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EFFECT OF CEREAL BASED TRADITIONAL MALTING TECHNOLOGY ON NUTRITIONAL QUALITY OF IRON-RICH BEAN FLOUR

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ABSTRACT

Iron deficiency is a major public health challenge affecting the health of about 18 and 13.8% of children and women globally, respectively. About 43% children and 29% women in sub-Saharan Africa suffer from Iron Deficiency Anemia. In Uganda, recent demographic health statistics indicate that the prevalence of anaemia among children of 6-59 months stands at 53%; while that for women of child bearing age stands at 32%. Biofortified iron-rich bean varieties have been developed and adopted in Uganda to contribute to alleviation of iron deficiency challenges. The objective of this study was to investigate the effect of traditional malting technology on nutritional quality of biofortified iron-rich beans (*Phaseolus vulgaris* L.). The study examined the effect of the traditional malting technology on: (i) the contents of anti-nutritional factors (phytates, oxalates, polyphenols, tannins, trypsin inhibitor activity); (ii) digestibility of protein and bioavailability of iron and zinc; and (iii) retention of proximate constituents and mineral micronutrient contents in bean varieties. We used three bean varieties, namely NAROBAN 1, hereafter referred to as NB1, NAROBAN 2 (NB2), and NAROBAN 3 (NB3), all of which are widely produced and consumed in Acholi sub-region. Application of paired t-test revealed that the traditional malting technology reduced only the content of oxalates by 42.3-54.8 % and trypsin inhibitor activity by 6.2-34.6 %, from the three varieties. The content of total phenols was reduced by 22.3 % only in NB 1 ($P < 0.05$). The traditional malting technology improved protein digestibility for the three varieties by 38-43.6 % ($P < 0.05$). Bioavailability of iron improved from in NB1 by 26.7 % and NB2 by 11.5 %; while that of zinc improved for only NB2 by 51.5 % ($P < 0.05$). The contents of micronutrients and proximate constituents were not adversely affected by the traditional malting technology, except for phosphorus in NB1 and magnesium in NB3. Overall, traditional malting technology is effective at reducing trypsin inhibitors and oxalates, and improving protein digestibility and iron bioavailability.

Key Words: Anti-nutritional factors, bio-fortified, *Phaseolus vulgaris*

RÉSUMÉ

La carence en fer est un défi majeur de santé publique affectant la santé d'environ 18 et 13,8 % des enfants et des femmes dans le monde respectivement. Environ 43 % des enfants et 29 % des femmes en Afrique subsaharienne souffrent d'anémie ferriprive. En Ouganda, des statistiques démographiques récentes sur la santé indiquent que la prévalence de l'anémie chez les enfants de 6 à 59 mois est de 53 % ; tandis que celui des femmes en âge de procréer est de 32 %. Des variétés de haricots biofortifiées riches en fer ont été développées et adoptées en Ouganda pour contribuer à atténuer les problèmes de carence en fer. L'objectif de cette étude était d'étudier l'effet de la technologie de maltage traditionnel sur la qualité nutritionnelle des haricots (*Phaseolus vulgaris* L.) biofortifiées riches en fer. L'étude a examiné l'effet de la technologie de maltage traditionnel sur : (i) la teneur en facteurs anti-nutritionnels (phytates, oxalates, polyphénols, tanins, activité inhibitrice de la trypsine) ; (ii) digestibilité des protéines et biodisponibilité du fer et du zinc ; et (iii) la rétention des constituants proches et des teneurs en micronutriments minéraux dans les variétés de haricots. Nous avons utilisé trois variétés de haricots, à savoir NAROBAN 1, ci-après dénommées NB1, NAROBAN 2 (NB2) et NAROBAN 3 (NB3), qui sont toutes largement produites et consommées dans la sous-région Acholi. L'application du test t apparié a révélé que la technologie de maltage traditionnelle ne réduisait que la teneur en oxalates de 42,3 à 54,8 % et l'activité inhibitrice de la trypsine de 6,2 à 34,6 %, pour les trois variétés. La teneur en phénols totaux a été réduite de 22,3 % uniquement dans le NB 1 ($P < 0,05$). La technologie de maltage traditionnel a amélioré la digestibilité des protéines pour les trois variétés de 38 à 43,6 % ($P < 0,05$). La biodisponibilité du fer s'est améliorée de 26,7 % dans NB1 et de 11,5 % dans NB2 ; tandis que celle du zinc s'est améliorée pour seulement NB2 de 51,5 % ($P < 0,05$). La teneur en micronutriments et en constituants proches n'a pas été affectée par la technologie de maltage traditionnelle, à l'exception du phosphore dans NB1 et du magnésium dans NB3. Dans l'ensemble, la technologie de maltage traditionnelle est efficace pour réduire les inhibiteurs de la trypsine et les oxalates et améliorer la digestibilité des protéines et la biodisponibilité du fer.

Mots Clés : Facteurs anti-nutritionnels, bio-fortifiés, *Phaseolus vulgaris*

INTRODUCTION

Malnutrition, particularly involving micronutrient deficiencies, still persists as a public health burden with about 30% of the population found in sub-Saharan countries. Children below 5 years, and pregnant and women of child bearing age (Hadgu *et al.*, 2017; Aslam *et al.*, 2018) are the most vulnerable to this nutritional vice. These groups are highly exposed to hidden hunger, particularly iron deficiency anemia, zinc and vitamin A deficiencies (Kumssa *et al.*, 2015; Platel and Srinivasan, 2015).

Over 18% of children under 5 years suffer from iron deficiency anemia; while 17% experience inadequate zinc intake globally (Harding *et al.*, 2017). On the other hand, 13.8% of women of child bearing age suffer

from iron deficiency anemia, and 25% inadequate zinc nutrition globally (Balk *et al.*, 2019). The prevalence of micronutrient deficiency is higher in developing countries with 43% of children under 5 years and 29% women of child bearing age (Harika *et al.*, 2017).

The high prevalence of iron deficiency in sub-Saharan Africa is mainly due to locational and demographical disparities, whereby those living in resource constrained rural settings are the most affected (Hassen *et al.*, 2020; Ohanenye *et al.*, 2021). Disparities in locational and demographic settings affect accessibility to good quality food and purchasing power of such foods, with rural populations resorting to consuming starchy foodstuffs of plant origin (Kennedy *et al.*, 2013; Masibo, 2013).

Plant-based foods such as cereals and legumes have high levels of anti-nutritional factors, which compromise their nutritional quality (Guillamón *et al.*, 2008; Fekadu, 2014). The high levels of anti-nutritional factors such as phytates, trypsin inhibitors, oxalates and tannins form complexes with nutrients and render them unavailable for metabolism; hence compromising the contribution of plant-based foods to micronutrient needs of the households (Kiranmayi, 2014; Petry *et al.*, 2014).

Strategies for addressing the challenges of micronutrient deficiencies such as physical fortification and biofortification of food with iron have been pursued for some time and evidence attests to their effectiveness at improving health and nutritional status of vulnerable people, particularly in developing countries (Bailey *et al.*, 2015; Losso *et al.*, 2017). However, these strategies present a number of challenges such as (i) inadequate consumption due to the varied nutrient needs of population groups, (ii) widely used strategies often require complicated processing and technologies that are not readily available, which may increase the cost of food for low income families in developing countries and (iii) organoleptic alterations of fortified products. Others include inadequate retention and bioavailability of added nutrients, lack of health education resulting in no awareness of benefits of fortified food consumption; and the inability of these strategies to reduce anti-nutritional factors (Singh *et al.*, 2016; Yadav *et al.*, 2020; Olson *et al.*, 2021).

A number of processing techniques have been investigated for ability to eliminate anti-nutritional factors from plant based foods; these include soaking (Bolade, 2015), germination/malting (Fouad and Rehab, 2015), extrusion (Kaur *et al.*, 2012), hydrothermal treatment (Pompeu *et al.*, 2015), dehulling (Mang *et al.*, 2015) and fermentation (Platel and Srinivasan, 2015). Alowo *et al.* (2018) documented the traditional malting technology practiced in Acholi sub-region of Uganda and demonstrated its potential to improve

nutritional quality of millet-sesame-soy complementary food composite formulae. Alowo *et al.* (2018) used native cereals and legumes that were subjected to traditional malting, a process that may bear on the nutritional quality of beans biofortified with iron. The objective of this study was to investigate the effect of traditional malting technology on nutritional quality of biofortified iron-rich beans.

MATERIALS AND METHODS

Iron-rich beans of three varieties (NAROBAN 1, 2 and 3) obtained from National Agriculture Research Organisation (NARO), Namulonge were used in this study. The seeds were hand cleaned separately using a winnower before being subjected to the traditional malting as described by Adebisi *et al.* (2018). Thus, treatments included the three bean varieties plus an unmalted controls; all laid out in a completely randomised design, with four replicates. The malted and unmalted samples were each milled separately using a locally fabricated grinding machine (Tonnet locally manufactured in Kampala, Uganda). Then, each sample was subjected to laboratory analysis for phytates, tannins, trypsin inhibitors, total phenolics, oxalates, protein digestibility, and *in-vitro* bioavailability of iron and zinc.

Phytates were determined by Anion Exchange Chromatographic Elution technique, followed by spectrophotometric method which involved the recovery of the column using standard sodium phytate solution of concentration $2.8 \mu\text{g ml}^{-1}$ (Sivakumaran and Herath, 2017). Tannins were determined by Vanillin-HCl method as described by Adeleke *et al.* (2017); while trypsin inhibitor activity and total phenolics were determined according to the methods previously followed by Alowo *et al.* (2018) with slight modifications whereby; the absorbance of the various tannic acid concentrations were used to obtain a regression equation that was used to determine

tannic acid in each sample extract. Oxalates were determined according to a method described by Oluwaniyi and Oladipo (2017).

Protein digestibility was determined following the enzymatic method described by Ojo *et al.* (2017); while *in-vitro* bioavailability of iron and zinc (simulated peptic and pancreatic digestion) were determined according to the enzymatic method described by Alowo *et al.* (2018). Moisture content was determined using the air oven method (Nwadike *et al.*, 2018); while ash content, crude protein and fat content were determined by the Kjeldahl method (Oghbaei and Prakash, 2016). Total carbohydrate was determined by summing up the percentages of moisture, ash, crude protein, fat, crude fibre and subtracting from 100% (Yixiang *et al.*, 2016). Mineral elements; calcium, magnesium, phosphorus, iron, manganese, potassium, sodium and zinc were determined according to the AOAC (1990) method 975.03 as previously applied by Saupi *et al.* (2009).

Data collected were subjected to normality test using Kolmogorov-smirnov. A paired sample t-test was used to compare the treatment means. One-way analysis of variance (ANOVA) was used to compare the level of these constituents among the three varieties before and after malting. These analyses were performed using the Statistical Package for Social Sciences, version 20.0.

RESULTS

Status of anti-nutritional bean factors.

Results of the effect of malting on the contents of anti-nutritional factors in biofortified iron-rich beans are presented in Table 1. Generally, malting significantly reduced ($P < 0.05$) the contents of total phenolics, trypsin inhibitors and oxalates depending on the variety. The reduction in the level of phenolics was only observed for NAROBAN 1 (22.32%), but not for other varieties. In the case of oxalates, the reduction ranged between 42.33 and 54.80% and was highest for NAROBAN 2, and lowest for NAROBAN 3. As for trypsin inhibitors,

TABLE 1. Effect of malting on content of anti-nutritional factors in iron-rich beans segregated varieties in Uganda

Parameter	Varieties/malting					
	NAROBAN 1		NAROBAN 2		NAROBAN 3	
	Malted	Control	Malted	Control	Malted	Control
Total phenolic (GAE mg 100 g ⁻¹)	26.86 ± 0.36 ^a	34.58 ± 0.00 ^b	35.09 ± 0.23 ^a	36.36 ± 2.51 ^a	34.16 ± 0.12 ^a	34.83 ± 1.07 ^a
Phytic acid (mg 100 g ⁻¹)	467.5 ± 6.36 ^a	467.5 ± 0.71 ^a	467 ± 5.66 ^a	468.5 ± 0.71 ^a	468 ± 1.41 ^a	465 ± 0.00 ^b
Tannic acid (mg 100 g ⁻¹)	2.17 ± 0.35 ^b	1.35 ± 0.0 ^b	1.75 ± 0.11 ^a	1.43 ± 0.21 ^a	1.5 ± 0.31 ^a	0.69 ± 0.14 ^b
Oxalates (mg 100 g ⁻¹)	5.99 ± 0.14 ^a	12.16 ± 0.13 ^b	5.79 ± 0.12 ^a	12.81 ± 0.26 ^b	6.69 ± 0.09 ^b	11.60 ± 0.13 ^b
TIU (mg 100 g ⁻¹)	62.2 ± 0.28 ^a	67.80 ± 0.14 ^b	43.40 ± 0.28 ^a	66.40 ± 0.14 ^b	61.45 ± 0.21 ^a	65.5 ± 0.14 ^b

Values show mean ± SD (n=6). For each variety and each parameter, means in the same row followed by different superscripts are significantly different ($P < 0.05$). TIU = Trypsin Inhibitor Units. GAE = Gallic Acid Equivalent

the level of reduction ranged between 6.2 and 34.6%; and was highest in NAROBEAN 2, and lowest in NAROBEAN 3. Overall, the contents of phytic acid and tannic acid were not significantly affected by malting ($P>0.05$).

Data on anti-nutritional factors (total phenols, tannins, trypsin inhibitors and oxalates) among the three varieties of beans before malting are presented in Figure 1. Among the four anti-nutritional factors investigated, trypsin inhibitors was the highest, followed by total phenolics, oxalates and tannins in decreasing order of magnitude. However, there was no evidence of varietal effect on the levels of anti-nutritional factors in raw beans ($P>0.05$).

Data comparing the content of each anti-nutritional factor among the three bean varieties, following application of malting treatment are presented in Figure 2. Generally, the levels of anti-nutritional factors varied with bean variety, but was also dependent on the type of anti-nutritional factor. The level of total phenolics in NAROBEAN 1 was significantly lower ($P<0.05$) than detected in NAROBEAN 2 and 3. The levels of trypsin inhibitor activity and concentration of oxalates was identical in NAROBEAN 1 and 2 ($P>0.05$), but significantly lower and higher in NAROBEAN 3, respectively ($P<0.05$). The content of phytic acid was identical among the three varieties irrespective of the malting treatment ($P>0.05$) (Table 1).

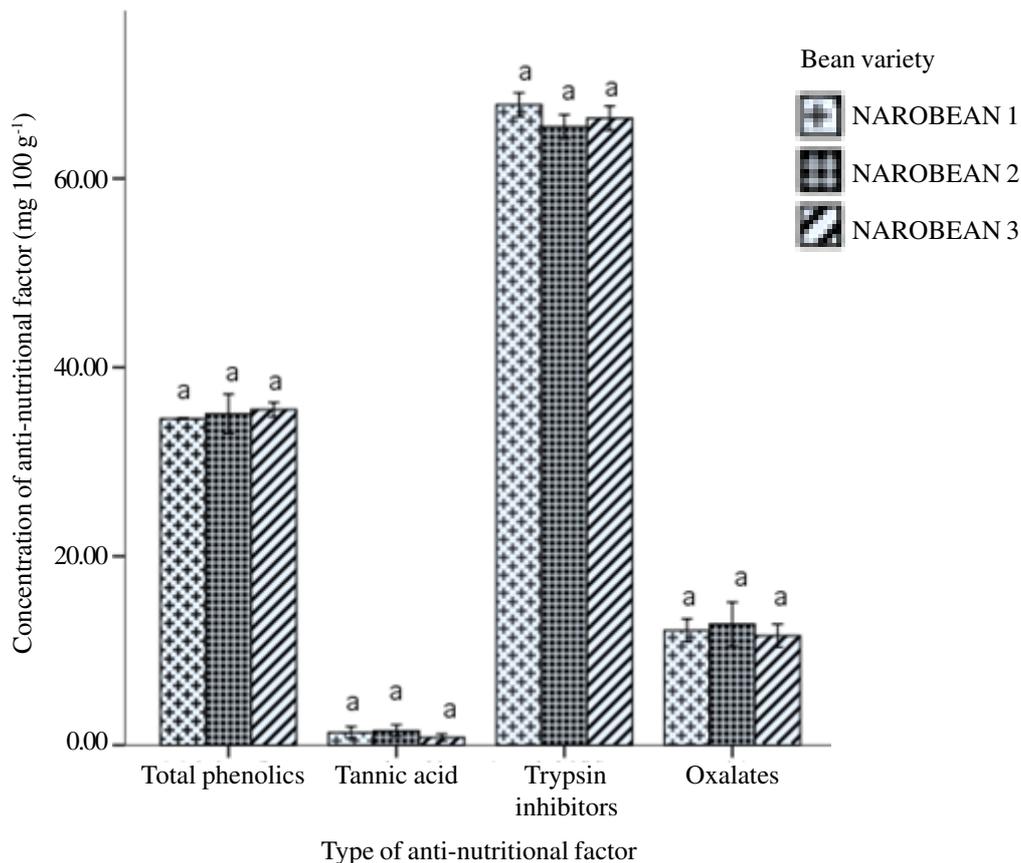


Figure 1. Initial contents of anti-nutritional factors among varieties before malting. Error bars show standard deviation ($n=6$). For each parameter, bars not sharing a common superscript letter are significantly different ($P<0.05$).

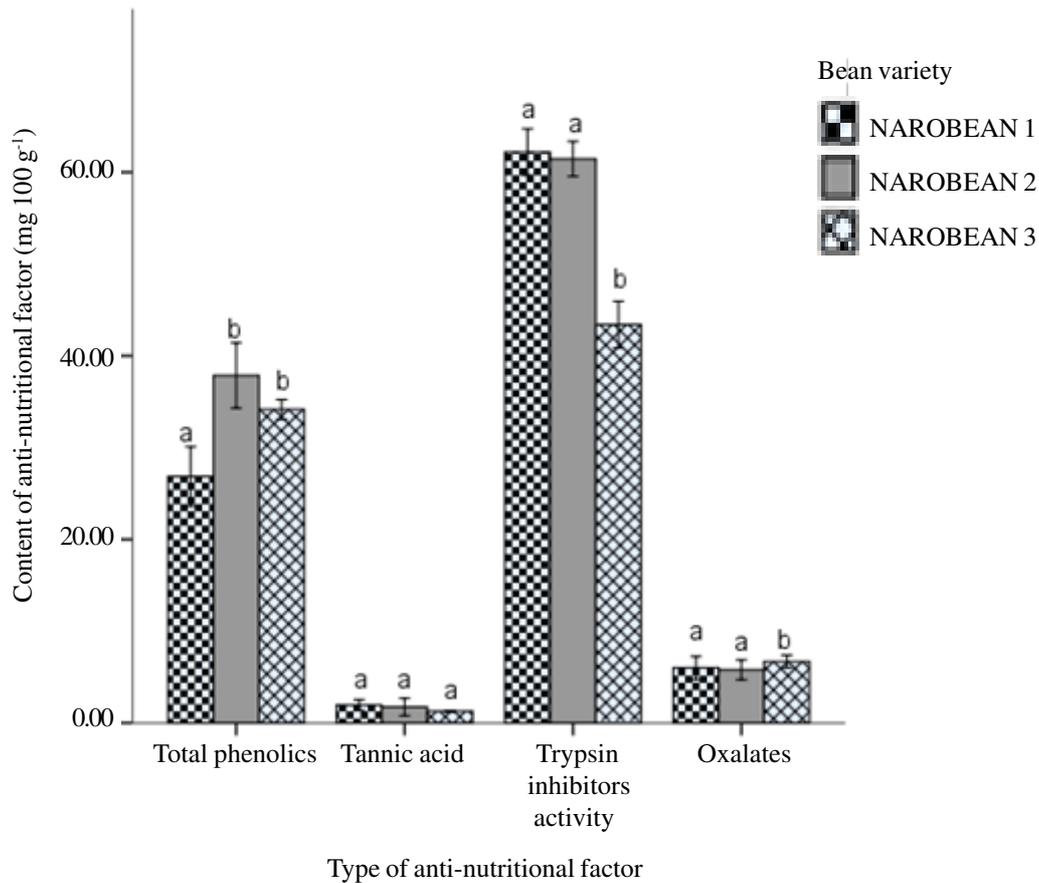


Figure 2. Contents of anti-nutritional factors among varieties after malting. Error bars show standard deviation (n=6). For each parameter, bars not sharing a common superscript letter are significantly different ($P<0.05$).

***In-vitro* protein digestibility and micronutrient bioavailability.** The results of *in-vitro* protein digestibility and micronutrient (iron and zinc) bioavailability of are presented in Table 2. Malting significantly ($P<0.05$) increased the proportion of digestible protein in all the three varieties of beans studied. The quantitative increase in digestibility of the nutrient was 38% for NAROBAN 1, 43.62% for NAROBAN 2 and 39% for NAROBAN 3. With respect to minerals, the proportion of bioavailable zinc in NAROBAN 2 increased by 51.49% as a result of malting ($P<0.05$). However, the treatment had no significant effect ($P<0.05$) on Zn bioavailability in rest of the varieties.

For iron, malted samples had significantly higher levels of bioavailable iron in NAROBAN 1 and 3 by 26.74 and 11.5%, respectively ($P<0.05$). However, the level of improvement in the bioavailability of both micronutrients in NAROBAN 1 was twice that of NAROBAN 3.

Figure 3 shows the levels of Zn and Fe bioavailability before and after malting. Although the levels of iron bioavailability were identical in all the three varieties of beans; those of zinc were identical in NAROBAN 1 and 2 before malting. After malting, bioavailability of Zn was highest in NAROBAN 2 by about 4.5% and lowest in NAROBAN 1.

TABLE 2. *In-vitro* protein digestibility and bioavailability of iron and zinc from malted and unmalted iron-rich beans segregated by variety

Parameter	Treatment					
	NAROBEAN 1		NAROBEAN 2		NAROBEAN 3	
	Malted	Control	Malted	Control	Malted	Control
% digestible protein	79.75 ± 0.06 ^a	41.83 ± 2.71 ^b	86.98 ± 4.56 ^a	43.36 ± 6.88 ^b	79.66 ± 8.31 ^a	40.77 ± 5.79 ^b
% bioavailable zinc	7.39 ± 0.40 ^a	5.96 ± 1.42 ^a	13.09 ± 0.69 ^a	6.35 ± 0.19 ^b	9.04 ± 0.68 ^a	8.64 ± 0.23 ^a
% bioavailable iron	4.30 ± 0.07 ^a	3.15 ± 0.07 ^b	4.30 ± 0.07 ^a	3.85 ± 0.07 ^a	4.85 ± 0.03 ^a	4.35 ± 0.07 ^b

Values show mean ± SD (n =6). For each variety and each parameter, means in the same row followed by different superscripts are significantly different (P<0.05)

Figure 4 shows the effect of malting on the digestibility of protein in iron-rich beans. Although there were significant differences with the treated and untreated groups, malting caused a significant difference with the control treatment, with values of the latter being nearly two folds.).

Effect of the traditional malting on nutrient retention. Results of the effect of malting on proximate composition of iron-rich beans are presented in Table 3. Generally, the effect was not uniform, but was dependent on proximate parameter and variety. In terms of proximate parameters, malting had mixed effects on moisture and a positive effect on crude fibre, ash and crude protein contents (P<0.05). In the case of moisture, its content in flour made from malted beans was significantly higher than in unmalted samples of NAROBEAN 2 by about 2.1%. However, the reverse was true for NAROBEAN 3 by about 6%. For crude fibre, the content of proximate constituent was higher in malted samples of NAROBEAN 2 and 3 by 0.8 and 1.4%, respectively; while ash content was higher in malted than unmalted samples of NAROBEAN 2 by 0.6%. However, the content of crude protein was significantly higher in malted than unmalted samples of NAROBEAN 1, 2 and 3 by 2.81, 1.94 and 3.7%, respectively. With exception of carbohydrate and crude fat for which malting had no positive effect across varieties, the effect of malting was registered largely on proximate parameters of NAROBEAN 2 and 3, but was limitedly apparent for NAROBEAN 1.

Figures 5 and 6 present results comparing initial contents of the proximate constituents among varieties, before and after malting, respectively. In the case of unmalted beans (Fig. 5), there was no significant difference in ash content, crude fat and moisture content among the three varieties (P>0.05). However, variety effect was observed in terms of the contents of crude fibre, protein and total carbohydrate. Significant differences were observed in the level of crude fibre content

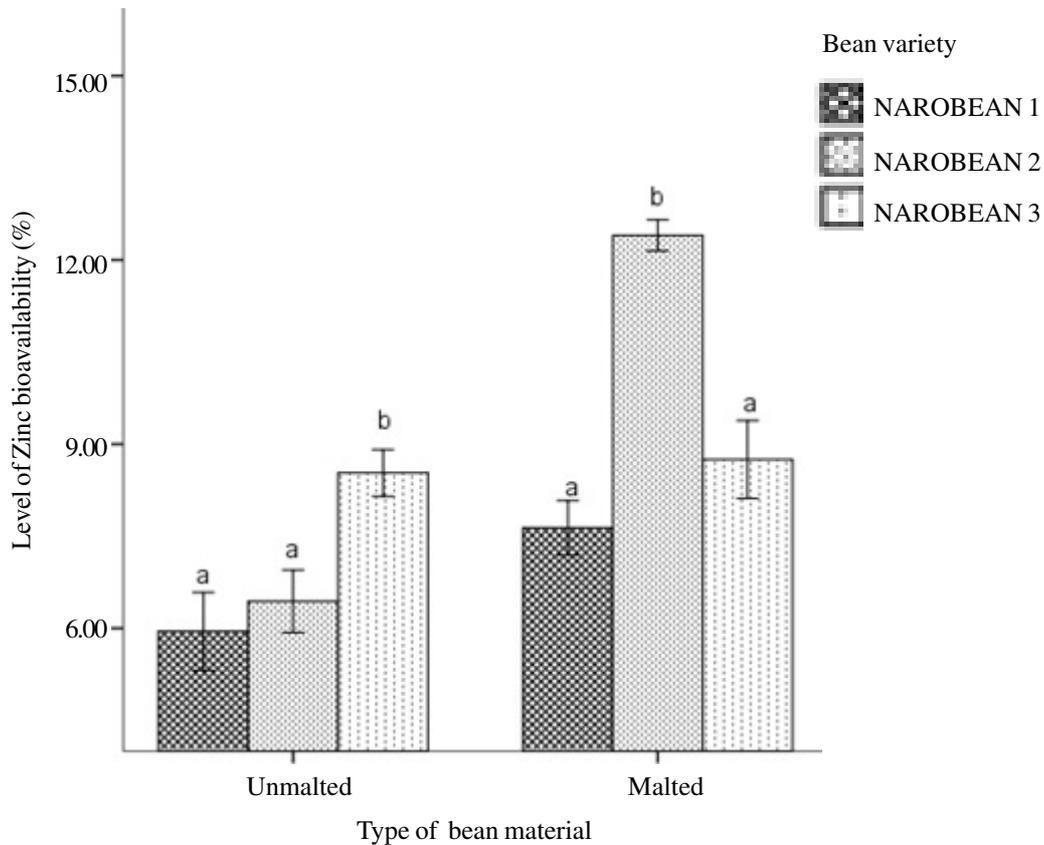


Figure 3. Zinc bioavailability among three bean varieties before and after malting. Error bars show standard deviation (n=6). For each parameter, bars not sharing a common superscript letter are significantly different ($P<0.05$).

among the three varieties of beans, with NAROBAN 3 having the highest level of the proximate component; followed by NAROBAN 1 and 2 in a decreasing order of magnitude. NAROBAN 1 had significantly lower levels of crude protein than the others that had identical levels; while NAROBAN 3 had significantly lower levels of carbohydrate content than the other varieties that had identical level of the macronutrient ($P<0.05$).

In the case of malted beans, the effect of variety was significantly apparent for all constituents, except crude fat and crude protein (Fig. 6). In situations where variations among varieties were significant, NAROBAN 3 had significantly higher levels of crude fiber than the other varieties; while NAROBAN 1

had higher levels of ash content compared to the other two varieties. In the same vein, NAROBAN 2 had lower levels of carbohydrate compared to the other varieties while moisture content was significantly different among all the three varieties. Overall, NAROBAN 2 had the highest level of the proximate constituent; followed by NAROBAN 1 and 2 in decreasing order of magnitude.

The effect of malting on micronutrient contents was largely observed in a few isolated cases and mixed in nature (Table 4). Whereas the levels of all the micronutrients were largely unaffected, irrespective of variety, the effects were only observed for phosphorus and iron in NAROBAN 2, sodium in NAROBAN 1

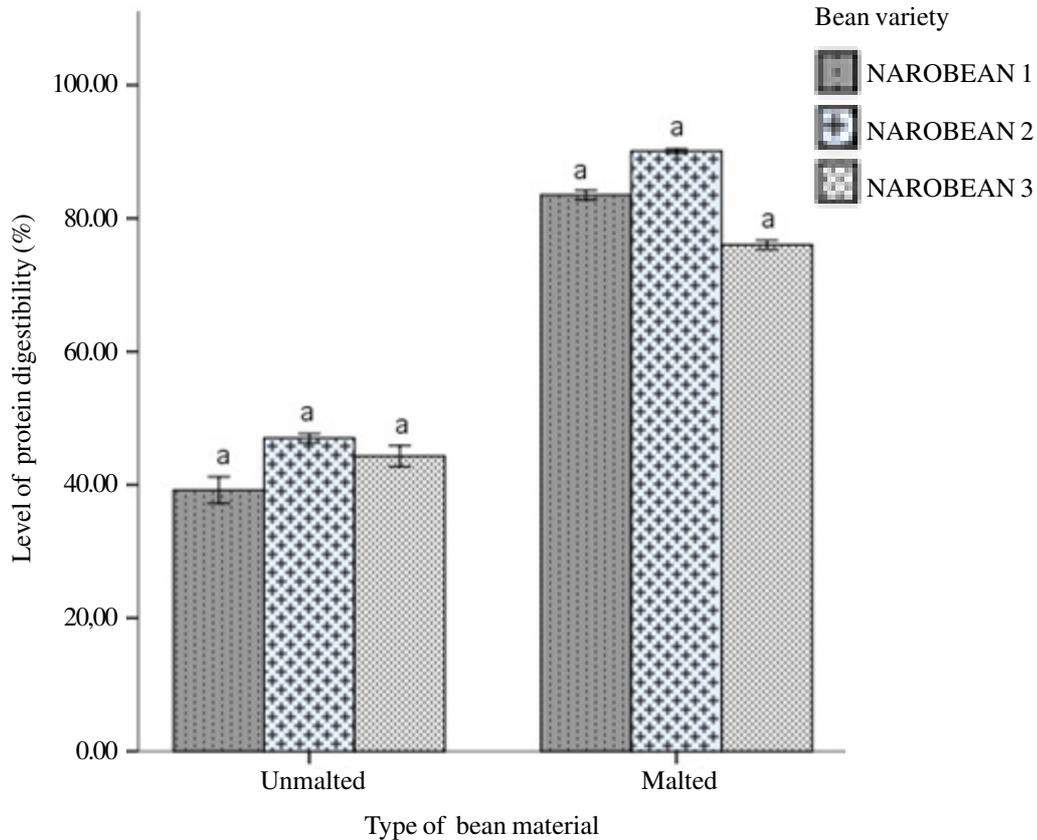


Figure 4. Protein digestibility among bean varieties before and after malting. Error bars show standard deviation ($n=6$). For each parameter, bars not sharing a common superscript letter are significantly different ($P<0.05$).

and magnesium in NAROBAN 3. For NAROBAN 1, the content of sodium was significantly higher in malted samples than in the control by about $2 \text{ mg } 100 \text{ g}^{-1}$.

For NAROBAN 3, the content of magnesium was significantly lower in malted than unmalted samples by about $7.5 \text{ mg } 100 \text{ g}^{-1}$. However, for NAROBAN 2, the effect of malting on phosphorus and iron was negative and positive, respectively. The contents of phosphorus and iron was lower and higher in malted and unmalted samples by 20 and $5.6 \text{ mg } 100 \text{ g}^{-1}$, respectively.

Figures 7-11 show the initial contents of micronutrients among varieties before and after application of malting treatment. Initially before malting, the levels of sodium, iron and zinc were identical in all the three varieties of

beans except for NAROBAN 2 which had significantly higher levels of sodium and lower levels of iron than the other two varieties ($P<0.05$) (Fig. 7). Furthermore, except for potassium which was present at identical levels ($P>0.05$), and phosphorus which was identically lower in NAROBAN 1 and 3 compared to NAROBAN 2, NAROBAN 1 had significantly higher levels of manganese, magnesium and calcium than NAROBAN 3 and 2 by 6, 5 and $0.01 \text{ mg } 100 \text{ g}^{-1}$, respectively ($P<0.05$). After malting (Fig. 8), the levels of zinc remained identical among the three varieties of beans ($P>0.05$). On the other hand, the level of sodium in NAROBAN 1 was significantly higher than detected in the other two varieties; while NAROBAN 2 had exceptionally higher levels of iron compared

TABLE 3. Proximate composition of malted and unmalted beans segregated by variety

Parameter	Treatment					
	NAROBAN 1		NAROBAN 2		NAROBAN 3	
	Malted	Control	Malted	Control	Malted	Control
Carbohydrate (%)	54.59±0.40 ^a	57.20±0.35 ^a	51.05±0.16 ^a	56.20±0.45 ^a	54.85±0.01 ^a	53.47±0.41 ^a
Moisture (%)	8.55 ± 0.21 ^a	9.35 ± 0.07 ^a	11.35 ± 0.07 ^a	9.25 ± 0.07 ^b	4.75 ± 0.07 ^a	10.75 ± 0.07 ^b
Crude fat (%)	1.27 ± 0.12 ^a	1.22 ± 0.04 ^a	1.20 ± 0.14 ^a	1.39 ± 0.16 ^a	1.025 ± 0.13 ^a	1.47 ± 0.26 ^a
Crude fibre (%)	4.40 ± 0.07 ^a	4.55 ± 0.17 ^a	4.52 ± 0.18 ^a	3.78 ± 0.13 ^b	6.19 ± 0.02 ^a	4.76 ± 0.16 ^b
Ash (%)	6.52 ± 0.11 ^a	5.78 ± 0.13 ^a	5.76 ± 0.15 ^a	5.19 ± 0.13 ^b	5.53 ± 0.08 ^a	5.68 ± 0.28 ^a
Crude protein (%)	24.73 ± 0.05 ^a	21.92 ± 0.08 ^b	26.13 ± 0.28 ^a	24.19 ± 0.09 ^b	27.64 ± 0.06 ^a	23.88 ± 0.47 ^b

Values show mean ± SD (n=6). For each variety and each parameter, means in the same row followed by different superscripts are significantly different (P<0.05)

TABLE 4. Micronutrient profile of malted and unmalted Iron rich beans segregated by variety

Parameter	Treatment					
	NAROBAN 1		NAROBAN 2		NAROBAN 3	
	Malted	Unmalted	Malted	Unmalted	Malted	Unmalted
P (mg 100 g ⁻¹)	201.48 ± 13.01 ^a	152.74 ± 3.50 ^a	161.68 ± 9.83 ^a	181.57 ± 8.82 ^b	155.09 ± 1.79 ^a	152.87 ± 12.28 ^a
Fe (mg 100 g ⁻¹)	7.91 ± 0.11 ^a	7.79 ± 1.45 ^a	11.37 ± 0.03 ^a	5.74 ± 0.49 ^b	8.26 ± 0.02 ^a	7.38 ± 0.73 ^a
Zn (mg 100 g ⁻¹)	2.78 ± 0.13 ^a	3.01 ± 0.05 ^a	3.38 ± 0.92 ^a	2.60 ± 0.59 ^a	3.03 ± 0.89 ^a	3.29 ± 0.12 ^a
Mg (mg 100 g ⁻¹)	109.46 ± 3.87 ^a	113.33 ± 0.69 ^a	98.97 ± 1.75 ^a	97.42 ± 8.33 ^a	95.33 ± 0.93 ^a	102.79 ± 2.46 ^b
Mn (mg 100 g ⁻¹)	0.14 ± 0.01 ^a	0.16 ± 0.01 ^a	0.14 ± 0.00 ^a	0.13 ± 0.01 ^a	0.15 ± 0.00 ^a	0.14 ± 0.00 ^a
Ca (mg 100 g ⁻¹)	71.80 ± 2.36 ^a	72.15 ± 2.05 ^a	60.40 ± 0.47 ^a	52.87 ± 6.18 ^a	58.25 ± 2.98 ^a	60.25 ± 6.40 ^a
K (mg 100 g ⁻¹)	7218.32 ± 62.57 ^a	6602.69 ± 38.48 ^a	6788.32 ± 228.39 ^a	6488.43 ± 356.43 ^a	5899.24 ± 239.05 ^a	6718.99 ± 118.10 ^a
Na (mg 100 g ⁻¹)	4.97 ± 0.18 ^a	2.98 ± 0.03 ^b	3.64 ± 0.25 ^a	3.51 ± 0.02 ^a	3.49 ± 0.11 ^a	2.73 ± 0.27 ^a

Values show mean ± SD (n =6). For each variety and each parameter, means in the same row followed by different superscripts are significantly different (P<0.05)

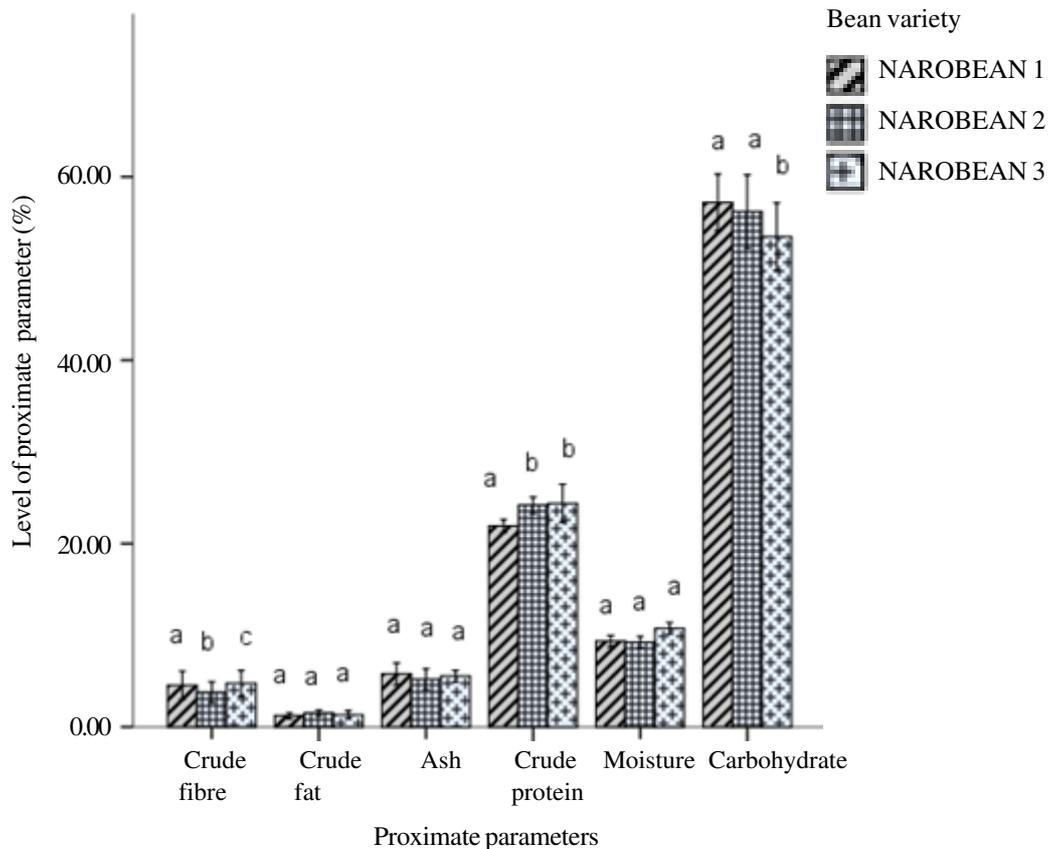


Figure 5. Initial contents of the proximate constituents among varieties before malting. Error bars show standard deviation (n=6). For each parameter, bars not sharing a common superscript letter are significantly different ($P < 0.05$).

to the other two varieties. The concentration of potassium was found to be identical in NAROBAN 2 and 3, but significantly higher in NAROBAN 1 by $440 \text{ mg } 100 \text{ g}^{-1}$; while no effect of variety was observed on the level of manganese ($P > 0.05$). However, the levels of calcium, phosphorus and magnesium had the same trend. The content of all the three mineral micronutrients was highest in NAROBAN 1 but identical and lower in NAROBAN 2 and 3. The magnitude of the difference was 11.4, 398 and $13.5 \text{ mg } 100 \text{ g}^{-1}$ for calcium, phosphorus and magnesium, respectively.

DISCUSSION

Anti-nutritional bean factors. The effect of the traditional malting technology on the anti-

nutritional factors differently is well illustrated by the significant reduction in the contents of oxalates and level of trypsin inhibitor activity, irrespective of the variety (Table 1); as well as lack of effect on phytic acid and tannic acid (Table 1). Many authors have reported decreases in anti-nutritional factors in legumes and cereals due to soaking, germination, fermentation, malting and boiling (Omer *et al.*, 2016; Kilonzi *et al.*, 2017; Mohammed *et al.*, 2017). The significant reduction of oxalates and total phenolics exhibited in the current study could be attributed to activation of endogenous oxidase enzymes (oxalate oxidase and polyphenol oxidase); which break down oxalic acid into carbon dioxide and hydrogen peroxide, and converts total phenolics to o-quinones that can easily be leached into the

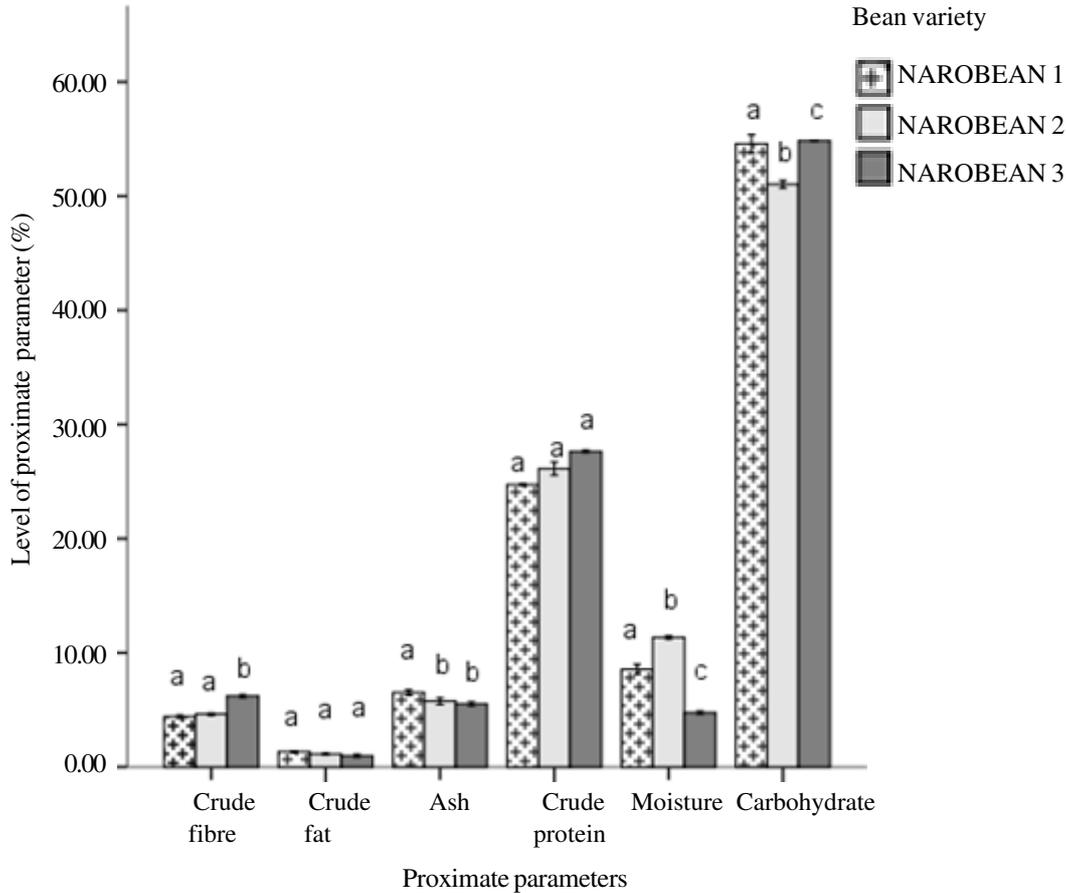


Figure 6. Proximate constituents among bean varieties after malting. Error bars show standard deviation (n=6). For each parameter, bars not sharing a common superscript letter are significantly different (P<0.05).

soak water, respectively (Baranwal, 2017; Sadawarte *et al.*, 2018).

The significant reduction in the contents of oxalates and trypsin inhibitor activity, following application of malting is consistent with the findings of other studies conducted under controlled conditions (Suma and Urooj, 2017; Abbas and Ahmad, 2018; Asouzu and Umerah, 2020). Reduction of trypsin inhibitors and oxalates is important for the final consumers because when present in the diet, they tend to reduce digestion and absorption of dietary proteins and mineral absorption thereby leading to reduced bioavailability, and hence compromised levels of nutrient uptake (Aviles-Gaxiola *et al.*, 2017; Ojha *et al.*, 2018).

Reduction in the levels of oxalates and trypsin inhibitors, following malting has been attributed to the fact that the compounds are predominantly found in the seed coat, are water soluble and consequently can easily leach into the liquid medium during steeping (Ojo *et al.*, 2017). Reduction in levels of trypsin inhibitor activity may also be due to breakdown of cellular proteins and enzymes by proteases during germination to provide free amino acids to support autotrophic growth of the seedling (Aviles-Gaxiola *et al.*, 2017). In the context of protein, iron and zinc nutrition, tannins when present in high amounts, inhibit the activities of trypsin, chymotrypsin and interfere with dietary iron

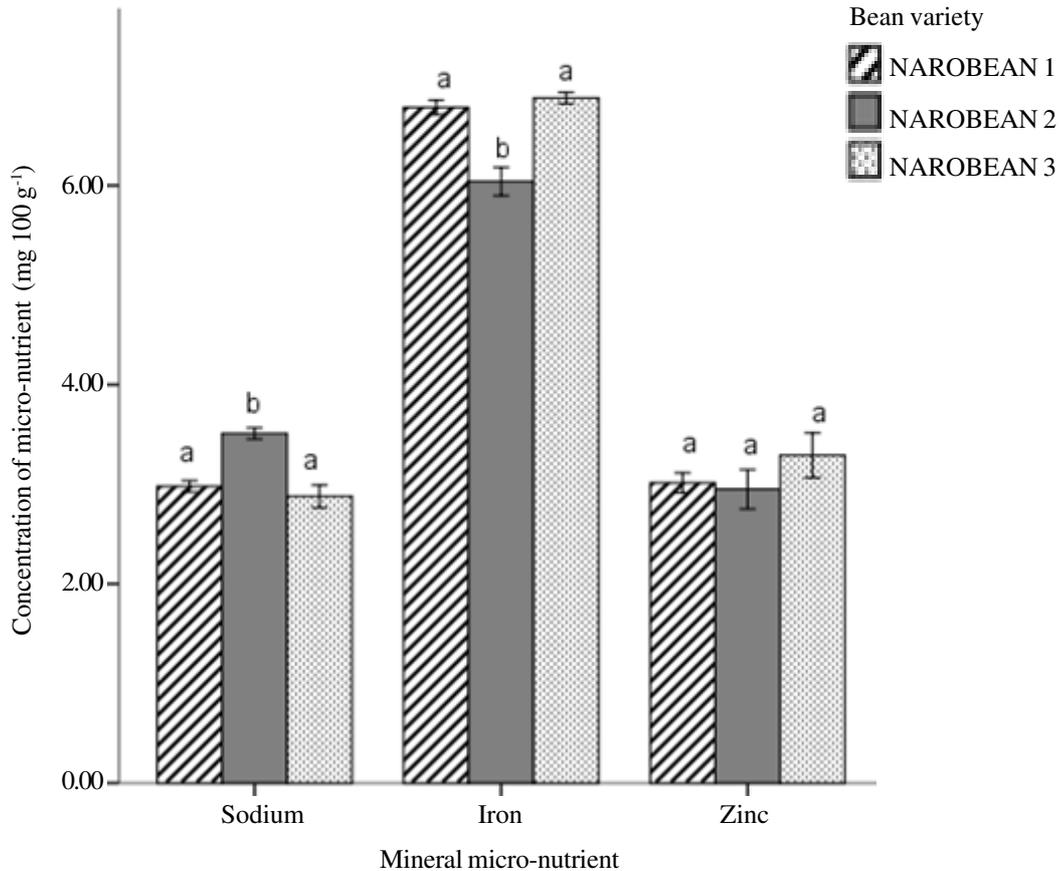


Figure 7. Contents of sodium, iron and zinc in unmalted bean varieties. Error bars show standard deviation (n=6). For each parameter, bars not sharing a common superscript letter are significantly different ($P < 0.05$).

and zinc absorption (Igwenyi *et al.*, 2013; Fekadu, 2014). Nonetheless, the lack of effect of traditional malting technology on tannin contents of the iron-rich bean varieties examined in the study may be inconsequential since the level of the anti-nutrient in unmalted samples were lower than 0.3%, the maximum for legumes specified by Codex (1990) and Asouzu and Umerah (2020).

Digestibility of proteins and bioavailability of iron and zinc. Malting increased the proportion of digestible protein across the iron-rich beans three varieties (Fig. 4), and the levels of improvement compare well with the range of values reported in literature for studies

conducted with other bean varieties under controlled experimental conditions (Nkundabombi *et al.*, 2015; Joshi and Varma, 2016; Oghbaei and Prakash, 2016a). However, improvements in protein digestibility observed in this study were lower than what Omer *et al.* (2016) reported (68.26%) for lupin seeds and Osman (2007) recorded (88.17%) for *Dolichos lablab* beans. The disparity between the results of this study and those reported by could be due to natural differences in the contents of anti-nutritional factors that affect protein digestibility. This is because the increase in protein digestibility in the previous study by Abbas and Ahmad (2018) was attributed to reduced trypsin and chymotrypsin

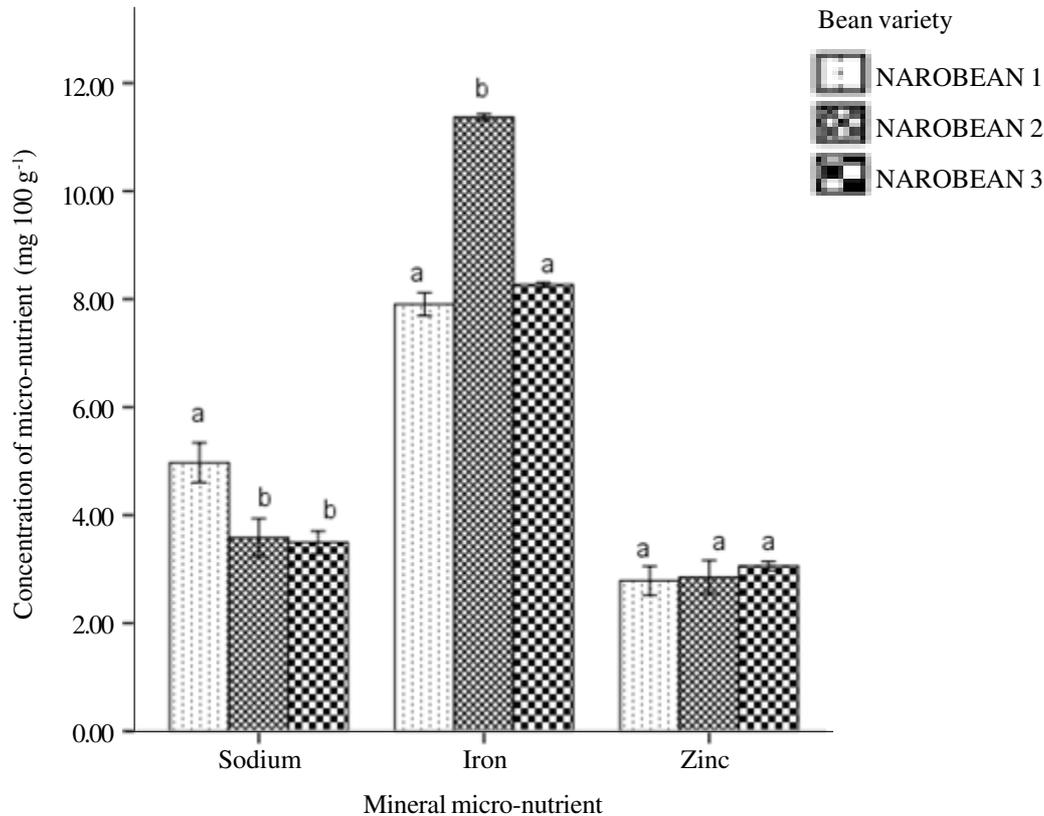


Figure 8. Contents of sodium, iron and zinc in malted bean varieties. Error bars show standard deviation (n=6). For each parameter, bars not sharing a common superscript letter are significantly different ($P < 0.05$).

inhibitory activity. On the other hand, as previously adduced by other reports (Baranwal, 2017; Alowo *et al.*, 2018; Nkhata *et al.*, 2018), the observed increase in protein digestibility may also be attributed to the degradation of long-chain polypeptides into simpler forms that can be easily digested. The lack of varietal effect among the three iron-rich bean varieties before and after malting, could be attributed to the identical state of trypsin inhibitor activity and the concentration of tannins in the raw and malted beans. The need to understand protein digestibility is important because its availability is not equivalent to its bioavailability (Ojo *et al.*, 2018). Besides, foods with high protein digestibility have better potential nutritionally compared to those of low digestibility due to

the ability of the former to provide more amino acids for absorption (Usman *et al.*, 2018).

Previous studies indicate that malting enhances iron and zinc bioavailability due to reduction of tannic or phytic acid content of plant foods (Hegazy *et al.*, 2017; Ghavidel and Prakash, 2007). Findings from our study indicate that malting had varying effect on bioavailability of iron and zinc across the three varieties of iron-rich beans studied (Fig. 4). Fundamentally, malting is believed to activate phytases, which in turn hydrolyze phytates; hence making iron and zinc more bioavailable (Joshi and Varma, 2016). Thus, variations in the extent of the effect of malting observed in the present study could be attributed to the varying effects malting had on anti-nutritional factor levels among the three iron-rich bean

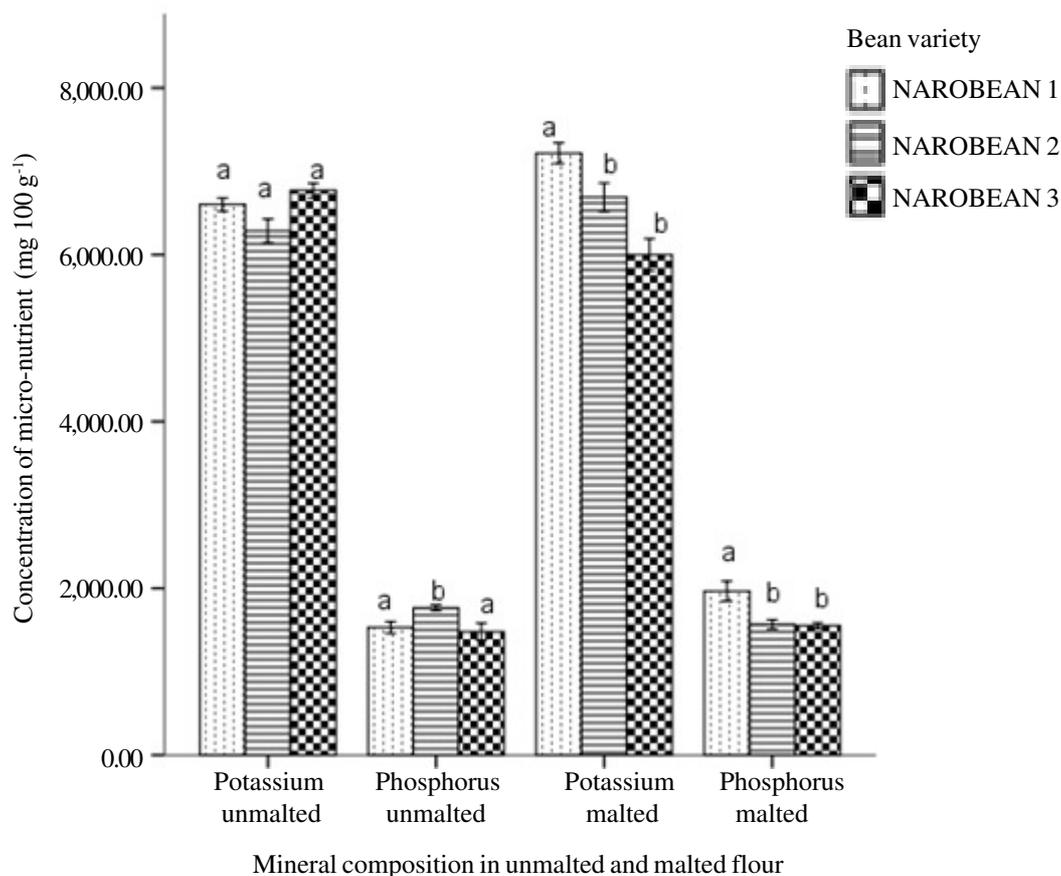


Figure 9. Content of potassium or phosphorus among varieties before and after malting. Error bars show standard deviation (n=6). For each parameter, bars not sharing a common superscript letter are significantly different ($P < 0.05$).

varieties. This is because, with the exception of zinc in NAROBAN 3, bioavailability levels of iron and zinc were identical among the unmalting samples of the three iron-rich bean varieties.

The levels of mineral bioavailability observed for iron-rich beans in this study somewhat mirror those reported by Luo *et al.* (2014) for faba beans and Hemalatha *et al.* (2007) for green gram; but were lower than the values reported by Salem *et al.* (2014) for lentil, chick pea, faba beans and white beans, and Nakitto *et al.* (2015) for K131 beans.

The significant malting effect on mineral bioavailability of the iron rich bean varieties (Fig. 3), suggests that malting is somewhat dependent of the initial bioavailability levels in

unprocessed beans with respect to iron and zinc, respectively. This is because, for unmalting materials, bioavailability of iron was highest in NAROBAN 1; followed the same trend after malting. However for zinc, bioavailability of the mineral was highest in NAROBAN 1 and NAROBAN 2 before and after malting, respectively. The increases in zinc bioavailability in NAROBAN 2 and iron bioavailability in NAROBAN 3 are in agreement with earlier studies (Afify *et al.*, 2011; Salem *et al.*, 2014; Desalegn, 2015). Contrary to our findings, Hemalatha *et al.* (2007) showed that malting green gram significantly decreased the zinc bioavailability. Luo *et al.* (2014) also found that malting significantly decreased the bioavailability of

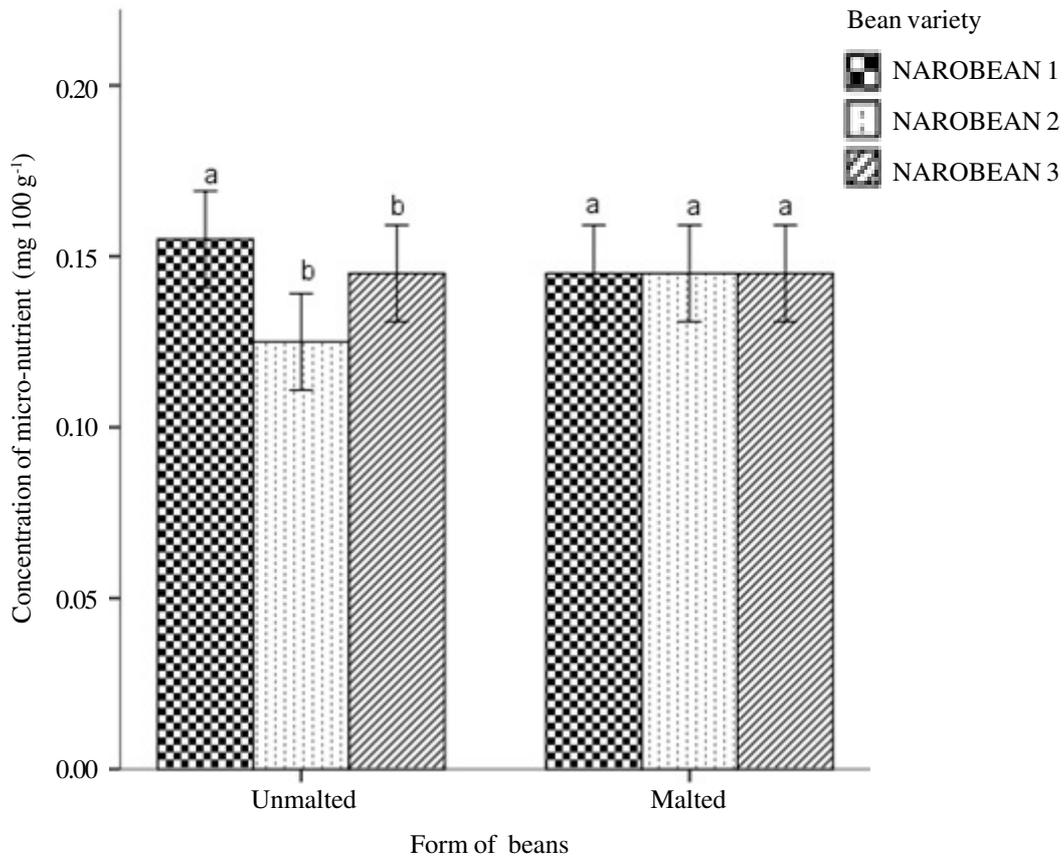


Figure 10. Content of manganese among varieties before and after malting. Error bars show standard deviation (n=6). For each parameter, bars not sharing a common superscript letter are significantly different ($P < 0.05$).

zinc from faba bean and soybean. Moreover, a review by Singh (2018) indicated that zinc bioavailability varied with bean variety (a fact which was confirmed in the current study) and the molar ratio of phytic acid to zinc (Rousseau *et al.*, 2019). Future studies should check whether variability due to molar ratio of phytic acid to zinc also applies to iron-rich beans.

Nutrient retention in iron-rich beans. The observed increase in protein content was expected based on existing literature (Nkhata *et al.*, 2018). This was illustrated in previous studies where protein content was reported to be higher in malted beans than in raw samples (Myrene, 2014; Nakitto *et al.*, 2015;

Nkundabombi *et al.*, 2015). The increase in protein content after malting can be attributed to synthesis of amino acids during germination and increase in the level of microbial proteins due to proliferation of microorganisms during the malting process (Okporo *et al.*, 2016; Yadav, 2016). Increase in protein after malting is positively correlated with improved protein digestibility since the free protein is not bound to the anti-nutritional factors.

Results for moisture indicate that the initial content of the proximate parameter was retained in NAROBAN 1, but increased and decreased significantly in NAROBAN 2 and NAROBAN 3, after malting. The findings of this study are in line with the results of Sadawarte *et al.* (2018), Myrene (2014) and

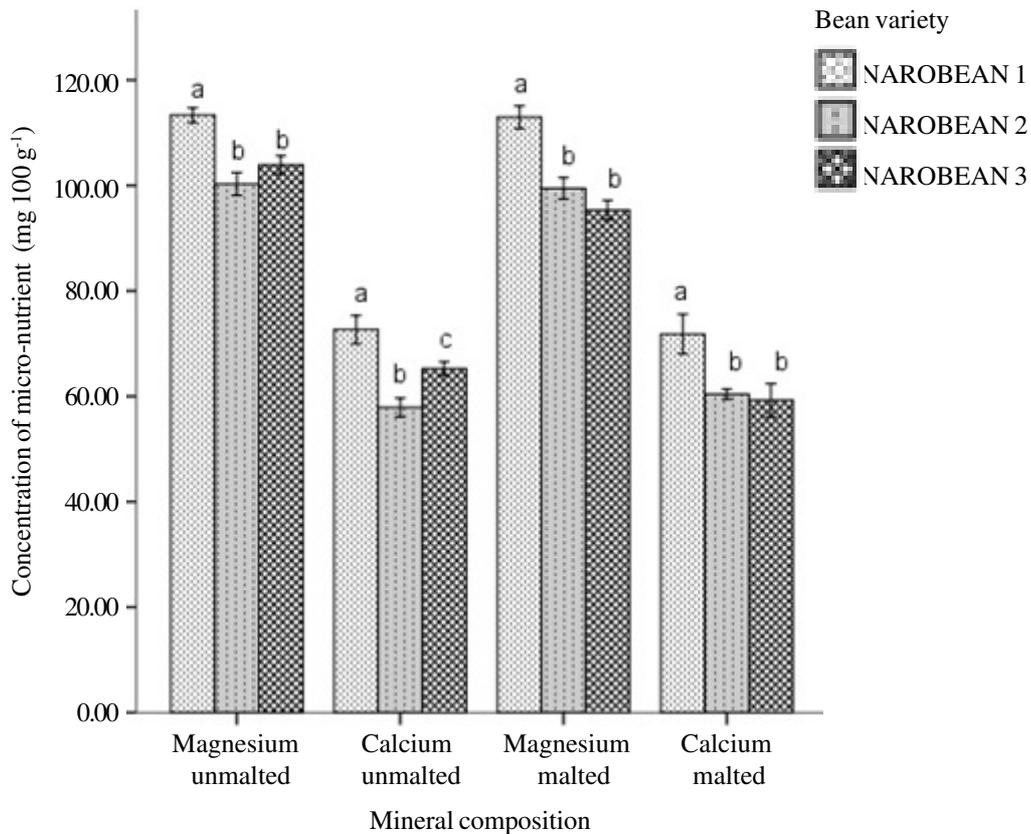


Figure 11. Content of magnesium and calcium before and after malting. Error bars show standard deviation (n=6). For each parameter, bars not sharing a common superscript letter are significantly different ($P < 0.05$).

Ghavidel and Prakash (2007) for other legume grains such as green gram, cowpea, lentil, chickpea and horse gram. Despite the mixed effect of malting, the moisture contents of flours from unmalting (9.25 - 10.75%) and malting beans (4.75 - 11.35%) were within the acceptable range for commercial flours (Buckman *et al.*, 2018). This implies that improvement in nutritional quality of iron-rich beans as a result of application of traditional malting technology may not lead to product quality deterioration during storage (Worku and Sahu, 2017; Baruah *et al.*, 2018; Obinna-chem *et al.*, 2019).

This study revealed that malting significantly led to increase in crude fiber content of flour made from NAROBAN 2 and 3; while no effect was observed for

NAROBAN 1 (Table 3). The higher level of crude fiber in flour from NAROBAN 2 and 3 following malting could be attributed to the thickness of seed coat, because it is present mainly in the outer seed testa and probably due to apparent increase in utilisation of other constituents such as starch during germination. (Bulbula *et al.*, 2018). Future studies should investigate the intrinsic factors present in NAROBAN 1 that limited the increase in crude fiber. The increase in crude fiber in NAROBAN 2 and 3 is comparable to the findings of Ghavidel and Prakash (2007) for green gram, cowpea, lentil and chickpea, and that of Saxena and Vyas (2016) and Megat *et al.* (2016) for soybean. Contrary to the results of the present study, decreased crude fiber content have been reported by Joshi and

Rahal (2018) for black soybean following malting. Moreover, the level of crude fiber with or without malting are in agreement with the range recommended for infant weaning food as reported by Fikiru *et al.* (2017). According to WHO guidelines on complementary feeding of children, dietary fiber and other non-absorbable carbohydrate that are partially fermented by the intestinal flora should not exceed 5 g 100 g⁻¹ on dry weight basis (WHO, 2001); and hence the levels of the fiber in the flours are in compliance with this guidelines. Fiber plays a role in the increased utilisation of nitrogen and absorption of some other micronutrients such as iron, calcium, selenium, copper, zinc, magnesium, manganese, phosphorus and chromium (Adams *et al.*, 2018; Laleg *et al.*, 2018; Obinna-echem *et al.*, 2019).

From Table 3 also showed that the chemical composition of bean malt increased in ash content of only NAROBAN 2 while the levels of ash were identical in NAROBAN 1 and 3 before and after malting. The differences observed in the ash content of the different bean varieties could be due to differences in the seed composition of the bean varieties (Onwurafor *et al.*, 2020).

The losses incurred in the content of magnesium and phosphorus after malting (Table 4) could have resulted from loss through utilisation of other nutrients by the growing embryo (Udeh *et al.*, 2018). It is important to note here that iron-rich beans were developed to enhance intake of iron to alleviate challenges of iron deficiency. Therefore, the lack of effect on the content of iron and the positive effect on its bioavailability (except for NAROBAN 2) indicates that the traditional malting technology can enhance intake of iron from iron-rich beans.

CONCLUSION

This study has demonstrated that the traditional malting technology practised in

Acholi sub-region of Uganda: (i) effectively reduces trypsin inhibitors and oxalates, but is largely ineffective on total phenolics, phytic and tannic acids; (ii) largely improves protein digestibility and iron bioavailability, but has limited effect on bioavailability of zinc; and (iii) has limited negative effects on proximate composition and micronutrient contents of the three iron-rich bean varieties studied. Therefore, future studies should improve the efficacy of the traditional malting technology to reduce anti-nutritional factors and improve further the nutrition profile of biofortified iron-rich beans.

Furthermore, the fundamentals underpinning the differential effect of the traditional malting technology on anti-nutritional factors and bioavailability of nutrients from iron-rich beans as a function of variety should be investigated.

Lastly, future studies should investigate whether the increase in protein digestibility following malting is due to the reduction in anti-nutritional factors or confounded by accumulation of microbial protein.

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