standard (ppm) | - | 0.375 | 0.750 | 1.500 |
| Working standard (ml) | - | 1.0 | 1.0 | 1.0 |
| Deionized water (ml) | 2.0 | 1.0 | 1.0 | 1.0 |
| Total volume (ml) | 3.0 | 3.0 | 3.0 | 3.0 |
| Final concentration (ppm) | 0 | 0.125 | 0.250 | 0.500 |

## 2.3 Analysis

The absorbance values of standard solutions and digested solutions were determined by flameless mode (52), using the instrumental settings and conditions as followed:-

| | | |
|---|---|---|
| Lamp current | 5 | mA |
| Wave length | 324.7 | nm |
| Spectral band width | 0.5 | nm |
| Sample size | 5 | µl |
| Dry | 90°C, 50 | sec |
| Ash | 900°C, 30 | sec |
| Atomize | 2000°C, 2 | sec |
| Ramp rate | 500°C/sec | |

## 2.4 Calculation

Standard curve (Figure 2) was prepared by plotting concentration against absorbance. Concentrations of copper in the digested solutions were obtained from the standard curve by extrapolating the absorbance against the concentration. Then, the concentrations of copper in the samples were calculated from:-

$$\text{Cu } (\mu g/g) = \text{concentration (from standard curve)} \times \text{dilution factor} \times \frac{25}{2}$$

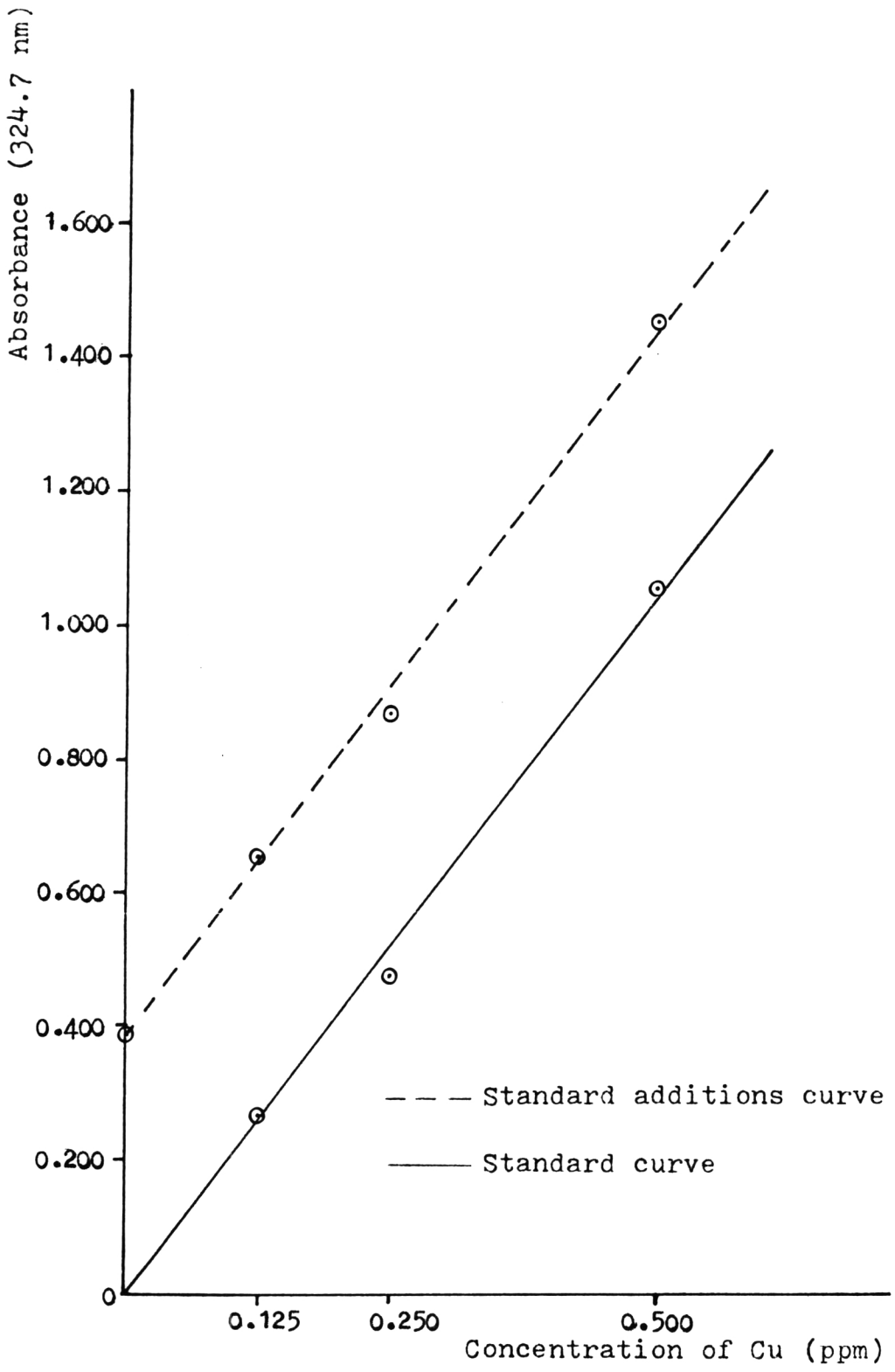


Figure 2 Standard curve for Cu