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Food Science & Nutrition Technology
ISSN: 2574-2701

Impacts of Fermentation Time on Phytate and Some Minerals Bioavailability of Maize and Haricot Bean Flour

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Research Article
Volume 8 Issue 1
Received Date: April 19, 2023
Published Date: May 24, 2023
DOI: 10.23880/fsnt-16000299

Abstract

Less in nutrient bio availability due to the presence of ant nutritional factors are common problem in plant based diet. Fermentation is a feasible food processing approach to improve nutrient bioavailability in plant based diets. The objective of this study was to estimate some minerals bioavailability in fermented maize and haricot bean flour. Maize and haricot bean flour were subjected to three fermentation time (0, 24 and 36 hours) at room temperature. Phytate, calcium, iron, zinc and their molar ratio were determined using official methods. The results showed that significant reduction of Phytate and calcium content were obtained in the maize and haricot bean flour as fermentation time increased. Moreover, iron in mg/100g ranged from 3.95±0.10 to 3.20±0.21 in maize flour and 7.38 ±0.34 to 5.20±0.16 in haricot bean flour. Zinc content in (mg/100g) increased from 2.79±0.03 for unfermented maize flour to 3.47±0.10 for 24 hours fermented followed decreased to 2.95±0.29 in maize flour fermented for 36 hours at room temperature. The molar ratios of the phytate to calcium and phytate to zinc in this study resulted improved calcium and iron bioavailability in maize and haricot bean flour. However, poor and good for iron bioavailability in haricot bean and maize flour, respectively, were noted in this study. Therefore, use of fermented maize and haricot bean flour should be recommended, in the production of nutritious and safe food products for targeted age group.

Keywords: Phytate; Calcium; Iron; Zinc; Molar Ratio

Background and Justification

Nutrient bioavailability refers to amount of nutrient that is absorbed, ready to provide the necessary benefits for the physiological functions and stored as energy form in our body. Foods and diets of plant origin have been shown less in nutrient bio availability due to the presence of antinutritional factors [1]. Plenty of antinutritional factors like phytate, tannins, polyphenol, oxalate and trypsin inhibitors are present in food crops. Among predominant antinutritional factors in plant based diets, Phytate has been significantly, affects bioavailability of many minerals like calcium, iron, copper, zinc and manganese [2]. Phytate/Phytic acid is potential antinutritional factors, source of inositol, and contributed to 70% of total phosphorus stored in plant seeds [3]. It is prevalent anti-nutritional factors in most of the cereals and has strong ability to chelate minerals ions, like Zinc, Calcium and iron thereby making them poorly absorbed by human body [4].

Phytate also found in large quantities in haricot beans and known to form complexes with certain minerals and leads to lower bioavailability of mineral ions in humans [5]. It is insoluble form of negatively charged salt and inhibit absorption of essential nutrients in the human intestine, mainly affects pregnant women that leads to prevalent micronutrient deficiency [6,7]. Bioavailabilities of the total minerals present in food stuffs are need serious attention than their concentration. Cereals and legumes are the most widely produced, consumed and staple crops worldwide and most frequently fermented to various food products [8,9]. Cereals are rich in energy and other caloric nutrients while legumes are good source of protein and minerals used in body building and cell maintenance.

Maize (Zea mays L.) is widely produced cereal crop in the world and is providing great benefits for human consumption, animal feed and various industrial inputs [10]. According to Suri and Tanumihardjo, [11] whole maize kernel contained different concentration of phosphorus, calcium, potassium, iron, and zinc. In other words haricot beans (Phaseolus vulgaris L.) are one of the legumes crop which are good sources of nutrients and have a low glycemic index, ranging from 26/42% relative to glucose [12].

Even though the amount of minerals presents in the maize and haricot beans are high, their bioavailability are low due to inhibition activity of various antinutritional factors. Different economically important biochemical and thermal food processing techniques have been used for substantial reduction of antinutritional factors found in cereals and legumes food crops [13-15]. Soaking, dehulling, cooking, boiling, autoclaving, microwave heating, germination and fermentation are considerable and easily applicable food processing techniques used in the reduction of such antinutritional factors [16-18].

Fermentation is among biochemical process that desirably enhances food constituents by increasing the palatability, sensory properties and alters nutritional constituents [19]. It also played an important role to increase bioavailability of minerals in cereals, legumes and other crops. During fermentation of cereals, legumes and other crops, optimum pH conditions for the phytase enzyme activity was created for degradation of phytate, presented in the form of complexes with proteins and polyvalent cations such as zinc, calcium, magnesium, and iron [20]. This attributed to enhancement of minerals bioavailability and favorability of minerals absorption in human body. Therefore, the objective of this study was to evaluate phytate and bioavailability of calcium, iron and zinc presented in maize and haricot bean flour fermented under different fermentation time.

Materials and Methods

Sample Collection

Maize (Zea mays L.) and Haricot bean (Phaseolus vulgaris L.) were obtained from Melkassa Agricultural Research Center of Ethiopian Institute of Agricultural Research. Collected samples were transported to Food Science and nutrition, laboratory of Melkassa Agricultural Research Center. Any post-harvest pesticides was not applied to obtained maize and haricot bean grain sample. Maize (Melkassa 2 variety) and Haricot bean (Awash 1 variety) were used in the experiment.

Experimental Design and Plan

The experiment was organized in a single factorial arrangement consisting of one factor, fermentation time. Maize and haricot flour were subjected to different fermentation time separately. The details of treatment combination were performed as indicated below. Fermentation has three levels (0 h, 24 h and 36 h). The experiment was organized in the total of six treatments as shown in the Table 1 below.

Ingredients	**Fermentation time**		
	F0	**F1**	**F2**
M	M*F0	M*F1	M*F2
HB	HB*F0	HB*F1	HB*F2

Table 1: Experimental plan.

Where, h= hour, F0= Fermentation for 0 h, F1 = Fermentation at room temperature for 24 h, F2 = Fermentation at room temperature for 36 h, M= 100% Maize flour, HB = 100% Haricot bean flour

Sample Preparation

Preparation of Maize Flours

Maize grain about nine kilogram was sorted, cleaned and abundantly washed by immersion in cold tap water stirred by hand and screened out of the water to remove impurities. The kernels then sun dried and milled using cyclone milling machine with 30-1060 model into flour using laboratory miller machine to sieve size of 0.5 mm. Finally, the maize flour was packaged and stored at room temperature in glass bottles (container) until used for phytate and minerals analysis.

Production of Haricot Bean Flour

Haricot bean seeds, about nine kilogram were sorted by removing dirt and broken beans. The clean beans were soaked in tap water (1:5 w/v) for 12 h at room temperature (28 ± 2°C). Soaked haricot bean seeds were dehulled manually by rubbing between palms. The dehulled beans were spread on a tray and dried in hot air oven drying with 3010 -019EN55014/EN55014S/N model at 60°C to a constant weight. The dried haricot bean seeds were subjected to roasting. Haricot bean roasting was carried out following the method described by Oraka and Okoye [21]. The dried beans were then roasted in an open frying pan with constant stirring on a electric stove using a moderate heat (130-140 ^{0}C) for 15 –20 min. The roasted beans were milled into flour, packed in a polythene bag and sealed. The beans flour was kept in the refrigerator before used for food product development and experimental analysis.

Phytate Determination

Phytate was determined according to procedure described by Latta and Eskin [22] as modified by Vaintraub and Lapteva [23]. About 0.1g of dried flour samples was extracted with 10ml of 2.4% HCl in a mechanical shaker for 1hour at room temperature and centrifuged at 3000rpm for 30 minutes. The clear supernatant was used for phytate estimation. A 2ml of Wade reagent (containing 0.03% solution of FeCl3.6H2O and 0.3% of sulfosalicilic acid in water) was added to 3ml of the sample solution (supernatant) and the mixture was mixed on a Vortex for 5 seconds. The mixtures were centrifuged for 10 minutes and the absorbance of the sample was measured at 500nm by using UV-VIS spectrophotometer while water used as a blank. A standard curve was made from absorbance versus concentration and the slope and intercept were used for calculation

Phytate in mg/100g = (absorbance-intercept) 3 / (slope * density * weight of sample *10)

Minerals Determination

Calcium, iron and zinc of unfermented and fermented maize and haricot bean flour were determined using atomic absorption spectrophotometer [43].

Minerals Bioavailability

Mineral bioavailability was estimated by determining molar ratios of phytate/ minerals using formula described by Norhaizan and Nor Faizadatul Ain [24]. The molar ratios of phytate to Calcium (phy: Ca), phytate to iron (phy:Fe), and phytate to zinc (phy:Zn) were calculated as following formula

$$\text{Molar ratio} = \frac{\text{mole of phytate}}{\text{mole of mineral}} \quad (1)$$

$$\text{Mole of phytate} = \frac{\text{mass of phytate (mg)}}{\text{molar mass of phytate}\left(\frac{\text{g}}{\text{mol}}\right)} \quad (2)$$

$$\text{Mole of minerals} = \frac{\text{mass of mineral (g)}}{\text{molar mass of mineral}\left(\frac{\text{g}}{\text{mol}}\right)} \quad (3)$$

The molar mass in (g/mol) 660, 40, 56 and 65 were referred for phytate, calcium, iron and zinc, respectively.

Fermentation

The fermentation was carried out according to Griffith, et al. [25]. Flour obtained from each raw material was mixed with distilled water in the ratio of 1:4 (w/v) to make dough. It was allowed to ferment in a cleaned plastic container covered with plastic material at room temperature for 24 hr and 36 hr fermentation period. The dough fermentation was preceded by adding 5% of ersho, starter culture obtained from previously fermented maize and haricot bean flour. The fermented dough was dried in laboratory oven dry at 70^{0}C for 16 hr. The dried slurries were milled to a fine powder using a laboratory miller machine to 0.5 mm sieve size and kept at 4 ^{0}C for phytate, calcium, and iron and zinc analysis [26].

Data Analysis

The collected data was analyzed using SAS Statistical software. One way analysis of variance (ANOVA) was used for statistical data analysis. The critical difference at $P< 0.05$ was estimated and used to find the significant difference among the sample mean.

Results and Discussions

Phytate

Ranged Phytate content obtained in the maize flour was from 1.61±0.25 to 2.39±0.14 mg/100g in this study (Table 2). The highest phytate value 2.39±0.14 mg/100g was found in unfermented maize flour while the lowest value, 1.61±0.25 mg/100g was noted for maize flour fermented for 36 hour. Similar trends of reduction in phytate content were recorded for haricot bean flour. The highest phytate content 240.03±1.25 mg/100g was recorded for unfermented haricot bean flour while the lowest value, 90.96 ±2 mg/100g was obtained in haricot bean flour fermented for 36 hour. Substantial reduction of phytate in maize and haricot bean flour in the present study was in line with related research finding reported by Onuoha, et al. [27] for pearl millet fermented under natural and using pure starter culture for 4 days. This significant reduction of phytate in maize and haricot bean was contributed by activities of phytase enzyme, activated during fermentation process.

	Maize flour				Haricot bean flour			
Fermentation time in hour	**Phytate**	**Calcium**	**Iron**	**Zinc**	**Phytate**	**Calcium**	**Iron**	**Zinc**
0h	2.39±0.14^{a}	34.50±0.92^{a}	3.95±0.10^{a}	2.79±0.03^{b}	240.03±1.25^{a}	75.36±1.19^{a}	7.38±0.34^{a}	6.28±0.18^{a}
24h	2.13±0.11^{a}	25.33±0.91^{b}	3.36 ±0.31^{b}	3.47±0.10^{a}	107.53±1.84^{b}	59.51±0.8^{b}	5.37±0.95^{b}	6.03±0.18^{a}
36h	1.61±0.25^{b}	23.53±0.16^{c}	3.20±0.21^{b}	2.95±0.29^{b}	90.96±2^{c}	57.44±0.45^{c}	5.20±0.16^{b}	5.57±0.76^{a}
CV	8.76	2.72	6.34	5.9	1.18	1.36	9.89	7.81
LSD	0.358	1.5086	0.4439	0.3621	3.4516	1.7401	1.1822	0.9303

Table 2: Phytate and some minerals content of unfermented and fermented Maize and Haricot bean Flour in (mg/100g). Means within the same column followed by the same letter are not significantly different (P>0.05), a>b>c, CV = Coefficient of variance, LSD = Least significant difference, h = hours.

Calcium

Calcium plays an important role in blood clotting, muscle contractions, optimum growth and development of infant and children [28]. The result in the present study showed that calcium content in maize flour significantly, decreased from 34.50 ±0.92 mg/100g to 23.53 ± 0.16 mg/100g as fermentation time increased (Table 2). This might be attributed to leaching of minerals into decanted fermentation water. Calcium content obtained in the haricot bean flour varied over 57.44 ±0.45 mg/100g to 75.36 ± 1.19 mg/100g in the current study.

Iron

Iron is important micronutrients for the formation of hemoglobin, the component of red blood cell that carries oxygen to body's organs and transport carbon dioxide from organs and tissue to lungs. Adequate iron in the diet is essential to reduce the incidence of iron deficiency anemia [29]. There were statistically, significant decrements ($P< 0.05$) of iron during the fermentation of maize and haricot bean flours as shown in Table 2. Fermentation of maize flour resulted in decrement of iron from 3.95±0.10 mg/100g for zero hour to 3.36±0.31 mg/100g and 3.20±0.21 mg/100g for 24 and 36 hours fermentation, respectively, while iron concentration decreased during fermentation of haricot bean dough from 7.38 ±0.34 mg/100g for zero hour to 5.37± 0.95 mg/100g and 5.20±0.16 mg/100g for fermented at ambient temperature for 24 and 36 hours, respectively. Significant reduction in some mineral contents of maize and haricot bean flour during fermentation in the present study was in agreement with research finding reported by Granito, et al. [30]; Adebiyi, et al. [31] for fermented common beans and Bambara ground nut. The decreases of those minerals were attributed to the leaching of mineral elements into decanted fermentation water and the utilization of mineral elements by microorganisms during fermentation [32].

Zinc

Zinc content of maize and haricot bean flour fermented at room temperature for different fermentation time was shown in Table 2. Zinc content of maize flour increased from 2.79±0.03 mg/100g for unfermented maize flour to 3.47±0.10 mg/100g for 24 hours fermented followed decreased to 2.95±0.29 in maize flour fermented for 36 hours at room temperature. Statistically, non-significant change was observed in zinc content of haricot bean flour as fermentation time progressed.

Molar Ratio

Molar ratio of phytate to calcium, phytate to iron and phytate to zinc of maize and haricot bean flour were presented in Table 3. According to Bhandari and Kawabata, [33] the bioavailability of minerals, like calcium, magnesium, iron and zinc decreased due to presence of phytate in given food. Molar ratios of phytate-to selected minerals of fermented maize and haricot bean can be used to predict the proportion of minerals absorbed in human body. Accordingly, molar ratio of phytate to calcium was varied over 0.0693± 0.0061 for unfermented and 0.0837±0.0025, 0.0683±0.0107 for 24 and 36 hour fermented maize flour, respectively.

As shown in table 3 molar ratio of phytate to calcium in haricot bean flour was ranged from 0.0960±0.0026 to 0.1931±0.0036. The recommended critical value for phytate to calcium >0.5 indicates poor calcium absorption [34]. In the present study molar ratio of phytate to calcium is less than critical value (0.5) which indicates, fermentation resulted good bioavailability of calcium in maize and haricot bean flour. This reflects that calcium in fermented maize and haricot bean flour is good for absorption. In similar manner molar ratio of phytate to iron was ranged from 0.5017±0.04 in 36 hours fermented to 0.6337±0.03 for unfermented maize flour as referred from Table 3.

	Maize flour			Haricot bean flour		
Fermentation time in hour	**Phytate :Ca**	**Phytate: Fe**	**Phytate: Zn**	**Phytate :Ca**	**Phytate: Fe**	**Phytate: Zn**
0h	0.0693± 0.0061^{ab}	0.6057± 0.0236^{a}	0.8570±0.0584^{a}	0.1931±0.0036^{a}	2.7653±0.1418^{a}	3.7640±0.0891^{a}
24h	0.0837±0.0025^{a}	0.6337±0.0341^{a}	0.6130±0.0401^{b}	0.1095±0.0033^{b}	1.7346±0.2927^{b}	1.7574±0.0422^{b}
36h	0.0683±0.0107^{b}	0.5017±0.0456^{b}	0.5470±0.0796^{b}	0.0960±0.0026^{c}	1.4868±0.0788^{b}	1.6338±0.2679^{b}
CV	9.84	6.13	9.15	2.43	9.64	6.91
LSD	0.0145	0.0711	0.1229	0.0064	0.386	0.3293

Table 3: Molar ratio of phytate to some minerals in unfermented and fermented maize and haricot bean flour.
Means within the same column followed by the same letter are not significantly different (P>0.05), a>b>c, CV = Coefficient of variance, LSD = Least significant difference, h = hours, Ca = Calcium, Fe = Iron, Zn = Zinc.

Statistically, the highest molar ratio of phytate to iron (2.7653±0.14) in haricot bean was noted for unfermented sample and the lowest value (1.4868±0.07) was recorded for 36 hours fermented haricot bean sample. Molar ratio of phytate to iron > 1 showed poor iron bioavailability [35]. The result of molar ratio of phytate to iron in the present study is less than 1 revealed good bioavailability for iron obtained in maize flour. This indicated as fermentation time increased bioavailability of iron enhanced. This is also contributed to degradation of phytate and liberating bonded minerals ion to phytate freely during fermentation [36,37].

In contrast to maize flour molar ratio of phytate to iron in haricot bean flour was greater than recommended critical value (>1) showed poor iron bioavailability. This might be attributed to higher concentration of phytate present in haricot bean flour which leads proportionally higher mole of phytate According to Hambidge, et al. (2008) [6] zinc absorption is highly affected as a result of phytate content found in the food stuff. Phytate to zinc molar ratios >15, referred of poor zinc bioavailability [38]. In the present study molar ratio of phytate to zinc in both maize and haricot bean samples fermented at ambient temperature for different fermentation time were in acceptable recommended critical value < 15 which reflects good indicator for zinc bioavailability. Related research finding showed that improved zinc bio availability was obtained in finger millet fermented for 24 hour [39] and also in cow pea fermented for 96 hours using Aspergillus oryzae as fermentation microorganism [40-42].

Conclusion

The present study has shown that Phytate significantly reduced as fermentation time increased in both maize and haricot bean flour. Fermentation strongly, improved calcium, iron and zinc bioavailability in maize flour. Good bioavailability of calcium and zinc were noted in haricot bean flour fermented under different fermentation time. The result of molar ratio of phytate to iron in the present study indicates Poor iron bioavailability in haricot bean flour.

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