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# Effect of processing on the level of zinc and phytic acid in quality protein maize flour for determination of bioavailability of zinc

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**Abstract**
Maize is the third most important crop after rice and wheat. It is rich in nutrients and also contains phytic acid concentrated in the germ that forms insoluble complexes zinc, thus reducing its bioavailability. Zinc is an essential component of a large number of enzymes participating in the synthesis and degeneration of carbohydrates, lipids, proteins and nucleic acids as well as in the metabolism of other micronutrients and its deficiency is an important issue these days. The zinc can be made bioavailable by reducing the phytic acid content of maize through different processing methods. Bioavailability is calculated by phytate: zinc molar ratio. This study reveals that boiled and alkali treated maize had medium zinc bioavailability.

**Keywords:** Zinc, phytic acid, quality protein maize, effect of processing, bioavailability

## Introduction
Maize (*Zea mays* L.) also known as corn, is one of the world's leading cereal grains along with rice and wheat. It contributes significantly to global grain pool of 2200 million metric tons annually in achieving food and nutritional security. It also provides nutrients for human and animals and serving as a basic raw material for the production of starch, oil, protein, alcoholic beverages, food sweeteners and more recently, fuel. The kernel of a maize plant consists of three main parts; the pericarp, endosperm and embryo. Maize grain is subdivided into distinct types based on endosperm and kernel composition, kernel colour, environment in which it is grown, maturity and its use [1]. There are 6 major varieties commercially grown speciality maize for human consumption including flint, floury, dent, pop, waxy and sweet corn [2]. the utilization pattern of maize in India include as a source of human food 25%, animal feed 12%, poultry feed 49%, industrial products mainly as starch (12%) and one per cent each in brewery and seed [3]. Maize varieties (yellow and white) were roasted for 17 minutes; and allowed to cool, and later milled into powder. The nutritional evaluation (proximate composition, mineral and antinutrient content determination) and antioxidant properties investigation (reducing power, free radicals scavenging ability and Fe2+ chelating ability) of the product was subsequently carried out. It was revealed that roasting caused a significant increase ($P$<0.05) in the crude fat, carbohydrate, Ca, Na, Mg and Zn content. Conversely, a significant decrease ($P$<0.05) was observed in crude protein, crude fibre, Fe and K content. A significant decrease in the phytate content was also observed. However, the reduced phytate content did not have sparing effect on Zn bioavailability. Roasting significantly ($P$<0.05) reduced the extractible phenol and flavonoid content of the maize varieties. However, roasting caused a significant increase in the ferric reducing antioxidant power of the maize varieties. Thus, roasting reduced the protein content of maize but also increased the energy value and antioxidant capacity as exemplified by high reducing power [4]. Zinc has an essential role in polynucleotide transcription and thus in the process of genetic expression. Its involvement in such fundamental activities probably accounts for the essentiality of Zinc for all life forms. Zinc plays a central role in the immune system, affecting a number of aspects of cellular and Humoral immunity [5]. The availability of Zinc from the diet can be improved by reductions in the phytate content and inclusion of animal protein sources. Lower extraction rates of cereal grains will result in lower Phytate content but at the same time the Zinc content is reduced, so that the net effect on Zinc supply is limited. The Phytate content can be reduced by activating the Phytase present in most Phytate-containing foods or through the addition of microbial or fungal Phytases. Phytases hydrolyse the Phytate to lower inositol phosphates, resulting in improved Zinc absorption [6, 7]. The activity of Phytases in tropical cereals such as maize and sorghum is lower than that in wheat and rye. Germination of cereals and legumes increases phytase activity and addition of some germinated flour to ungerminated maize or sorghum

followed by soaking at ambient temperature for 12-24 hours can reduce the phytate content substantially. Additional reduction can be achieved by the fermentation of porridge for weaning foods or doughs for bread making. Commercially available phytase preparations could also be used but may not be economically accessible in many populations [8]. Phytic acid binds essential micronutrients such as Fe and Zn in seeds and also complexes with micronutrients in other foods during intestinal digestion. These phytate-salt complexes are not absorbed across the intestinal mucosa resulting in low bioavailability of minerals. Accordingly, PA acts as an antinutrient for micronutrient availability and diets rich in PA and low in mineral micronutrients can cause health problems, typically Fe, Zn, and Mg deficiencies [9]. The consequences of micronutrient deficiencies can be severe for populations that are heavily dependent on cereal and legume diets rich in PA [9, 10]. Zinc appears to be the trace element whose bioavailability is most influenced by Phytic Acid. It has been shown in a series of *in vitro* and *in vivo* investigations that a higher molar ratio of PA compared with Zn coincides with a significant reduction in the bioavailability of Zinc. A reduction of Zinc bioavailability is to be expected with a molar PA:Zn ratio > 10-15 in the diet [11, 12]. Zinc inhibitors like phytates and fiber are present in higher amounts in plant foods, especially cereals and legumes, and influence zinc absorption. Phytates have been singled out as the most potent dietary inhibitor of zinc bioavailability [13, 14].

## Materials and methods

### Materials

#### Selection of raw materials

Among the quality protein maize varieties, 'Shaktiman5' was selected for the study.

#### Procurement of maize grains

For the study freshly harvested Quality protein maize variety; (Shaktiman5) was collected from the farmers of Bisanpur, Birauli, Samastipur in one lot. The quantity to be procured was 12 kg.

The collected maize grains were cleaned by isolating damaged and unhealthy seeds and remaining foreign matter. The remaining maize grains were 10 kilogram. The lot was divided into four equal sets in triplicate for processing. Each replicate of maize grains was of 2.5 kg.

#### Processing of maize grains

For the study, out of four sets (in triplicate), one set was kept as such as control (in triplicate). The other three sets (each in triplicate) were kept for processing. The processing methods applied were boiling, roasting and alkali processing.

#### Application of boiling method for maize grains

Both normal and quality protein maize grains (in triplicate) were boiled in double amount of water by weight for 30 minutes. Then it was oven dried for 10 hours at 60° C.

#### Application of roasting method for maize grains

Both the varieties of maize grains were roasted at 180° C for 20 minutes.

#### Application of alkali processing for maize grains

Both the varieties of maize grains were soaked for 5 minutes in double the amount of 1% lime water by weight and then heat treatment was given for 30 minutes at 85°C. Then, it was kept overnight. Next day, the grains were washed 4 times and kept in oven for 10 hours at 60°C for drying.

### Methods

#### Determination of zinc

The zinc was determined by using Atomic Absorption Spectrophotometer [15]. 0.5g of flour sample was weighed and taken in 100 ml conical flask. 10 ml diacid was added and left over night. Then it was kept on a hot plate and heated gently. Then it was heated vigorously till a colourless solution was obtained. The heating was discontinued when the volume was reduced to 1ml (1 Drop). Precaution was taken not to take up to dryness. Then it was cooled and some distilled water was added. Then it was transferred into a 50 ml volumetric flask. The volume was made by adding distilled water. The reading was taken by AAS.

#### Calculation

Zn in grain (ppm) = x × dilution factor
(x = reading noted from AAS)
Dilution Factor = 50/0.5 = 100
Zn (ppm) = x × 100

#### Determination of Phytic Acid

The Phytic Acid was determined by using the procedure that involves the use of spectrophotometer [16]. 0.5-1g of maize flour was taken. It was extracted with 20 ml, 0.5M- $HNO_3$ for 3-4 hours with continious shaking. Then it was filtered. 0.2 to 0.5g of filterate that was extracted was made upto suitable volume with water (20 ml). To 1.4 ml of the filterate, 1 ml of Ferric Amonium Sulphate solution (21.6 mg in 100 ml water) was added, mixed and placed in a boiling water- bath for 20 minutes. The contents were cooled and 5 ml of isoamyl alcohol was added and mixed. To this, 0.1 ml ammonia solution was added, shaken thoroughly and centrifuged at 3000 rpm for 10 minutes. The alcoholic layer was separated and the colour intensity was read at 465 nm against amyl alcohol blank after 15 minutes. Sodium phytate standards were run along with the sample. The results were expressed as mg Phytic acid/100g dry weight.

#### Evaluation of bio-availability of Zinc

For the determination of the bio-availability of zinc, the phytate: zinc molar ratio was calculated [17].

The mole of phytate and zinc was determined by dividing the weight of phytate and zinc (mg/100g) with its atomic weight (phytate: 660g/mol; Zn: 65g/mol). The molar ratio between phytate and zinc was obtained by dividing the mole of phytate with the mole of zinc.

Phytate/ zinc molar ratios

- Greater than 5: low zinc bio-availability
- Between 5-15: medium zinc bio-availability
- Less than 5: relatively good zinc bio-availability

#### Statistical Analysis

The data obtained upon the determination of quality parameters of maize flours had been analysed for statistical implications by using Mean, Standard Deviation and Paired 't' test to find out the effect of processing methods on the bio-availability of zinc in different maize varieties [18].

## Results and discussion

### Zinc in freshly harvested QPM flour before and after processing

The Zinc in QPM flour (control and after processing) was determined. The data obtained on Zinc in flour had quantitative changes after the application of different processing methods have been presented in Table 1 & 2.

The Zinc in QPM flour from freshly harvested maize grains before and after processing methods like boiling, roasting and alkali processing has been presented in Table 1. The freshly harvested maize grains i.e., raw maize grains had been taken as control. It was revealed from the table that control flour sample contained 3.78 mg/100g Zinc. In boiled maize flour sample, the Zinc content was 2.41 mg/100g. Roasted Maize sample contained 1.63 mg/100g Zinc. In alkali treated maize flour sample, the Zinc content was 1.89 mg/100g.

It can be observed in Table 1 that the Zinc content in control maize sample was the highest (3.78 mg/100g) followed by the boiled maize sample (2.41 mg/100g), alkali treated maize sample (1.89 mg/100g) and roasted sample (1.63 mg/100g).

The statistical analysis clearly shows that the Zinc content of the control maize sample was significantly higher than boiled maize sample ('t' value 137.65), roasted maize sample ('t' value 112.79) and alkali treated maize sample ('t' value 73.45) at 1% level of probability. Further, the Zinc content of boiled maize sample was significantly higher than the roasted maize sample ('t' value -31.35) and alkali treated sample ('t' value 15.67) at 1% level of probability. Whereas, the Zinc content of the roasted maize sample was significantly lower than the alkali treated maize sample ('t' value -18.11) at 1% level of probability.

**Table 1:** Zinc in freshly harvested QPM flour before and after processing.

| Maize grain Sample | Parameter (mg/100g) |
|---|---|
| | **ZINC** (Mean±S.D) |
| Control (A) | 3.78 ± 0.03 |
| Boiled (B) | 2.41 ± 0.05 |
| Roasted (C) | 1.63 ± 0.02 |
| Alkali treated (D) | 1.89 ± 0.04 |
| 't' value among maize samples | |
| A×B | 137.65** |
| A×C | 112.79** |
| A×D | 73.45** |
| B×C | 31.35** |
| B×D | 15.67** |
| C×D | (-) 18.11** |

Each value is the mean of six observations, $^{NS}$ Not significant
*Significant at 5% level of probability, **Significant at 1% level of probability

## Changes in Zinc content of QPM flour after processing as compared to control sample

The changes in Zinc content of QPM flour after application of different processing methods as compared to control sample can be observed in Table 2. The maximum percentage loss in Zinc content was 56.87percent in case of roasted maize sample followed by 50 percent in alkali treated and 36.24 percent in boiled maize sample.

It can be concluded from Table 2 that after processing the changes in Zinc content was significant. In case of roasted maize sample, the Zinc content significantly reduced followed by alkali treated maize sample and boiled maize sample. Definitely qualitative and quantitative changes occur after processing.

**Table 2:** Changes in Zinc content of QPM flour after processing as compared to control sample.

| Percentage change in maize grains | Parameter (g/100g) |
|---|---|
| | **ZINC** |
| Boiled (B) | 36.24↓ |
| Roasted(C) | 56.87↓ |
| Alkali treated (D) | 50.00↓ |

↓ Indicates decreasing trend

## Phytic acid in freshly harvested QPM flour before and after processing

The Phytic acid in QPM maize flour (control and after processing) was determined. The data obtained on Phytic acid in flour had quantitative changes after the application of different processing methods have been presented in Table 3 & 4.

The Phytic acid in QPM maize flour from freshly harvested maize grains before and after processing methods like boiling, roasting and alkali processing has been presented in Table 3. The freshly harvested maize grains i.e., raw maize grains had been taken as control. It was revealed from the table that control flour sample contained 291.18 mg/100g Phytic acid. In boiled maize flour sample, the Phytate content was 263.15 mg/100g. Roasted Maize sample contained 274.28 mg/100g Phytate. In alkali treated maize flour sample, the Phytic acid content was 267.13 mg/100g.

It can be observed in Table 3 that the Phytic acid content in the control maize sample was the highest (291.18 mg/100g) followed by the roasted maize sample (274.28 mg/100g), lime treated maize sample (267.13 mg/100g) and boiled maize.

The statistical analysis clearly shows that the Phytic acid content of the control maize sample was significantly higher than boiled maize sample ('t' value 54.79), roasted maize sample ('t' value 28.84) and alkali treated maize sample ('t'value63.71) at 1% level of probability. Further, the Phytic acid content of boiled maize sample was significantly lower than the roasted maize sample ('t' value -63.12) and alkali treated sample ('t' value -11.92) at 1% level of probability. Whereas, the Phytic acid content of the roasted maize sample was significantly higher than the alkali treated maize sample ('t' value 22.12) at 1% level of probability.

**Table 3:** Phytic Acid in freshly harvested QPM flour before and after processing.

| Maize grain Sample | Parameter (mg/100g) |
|---|---|
| | **PHYTIC ACID**(Mean±S.D) |
| Control (A) | 291.18 ± 1.17 |
| Boiled (B) | 263.15 ± 0.35 |
| Roasted (C) | 274.28 ± 0.47 |
| Alkali treated (D). | 267.13 ± 0.54 |
| 't' value among maize samples | |
| A×B | 54.79** |
| A×C | 28.84** |
| A×D | 63.71** |
| B×C | (-) 63.12** |
| B×D | (-) 11.92** |
| C×D | 22.12** |

Each value is the mean of six observations, $^{NS}$ Not significant
*Significant at 5% level of probability, **Significant at 1% level of probability

## Changes in Phytic acid content of QPM flour after processing as compared to control sample

The changes in Phytic acid content of QPM flour after application of different processing methods as compared to control sample can be observed in Table 4. The maximum percentage loss in Phytic acid content was 9.62percent in case of boiled maize sample followed by 8.25percent in alkali treated maize sample and 5.80percent in roasted maize sample.

It can be concluded from Table 4 that after processing the changes in Phyic acid content was significant. In case of boiled maize sample, the Phyic acid content significantly reduced followed by alkali treated maize sample and roasted

maize sample. Definitely qualitative and quantitative changes occur after processing.

**Table 4:** Changes in Phytic acid content of QPM maize flour after processing as compared to control sample.

| Percentage change in maize grains | Parameter (g/100g) |
|---|---|
| | Phytic Acid |
| Boiled (B) | 9.62↓ |
| Roasted(C) | 5.80↓ |
| Alkali treated (D) | 8.25↓ |

↓Indicates decreasing trend

**Bio-availability of Zinc in QPM flour before and after processing**

The Phytate: Zinc molar ratio in QPM flour (control and after processing) was calculated. The mole of Phytate and Zinc was determined by dividing the weight of Phytate and Zinc (mg/100g) with its atomic weight (Phytate: 660g/mol; Zinc: 65g/mol). The molar ratio between Phytate and Zinc was obtained by dividing the mole of Phytate with the mole of Zinc.

If the Phytate: Zinc molar ratio was greater than 5, there was low Zinc bioavailability. If it was between5-15, then there was medium Zinc bioavailability, if it was less than 5, there was relatively good Zinc bioavailability. The bioavailability of Zinc in the QPM flour before and after processing has been presented in Table 5.

The Phytate: Zinc molar ratio in QPM flour (control and after processing) was calculated and this helped us to evaluate the bioavailability of Zinc in QPM grains before and after the application of different processing methods like boiling, roasting and lime treatment which has been presented in Table 5. It was revealed from the Table that in the control maize sample the Phytate: Zinc molar ratio was found to be 7.6. In the boiled maize sample, the ratio was 10.75, in the roasted maize sample it was found to be 16.69 and in the alkali treated maize sample, it was found to be 13.93.

It can be observed from the Table 5 that the Phytate: Zinc molar ratio in the control maize sample was the lowest (7.6) followed by boiling maize sample (10.75), lime treated maize sample (13.93) and roasted maize sample (16.69).

This clearly shows that in QPM flour, the control maize sample, the boiled maize sample and the alkali treated maize sample had medium Zinc bioavailability whereas, the roasted maize sample had low bioavailability of Zinc.

**Table 5:** Bio-availability of Zinc in QPM flour before and after processing.

| Bioavailability of Zinc | (Phytate : Zinc Molar Ratio) |
|---|---|
| Control (A) | 7.6 |
| Boiled (B) | 10.75 |
| Roasted (C) | 16.69 |
| Alkali Treated (D) | 13.93 |

Phytate/ zinc molar ratios
Greater than 5: Low zinc bio-availability
Between 5-15: Medium zinc bio-availability
Less than 5: Good zinc bio-availability

## Conclusion

The Phytate: Zinc molar ratio in the control maize sample was the lowest (7.6) followed by boiling maize sample (10.75), lime treated maize sample (13.93) and roasted maize sample (16.69). The control maize sample, the boiled maize sample and the alkali treated maize sample had medium Zinc bioavailability whereas, the roasted maize sample had low bioavailability of Zinc.

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