

3 **The anti-nutritional effect of phytate on zinc, iron and calcium**
4 **bioavailabilities of some cereals staple foods in Zaria, Nigeria**

9 **ABSTRACT**

Aim of Study: To evaluate the effect of phytate on the bioavailabilities of zinc, iron and calcium of some cereals staple foods in Zaria, Nigeria.

Study Design: Experimental

Place of Study: Department of Biochemistry, Faculty of Sciences, Ahmadu Bello University Zaria, Nigeria.

Methodology: *Tuwon surfafen masara* was prepared by first milling the processed maize. *Tuwon masara* and Pap were prepared using standard local preparation methods. Phytic acid content was determined according to the method described by Reddy; The minerals were determined using atomic absorption spectrophotometry; AOAC 1990. Data was analysed with one way ANOVA and differences were considered significant at $P = .05$

Results: The ratios of Phytate:Ca, Phytate:Fe, Phytate:Zn for *Tuwon surfafen masara* were found to be 0.0026, 0.197 and 0.429 respectively. While that of *Tuwon masara* was 0.0044, 0.127 and 0.376 respectively. Accordingly pap showed a phytate to mineral ratios of 0.0025, 0.043 and 0.162 for Phytate:Ca, Phytate:Fe and Phytate:Zn respectively. The ratios of Phytate:Ca, Phytate:Fe and Phytate:Zn for local rice was found to be 0.0075, 0.110 and 0.625 respectively. While that of foreign rice was 0.0031, 0.046 and 0.266 respectively. The phytate to mineral ratios of all the staple foods in the present study falls below the critical values of >0.24 , >1 and >18 for Phytate:ca, Phytate:Fe and Phytate:Zn respectively which indicate good bioavailability.

Conclusion: The result obtained showed that the bioavailability of Ca, Fe and Zn in *Tuwon surfafen masara*, tuwo, Pap, local rice and foreign rice in Zaria, Nigeria is not affected by their phytic acid contents.

11
12 **Keywords:** *Bioavailability; Phytic acid; Calcium; Iron; Zinc*

13 **1. INTRODUCTION**

14

15 Malnutrition results from imbalance between the body's needs and intake of nutrients which can
16 lead to symptoms of deficiency, toxicity or obesity [1]. It includes moderate and severe
17 malnutrition. Malnutrition can result from inadequate food intake, malabsorption, abnormal
18 systemic loss of nutrients due to diarrhea, hemorrhage or renal failure, infection and addiction to
19 drugs [2].

20

21 Anti-nutritional factors are compounds which reduce the nutritional utilization or bioavailability
22 of nutrient in food substances and they play a vital role in determining the use of plants as human
23 food and animals feed. Apart from the cyanogenic glycosides, food poisoning arising from other
24 anti-nutritional factors has not been properly addressed in most part of the developing world [3].
25 People have died out of ignorance, poverty and inadequate nutritional information. There are
26 reports from time to time of death after consuming some certain species of beans even after
27 cooking. This is known to be due to the presence of toxic plants secondary metabolites also refer
28 to as anti-nutritional factors [3]. It is well established that plants generally contain these
29 compounds obtain from fertilizers, pesticides and several naturally occurring compounds [4].
30 Some of these plants secondary metabolites are known to be highly biologically active [5].
31 Among them is phytic acid whose effect on the bioavailability of some minerals is the main
32 focus of the present study.

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34 Phytic acid chelates metal ions, especially zinc, iron, and calcium, but not copper [6], forming
35 insoluble complexes in the gastrointestinal tract that cannot be digested or absorbed in humans
36 because of the absence of intestinal phytase enzymes [7], Phytate also complexes endogenously
37 secreted minerals such as zinc [8] and calcium [9], making them unavailable for reabsorption
38 into the body. At present, studies on the effect of phytate on the bioavailability of iron, zinc, and
39 calcium on cereals staple foods commonly consumed in Zaria, Nigeria has not been reported.
40 The aim of the present study is to investigate the possible anti-nutritional effect of phytate on
41 these mineral elements. This will serve as indicator of the need either to include phytate-reducing
42 local cereal processing method and/or fortification with minerals.

43

44 Minerals, classified as micronutrients are needed by our body in small amounts. Deficiency in
45 minerals, however, can have a major impact on health such as anemia and osteoporosis that
46 commonly occur in both developed and developing countries [10]. The deficiency of calcium
47 result in abnormalities in the bones and teeth, subnormal growth, low milk and egg production,
48 depressed appetite and fertility is also likely to be impaired. Studies in animals have clearly
49 shown that iron deficiency has several negative effects on some important functions [11].
50 Physical working capacity in rats has been shown to be significantly reduced in iron deficiency
51 which is especially valid for endurance activities [12, 13]. Result obtain from zinc
52 supplementation studies showed that a low zinc status in children not only affects growth but is

53 also associated with increased risk of severe infectious diseases [14]. Episode of acute diarrhea,
54 respiratory tract infection and malaria has also been reported in severe zinc deficiency. Decrease
55 in severity, duration, and reductions in incidence of diarrhea have been reported in a group of
56 zinc supplemented children.

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59 **3. Materials and methods**

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61 *3.1 Samples collection.* The rice and maize used in this work were bought from Samaru market,
62 Zaria.

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64 *3.2 Chemicals and reagents.* Concentrated sulphuric acid (H_2SO_4), concentrated nitric acid
65 (HNO_3), perchloric acid ($HClO_4$), ammonium thiocyanate (NH_4SCN), hydrochloric acid (HCl),
66 Iron (iii) chloride ($FeCl_3$), and deionized water.

67

68 *3.3 Sample Preparation.* *Tuwon surfafen masara* was prepared by first milling the processed
69 maize using a milling machine to produce maize flour, the maize flour was added to boiling
70 water with continuous stirring until a thick paste is formed which was allowed to cool at room
71 temperature for 3 hrs. *Tuwon masara* was prepared using the same method describe above only
72 that the corn used in this case was not processed. Pap was prepared by first soaking the corn for 8
73 hrs and milled, the milled corn was filtered and the filtrate was collected in a container and
74 allowed to stand for 24 hrs, the clear supernatant was discarded and the thick lower portion that
75 settled was then added to boiling water with continuous stirring to form a thick paste (Pap).
76 Local and foreign rice were washed and cooked at $100^\circ C$ for 45 min. About 200 g each of the
77 food samples were oven dried and grinded to powder for laboratory analysis.

78

79 *3.4 Determination of Phytate.* Phytic acid content of food samples was determined according to
80 the method described by Reddy et al. [15]; Exactly 40 g each of the powdered samples were
81 soaked in 100 mL of 2% hydrochloric acid for 5 hrs and filtered. 25 mL of the filtrate was taken
82 into a conical flask and 5 mL of 0.3% ammonium thiocynate (NH_4SCN) was added, the mixture
83 was titrated with a standard solution of $FeCl_3$ (0.25M), until a brownish yellow colouration
84 appear and persisted for 5 min. From the titre values obtained, phytic acid content was calculated
85 from the relation; 1 mL of 0.25 M $FeCl_3$ = 6.606 mg of phytate according to the method of
86 Reddy et al. [15].

87

88 *3.5 Determination of Minerals (Ca, Zn and Fe).* The minerals were determined using atomic
89 absorption spectrophotometry [16].

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91 *3.5.1 Digestion of Samples.* Exactly 500 mg each of the powdered samples were digested with 10
92 mL Conc. HNO_3 , 4 mL of 60% $HClO_4$, and 1 mL of Conc. H_2SO_4 . After cooling each of the
93 digest was diluted with 50 mL of deionized water. It was then filtered and the filtrate was made

94 up to 100 mL in a glass volumetric flask with deionized water [16]. All the minerals were
95 determined from the triple acid-digested samples by atomic absorption spectrophotometer
96 (AAS). The AAS analysis was carried out in NARICT Zaria, Nigeria and Central Laboratories
97 Complex Bayero University Kano, Nigeria. Exactly 1 mL each of the digested samples was
98 analysed using AAS for the various elements mentioned, and their respective absorbance
99 recorded. The various concentrations in part per million (ppm) of the minerals was extrapolated
100 from their standard calibration curves.

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102 *3.5.2 Preparation of Standard Curve for Various Elements.* A stock solution of calcium was
103 prepared by dissolving 2.49 g of dried calcium carbonate in 1000 mL volumetric flask and the
104 solution made up to the mark with distilled water. This solution contained 1000 ppm Ca^{2+} . From
105 this stock solution, calcium standard solutions were prepared and their absorption was taken, a
106 standard curve of absorbance against concentration for calcium was plotted. A stock solution
107 containing 1000 ppm of Fe^{2+} was prepared by dissolving 1 g of pure iron wire in 10 mL of
108 concentrated nitric acid, boiled in a water bath and diluted to 1000 mL. Standard solutions were
109 prepared and absorption taken and a standard curve for iron were plotted. Similarly, stock
110 solution containing 1000 ppm of Zn was prepared by dissolving 2.0 g of ZnCl_2 in 1000 mL
111 volumetric flask and the volume was made up to the mark. Standard solutions were prepared and
112 absorptions readings were taken and a standard curve of Zn was plotted.

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114 *3.6 Statistical analysis.* The data obtained were expressed as means \pm SD and analyzed with
115 SPSS version 17. One way analysis of variance (ANOVA) with subsequent Bonfferoni's *post*
116 *hoc* test was carried out. Differences were considered significant at $p \leq 0.05$. Data was presented
117 as tables as appropriate.

118 119 **4. Results and Discussion**

120 The term "bioavailability" is defined as the proportion of an ingested nutrient in food that is
121 absorbed and utilized through normal metabolic pathways [17]. Environmental conditions
122 (climate, soil, and irrigation), fertilizer applications, and stage of maturation influence the
123 phytate content of cereals, legumes, and oleaginous seeds [18]. Hence the need to determine the
124 phytic acid levels of food consumed in a specific region. Many techniques are used to determine
125 the bioavailability of minerals in foods with respect to the anti-nutritional effect of phytic acid,
126 one of the methods is by measuring the mole ratio of phytate to minerals in the food samples
127 [19]. Foods with ratios above the critical levels have a poor bioavailability for the mineral. The
128 result obtained in the present study showed that the phytic acid content of local rice (9.90
129 mg/100g) was greater than all the other foods analysed, Pap has the lowest phytic acid content of
130 (2.76 mg100/g) (Table 1)

131

132 **Table 1:** Phytate and mineral contents of commonly consumed staple cereal foods in Zaria,
 133 Nigeria

Samples	Phytate and mineral elements (mg/100g of sample)			
	Phytate	Calcium	Iron	Zinc
A	4.40 ± 0.95 ^d	100.70 ± 2.49 ^{cd}	1.90 ± 0.41 ^d	1.02 ± 0.00 ^{bcd}
B	6.05 ± 0.95 ^{ac}	83.68 ± 2.43 ^e	4.04 ± 0.41	1.58 ± 0.20 ^{ae}
C	2.76 ± 0.95 ^{ade}	66.66 ± 2.45 ^{ae}	5.46 ± 0.40	1.68 ± 0.00 ^{ae}
D	9.90 ± 1.65 ^{abce}	79.42 ± 2.46 ^{ae}	7.60 ± 0.41 ^a	1.56 ± 2.10 ^{ae}
E	5.50 ± 0.95 ^{cd}	105.00 ± 22.50 ^{bcd}	10.00 ± 0.31 ^a	2.04 ± 0.00 ^{abcd}

134 Data presented are means ± SD and analysed using one way ANOVA followed by Bonfferoni's *post hoc* test.
 135 Values with the same superscript to that of the samples differ significantly at (P = .05) along a column. Sample A;
 136 Tuwon surfafen Masara, Sample B; Tuwon masara, Sample C ; Pap, Sample D; Local rice, and Sample E; Foreign
 137 Rice

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139 According to Gibson et al. [20] soaking is one of the food preparation methods that can reduce
 140 phytic acid content. This could be the reason why Pap has the lowest phytic acid content (its
 141 preparation involves soaking of the maize for 8 hour). According to Gibson et al. [20], unrefined
 142 cereals and legumes have a high content of phytate, a potent inhibitor of mineral absorption
 143 compared to their refined counterpart. This is most likely the reason why Tuwon masara and
 144 local rice (unrefined) demonstrate significant (P = .05) high phytic acid content compared to
 145 Tuwon surfafen masara and foreign rice (refined) respectively (Table 1). In general the phytic
 146 acid levels obtained in the present study are lower than those previously reported for cereal foods
 147 [21, 22], this could be attributed to differences in geographical locations [18] and food
 148 processing methods [20]. Foreign rice showed the highest levels of calcium, iron and zinc; 105,
 149 10 and 2.04 mg/100g respectively (Table 1). The levels of calcium are lowest in Pap (66.66
 150 mg/100g) similarly; Tuwon Surfafen Masara records the lowest levels of iron (1.90 mg/100g)
 151 and zinc (1.02 mg/100g). The low levels of these minerals demonstrated by Pap and Tuwon
 152 surfafen Masara could be attributed to the food processing methods in their preparation which
 153 has to do with soaking and refinement respectively. The highest ratio for phytate:ca was
 154 demonstrated by Local rice (0.0075) and the lowest ratio was demonstrated by Pap (0.0025)
 155 (Table 2).

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159 **Table 2:** Phytate and minerals mole ratios of commonly consumed staple cereal foods
 160 in Zaria, Nigeria
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Sample	Phytate:Ca	Phytate:Fe	Phytate:Zn
Tuwon surfafen masara	0.0026	0.197	0.429
Tuwon masara	0.0044	0.127	0.376
Pap	0.0025	0.043	0.162
Local Rice	0.0075	0.110	0.625
Foreign Rice	0.0031	0.046	0.266

162
 163
 164 According to Emeta et al. [22] phytate:calcium molar ratios less than 0.17 are indicators of good
 165 bioavailability. The phytate:ca mole ratios obtained are far lower than the critical levels of 0.17
 166 which showed that the bioavailability of calcium is not affected by phytic acid in the food
 167 samples analysed. According to Hurrell [23], phytate:Fe mole ratios less than one (1) are
 168 desirable for iron bioavailability. The result in table 2 showed phytate:Fe ratios of less than 1 for
 169 all the food samples with the highest ratio (0.197) demonstrated by Tuwon surfafen masara.
 170 Accordingly, this result showed that the bioavailability of iron is not affected by phytic acid.
 171 Similar result was obtained for the phytate:zn ratio which were all below the critical levels of 18
 172 according to the classification of Hotz and Brown [24]. Local rice showed the highest levels of
 173 Phytate:zn of 0.625

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176 **5. Conclusion**

177 All the food samples analysed showed a phytate to mineral mole ratios below the critical values
 178 and therefore the bioavailability of Ca, Fe and Zn is assured. The phytate content of the food
 179 samples is low this may be due to the effect of cooking and other processing during food
 180 preparation. The consumption of these cereal foods: Pap, *Tuwon masara*, *Tuwon surfaffen*
 181 *masara*, local rice and foreign rice in Zaria, Nigeria will not result in the deficiency of Ca, Zn
 182 and Fe in the body with respect to the anti-nutritional effect of phytate

183 **Recommendations**

184 Further work should be carried out to evaluate the effect of other anti-nutrient such as oxalate,
 185 tannins, saponins and metal chelators on the bioavailabilities of Ca, Zn and Fe in cereal staple
 186 foods consumed in Zaria, Nigeria.

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