academicJournals

Vol. 12(34), pp. 5285-5293, 21 August, 2013
DOI: 10.5897/AJB12.2920
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Influence of biofortification with provitamin A on protein, selected micronutrient composition and grain quality of maize

Kirthee Pillay[1]*, Muthulisi Siwela[1], John Derera[2] and Frederick J. Veldman[1]

[1]Dietetics and Human Nutrition, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville, 3209, Pietermaritzburg, South Africa.
[2]African Centre for Crop Improvement, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, South Africa.

Accepted 8 August, 2013

Provitamin A-biofortified maize is currently being evaluated for use in the alleviation of vitamin A deficiency. Apart from the differences in provitamin A content, the nutritional composition of provitamin A-biofortified maize compared to white maize is hardly known. This study aimed to evaluate the protein and selected micronutrient composition of biofortified maize varieties and the quality of their grains. A total of 32 provitamin A-biofortified maize varieties was analysed for their starch, fat, protein and mineral content. The milling and storage quality of the biofortified maize grains were also assessed. When compared with the white maize variety, the biofortified varieties were higher in starch, fat and protein, but were lower in iron. The biofortified maize varieties were better sources of most of the essential amino acids relative to the white variety, but, similar to the white maize, they were deficient in histidine and lysine. Overall, the quality of the grains of the biofortified maize varieties was superior to that of the white maize grain, although, the biofortified grains were more susceptible to fungal invasion. This study indicates that, in terms of the nutrients assessed, provitamin A-biofortified maize is generally superior to white maize, except for minerals.

Key words: Biofortification, provitamin A, protein, micronutrient composition, maize.

INTRODUCTION

Vitamin A Deficiency (VAD) is a major health problem in the developing regions, particularly in sub-Saharan Africa (SSA) (WHO, 2009). Apart from VAD, the sub-Saharan Africa region is also plagued by protein energy malnutrition (PEM) and deficiencies in other micronutrients, especially iron and iodine (Bouis, 1996; WHO, 2002b; De Onis and Blössner, 2003). On the other hand, the sub-Saharan region of Africa is a leader in the consumption of white maize (IITA, 2010), which makes maize a strategic vehicle for delivering nutrients to poor communities (Ortiz-Monasterio et al., 2007). Unfortunately, white maize is devoid of vitamin A, contains poor quality protein and its mineral composition is not nutritionally adequate (FAO, 1992; Johnson, 2000). Like in most other cereals, the essential amino acids lysine and tryptophan are limiting in maize (Johnson, 2000). In addition to lacking vitamin A, maize, wheat and rice, the most widely consumed staple crops, are poor sources of iron and iodine (Bouis, 1996). Consequently, maize is one of the six staple crops that have been targeted for biofortification with provitamin A carotenoids by conventional breeding as part of an international effort to combat VAD

*Corresponding author. E-mail: Pillayk@ukzn.ac.za. Tel: + 27-033-2605674. Fax:+ 27-033-2606270.

(Tanumihardjo, 2008; HarvestPlus Brief, 2006).

While the nutritional composition of white maize and the factors affecting nutrient content are well documented (FAO, 1992; Johnson, 2000), published data on the overall nutritional composition of provitamin A-biofortified maize are scarce. It may be assumed that the nutritional composition of provitamin A-biofortified maize is similar to that of white maize, apart from the expected differences in provitamin A carotenoid content. However, hybridization of maize genotypes to produce provitamin A-biofortified maize, and the subsequent selection during breeding may result in a significant change in the nutritional composition of the maize, including nutrients other than provitamin A, similar to the changes in protein content reported during the breeding of white maize genotypes to produce low phytic acid maize (Raboy et al., 1989). Already, provitamin A biofortification of maize by conventional breeding has been found to cause a variation in the provitamin A composition of the maize varieties; orange varieties were found higher in provitamin A than the yellow varieties (Menkir et al., 2008; Li et al., 2007; Ortiz-Monasterio et al., 2007). The changes in the composition of nutrients other than provitamin A that might occur during biofortification may have a positive or negative impact on the nutritional value of the provitamin A-biofortified maize.

If provitamin A-biofortified maize were to be used as a food source, the quality of the biofortified grain should be of acceptable standard. However, the quality of the biofortified maize grain may be affected by changes in grain composition that could occur as a result of genetic manipulation or by conventional breeding as postulated earlier. Because the processing of maize grain into food products usually involves milling, it is important to assess the milling quality of provitamin A-biofortified maize grain. It is also important to assess the resistance of the provitamin A-biofortified maize grain to fungal invasion during storage as this may impact on grain quality and safety. The objectives of this study were to evaluate the influence of biofortification with provitamin A on protein and selected micronutrient composition of provitamin A-biofortified maize grain, and to assess the quality of the biofortified maize grains.

MATERIALS AND METHODS

Maize breeding

A total of 32 provitamin A-biofortified maize varieties was produced by conventional breeding. The experimental F_1 maize hybrids (varieties) were derived from recombinant inbred lines (hybrid parents) with deep orange grain colour. The F_1 hybrids with sufficient seed were then planted at Makhathini Research Station in KwaZulu-Natal, South Africa, to produce F_2 grain. Bulk grain of each of a reference white variety (CC-37) and a reference yellow/orange maize variety (commercial provitamin A variety) (10 MAK 7-10) obtained from Seed Co Ltd. (Zimbabwe), were also produced under the same conditions as the experimental varieties.

The maize was harvested manually and left to dry under ambient conditions (±25°C) for 21 days to simulate the farmers' production conditions. The maize was then threshed mechanically and the grain stored in a cold room (±4°C) before analysis.

Nutritional analysis

Although, a total of 32 provitamin A-biofortified orange maize varieties were produced for nutritional composition analysis; due to financial limitations, only seven of the 32 biofortified varieties were analysed for amino acid composition. However, all the 32 biofortified varieties were analysed for the levels of all the other nutrients included in the study (Table 1). Approximately, 10 kg grain of each maize variety was obtained after threshing. Each grain sample was mixed thoroughly and then a sub sample of approximately 2 kg grain (approximately 20% of the original grain sample) was taken and milled into whole grain flour. The flours of each maize variety were mixed thoroughly before the required amounts of flour samples were taken for the respective analysis. The moisture content of the samples was measured according to the Association of Official Analytical Chemists International (AOAC) Official Method 934.01 (AOAC, 2002). Starch content was determined according to the AOAC Method 979.10 (Glucoamylase Method) (AOAC, 2002). This method entails the gelatinization of starch in the sample by autoclaving followed by the enzymatic hydrolysis of the starch to glucose and then the determination of glucose content by the 'glucose oxidase' method. Sugars present in the samples were removed by refluxing the samples in 80% ethanol before determining the starch. The fat content of the samples was determined according to the Soxhlet procedure, using a Büchi 810 Soxhlet Fat extractor (Büchi, Flawil, Switzerland) according to the AOAC Official Method 920.39 (AOAC, 2002).

The crude protein content of the samples was measured with a LECO Truspec Nitrogen Analyser (LECO Corporation, St Joseph, Michigan, USA) using the Dumas Combustion method (AOAC Official Method 968.06) (AOAC, 2002). The amino acid profile of the samples was analysed by the Pico-Tag method using a waters breeze high-performance liquid chromatography (HPLC) with empower software (Waters, Millipore Corp., Milford, USA). Samples (400 mg) were hydrolysed with 6 N HCl for 24 h and then derivatized with phenylisothiocyanate (PITC) to produce phenyltiocarbamyl (PTC) amino acids, which were analysed by reverse phase HPLC (Bidlingmeyer et al., 1984). The iron and zinc contents of the samples were determined by atomic absorption (AA) spectroscopy (Giron, 1973). The Varian Spectr AA model of the atomic absorption spectrophotometer (Varian Australia Pty Ltd, Mulgrave, Victoria) was used to analyse for iron and the GBC 905AA spectrophotometer (GBC Scientific Equipment Pty Ltd, Dandenong, Victoria, Australia) was used to determine zinc. Phosphorus was determined according to the AOAC Official Method 968.08 (AOAC, 2002). Absorbance was measured with the Analytik Jena Spekol 1300 spectrophotometer (Analytik Jena AG, Achtung, Germany).

Grain quality analysis

Grain of seven of the experimental maize varieties as well as the white variety (CC-37) (reference) and the reference yellow/orange variety (10 MAK 7-10) was analysed for selected grain quality attributes, namely: hectolitre mass, stress cracks, milling index and resistance to fungal infection. Hectolitre mass is the mass of a hectolitre (100 L) of grain and is a measure of grain density. Grain density is routinely used to indicate grain milling quality, where a higher grain density indicates a better milling quality (Taylor and Duodu, 2009). The hectolitre mass of grain was measured using an apparatus that consisted of a hopper and a 0.5 L receiver (cup) following the American Association of Cereal Chemists (AACC) Method 55-10 Test Weight per Bushel (AACC, 2000). The hopper was filled with grain which was then emptied into the receiver until it

Table 1. Nutritional composition of provitamin A-biofortified maize grain varieties (dry weight).

Number	Variety	Moisture (%)	Starch (g/100 g)	Fat (g/100 g)	Protein (g/100 g)	Iron (mg/100 g)	Zinc (mg/100 g)	Phosphorus (mg/100 g)
1	CC-37[Ψ]	17.1[a](0.1)[b]	59.4 (0.3)	3.0 (0.0)	10.7 (0.1)	5.90 (0.00)	2.12 (0.08)	393.97 (17.17)
2	10 MAK 7-1	12.1(0.1)	**56.2** (0.2)	**3.9** (0.1)	**13.6** (0.5)	**4.63** (0.04)	2.24 (0.08)	378.18 (12.87)
3	10 MAK 7-2	11.3 (0.4)	**54.3** (0.1)	**4.7** (0.1)	**13.4** (0.1)	**4.91** (0.05)	2.27 (0.16)	380.22 (1.29)
4	10 MAK 7-3	11.9 (0.1)	**55.6** (0.9)	**5.2** (0.1)	**11.9** (0.1)	**4.67** (0.03)	1.83 (0.00)	355.25 (1.34)
5	10 MAK 7-5	10.5 (0.1)	**54.3** (0.0)	**4.3** (0.1)	**12.4** (0.1)	**5.03** (0.15)	1.97 (0.08)	360.68 (0.26)
6	10 MAK 7-7	9.4 (0.3)	**55.0** (0.3)	**4.3** (0.0)	**13.5** (0.1)	**4.88** (0.15)	2.23 (0.08)	396.66 (2.05)
7	10 MAK 7-8	11.3 (0.1)	**55.4** (0.7)	**4.2** (0.0)	**13.2** (0.1)	**4.96** (0.07)	**2.90** (0.08)	409.57 (11.08)
8	10 MAK 7-9	14.5 (0.4)	57.0 (0.0)	**4.0** (0.1)	**11.2** (0.0)	4.96 (0.07)	**2.71** (0.00)	396.52 (13.24)
9	10 MAK 7-10[*]	14.1 (0.1)	57.2 (0.3)	**3.9** (0.0)	**12.1** (0.1)	**4.52** (0.15)	2.25 (0.00)	**329.42** (7.01)
10	KPPVAH-1	13.4(0.0)	**70.3** (1.7)	**4.7** (0.1)	**14.2** (0.0)	**2.63** (0.08)	2.27 (0.00)	357.12 (9.63)
11	KPPVAH-2	12.8 (0.0)	**70.7** (0.3)	**4.5** (0.1)	**15.3** (0.0)	**2.11** (0.01)	2.21 (0.08)	389.31 (2.42)
12	KPPVAH-3	12.9 (0.1)	**72.0** (0.3)	**5.0** (0.1)	**13.5** (0.2)	**2.98** (0.11)	2.21 (0.08)	**338.08** (3.17)
13	KPPVAH-4	12.6 (0.0)	**70.8** (1.3)	**5.0** (0.0)	**12.9** (0.6)	**2.63** (0.11)	2.43 (0.08)	365.60 (1.41)
14	KPPVAH-5	13.1 (0.0)	**69.1** (0.3)	**4.4** (0.0)	**13.5** (0.2)	**2.63** (0.18)	2.21 (0.08)	366.17 (3.01)
15	KPPVAH-6	13.3 (0.0)	**71.9** (1.0)	**4.2** (0.2)	**12.5** (0.0)	**2.72** (0.07)	2.16 (0.16)	363.98 (1.58)
16	KPPVAH-7	13.1 (0.0)	**73.9** (1.3)	**4.9** (0.1)	10.3 (0.1)	**3.48** (0.00)	2.04 (0.00)	**339.84** (47.06)
17	KPPVAH-8	13.0 (0.1)	**72.3** (0.7)	**4.9** (0.1)	**12.3** (0.1)	**2.61** (0.00)	2.03 (0.33)	359.96 (11.98)
18	KPPVAH-9	13.5 (0.0)	**71.5** (1.3)	**4.5** (0.1)	**14.0** (0.1)	5.77 (0.04)	2.28 (0.17)	390.55 (4.08)
19	KPPVAH-10	12.7 (0.0)	**70.6** (0.8)	**5.0** (0.0)	**12.9** (0.1)	**2.78** (0.12)	2.09 (0.08)	366.11 (33.93)
20	KPPVAH-11	12.9 (0.0)	**70.2** (0.7)	**4.1** (0.0)	**13.5** (0.1)	**3.51** (0.13)	2.03 (0.00)	389.15 (8.80)
21	KPPVAH-12	13.2 (0.0)	**68.7** (1.1)	**4.5** (0.1)	**14.3** (0.1)	**3.34** (0.15)	2.33 (0.08)	**336.55** (7.68)
22	KPPVAH-13	12.6 (0.0)	**66.9** (0.9)	**5.1** (0.2)	**14.2** (0.1)	**3.28** (0.07)	2.20 (0.08)	**343.95** (6.58)
23	KPPVAH-14	12.9 (0.0)	**67.6** (0.0)	**5.7** (0.0)	10.3 (0.1)	**2.33** (0.01)	**1.75** (0.08)	**308.51** (12.03)
24	KPPVAH-15	12.7 (0.0)	**68.0** (0.9)	**5.4** (0.0)	**12.5** (0.0)	**2.64** (0.06)	2.09 (0.08)	367.66 (6.07)
25	KPPVAH-16	12.9 (0.0)	**65.7** (2.7)	**5.0** (0.1)	**11.4** (0.0)	**1.98** (0.11)	**1.75** (0.08)	**346.21** (4.98)
26	KPPVAH-17	12.5 (0.0)	**70.0** (1.1)	**5.3** (0.0)	**11.5** (0.0)	**2.11** (0.05)	1.97 (0.08)	368.01 (5.20)
27	KPPVAH-18	12.9 (0.0)	**70.0** (0.9)	**5.6** (0.0)	**12.0** (0.0)	**2.14** (0.01)	1.86 (0.08)	**350.06** (12.92)
28	KPPVAH-19	12.7 (0.1)	**68.1** (0.1)	**4.8** (0.0)	**13.1** (0.1)	**2.58** (0.13)	**2.55** (0.08)	393.54 (1.20)
29	KPPVAH-20	12.7 (0.1)	**68.4** (0.4)	**5.3** (0.2)	**12.9** (0.0)	**2.60** (0.14)	**2.55** (0.08)	382.06 (6.67)
30	KPPVAH-21	12.8 (0.0)	**67.5** (0.1)	**4.8** (0.0)	**13.0** (0.0)	**2.69** (0.13)	2.32 (0.08)	377.89 (0.16)
31	KPPVAH-22	12.8 (0.0)	**69.8** (1.0)	**4.9** (0.1)	**12.5** (0.0)	**1.90** (0.04)	2.26 (0.00)	356.96 (5.72)
32	KPPVAH-23	13.1 (0.1)	**71.1** (0.8)	**4.3** (0.0)	**12.0** (0.2)	**2.28** (0.09)	1.81 (0.00)	**296.44** (12.67)
33	KPPVAH-25	12.7 (0.1)	**70.9** (0.6)	**4.0** (0.0)	**13.6** (0.2)	**2.16** (0.11)	2.15 (0.16)	357.82 (15.03)
34	KPPVAH-26	13.3 (0.0)	**70.0** (0.4)	**4.7** (0.0)	**11.5** (0.0)	**2.07** (0.11)	2.27 (0.00)	**336.50** (6.78)
Grand mean		12.6[c] (0.9)[d]	**66.7** (6.3)	**4.7** (0.5)	**12.8** (1.2)	**3.21** (1.17)	2.18 (0.27)	363.28 (26.56)

[a] Mean; [b] Standard deviation; [c] Mean of provitamin A-biofortified varieties; [d] Standard error; Values in bold are significantly different from CC-37 (white maize) for that nutrient (Dunnett Test, p significant at < 0.05); [Ψ] White variety (reference); [*] Reference yellow/orange variety.

overflowed. The grain in the receiver was levelled off and weighed, and the net mass of the grain used to calculate hectolitre mass. Maize kernels can crack during artificial drying from stress caused by the uneven contraction of different parts of the endosperm (Taylor and Duodu, 2009). Hard grain is more susceptible to cracking than soft grain. Grain cracking can have negative effects on the grain such as grain losses due to breakage of cracked kernels during grain handling and processing and a reduction in milling quality (Taylor and Duodu, 2009). The stress cracks of the maize grain samples were analysed according to the Southern African Grain Laboratory (SAGL) Industry Accepted Method for Stress Crack Analysis of Maize Kernels (SAGL, 2001). A total of 100 sound kernels from each maize sample were selected and placed on a light board. The kernels were then visually inspected for cracks. The cracks in the kernel were seen as dark lines when light was transmitted through the grains. A kernel was reported as positive for stress cracking if one or more cracks were seen on it.

Milling index is an indicator of grain hardness and hence, milling quality (Taylor and Duodu, 2009). In this study, the milling index of the maize grain was determined according to the SAGL Industry Accepted Method for estimating milling index using near-infrared transmittance (NIT) (SAGL, 2007). The basis of the method is that the transmittance of light in the near-infrared wavelength through the grain is directly related to grain hardness and hence, milling quality (Taylor and Duodu, 2009). The method is calibrated against data obtained from pilot-scale roller milling trials, with milling index representing extraction rate (% meal obtained from milling the grain) (Taylor and Duodu, 2009). The milling indices of the maize

grain samples of this study were measured with the INFRATEC 1241 Grain Analyzer (Foss Tecator AB, Höganäs, Sweden) NIT machine, which had been calibrated as described earlier. Approximately, 500 g of a maize grain sample was loaded into the machine.

Fungi infecting the maize grains were enumerated, isolated and identified by the direct plating method (Rabie and Lübben, 1984; Rabie et al., 1997). According to this method, sample grains are evenly spread over three different growth media to ensure mycelia growth of all possible species present. The fungi, which use the growth media as nutrients, grow out of the test sample onto the medium. Potato dextrose agar (PDA) was used as a non-selective medium; malt salt agar (MSA) for the selective growth of *Aspergillus*, *Eurotium* and *Penicillum* spp.; and pentachlorobenzene agar (PCNB) for the selective growth of *Fusarium* spp. (Rabie and Lübben, 1984).

Kernels of each grain type were surface-disinfected by shaking them in a flask of 76% (v/v) ethanol and then rinsing them three times with sterile distilled water. Five kernels were placed on plates (10 each) of PDA, MSA and PCNB and incubated at 25°C for 2 to 14 days. Kernels with mycelia growth of any fungal type were counted and expressed as a percentage of the total kernels plated. The fungal colonies were isolated and purified on fresh PDA plates and then identified based on morphological features of their fruiting bodies (Dugan, 2006).

Statistical analyses

Predictive analytics software (PASW) statistics version 18.0 (IBM Corporation, New York) was used to analyse the data. The Dunnett test was used to compare the nutrient content of the maize variety CC-37 [white maize (reference)] with the nutrient content of the experimental provitamin A-biofortified maize varieties. Univariate analysis of variance (UNIANOVA) was used to analyse for differentces in the nutrient content of the maize varieties. Statistical significance was measured at the 0.05 level.

RESULTS AND DISCUSSION

The starch, fat and protein content of the biofortified maize varied across the varieties (Table 1). The provitamin A-biofortified maize grain varieties had significantly higher levels of starch, fat and protein relative to the white maize variety (CC-37) ($p<0.05$) (Table 1). The reference yellow variety (10 MAK 7-10) had higher levels of fat and protein relative to the white maize variety ($p<0.05$), but was lower in iron and phosphorus ($p<0.05$). The mean starch (66.7/100 g) and fat (4.7/100 g) values of the provitamin A-biofortified maize varieties of this study were similar to those of white maize found in the literature, 71.3/100 and 4.1/100 g, respectively (Johnson, 2000).

On the other hand, the mean protein content (12.8/100 g) of the biofortified maize varieties of this study was much higher than the documented values for white maize (Machida et al., 2010; Johnson, 2000). The finding that the provitamin A- biofortified maize varieties had a higher protein and fat content relative to the white maize is encouraging. This indicates that in addition to provitamin A, the biofortified maize varieties could also be used to improve protein, fat and overall energy intake and thereby serve as a valuable tool to fight PEM in SSA. The higher starch, protein, fat and energy content of the provitamin A-biofortified maize varieties may also help to improve the overall quality of food intake in SSA, where low protein and energy plant foods (including cereal grains) are leading staples. The biofortified maize varieties had a higher concentration of most of the essential amino acids relative to the white maize (CC-37) (Table 2a). However, the levels of histidine and lysine were generally lower in the biofortified maize varieties compared to the white maize (CC-37).

As for the non-essential amino acids, the levels of aspartic acid, glutamic acid, serine and alanine were generally higher in the biofortified maize varieties, whilst the levels of glycine and arginine were higher in the white maize (CC-37). Although, not corrected for protein digestibility, the concentrations of all the essential amino acids, except lysine, in all the biofortified maize varieties and the white variety were generally higher than the pattern of amino acid requirements for all age groups (Table 2b).

These results indicate that, as is the case with white maize (FAO, 1992), provitamin A-biofortified maize is deficient in some of the essential amino acids. In order to overcome the lysine deficiency in provitamin A-biofortified maize, biofortified maize food products should be consumed with food sources that are rich in lysine, using the concept of complementary proteins. With complementary proteins, the amino acid profiles complement each other in such a way that the essential amino acids missing from one food source are supplied by other food sources (Whitney and Rolfes, 2011). Alternatively, the protein quality of provitamin A-biofortified maize varieties could be improved by a marker-assisted backcross breeding programme or by genetic engineering to incorporate the limiting essential amino acids.

The iron content varied widely across the biofortified maize varieties, 1.90 to 5.77 mg/100 g (mean = 3.21 mg/100 g), whilst zinc content varied within a narrower range; 1.75 to 2.90 mg/100 g (mean = 2.18 mg/100 g) (Table 1). Provitamin A-biofortified maize samples from the International Maize and Wheat Improvement Center (CIMMYT) were found having iron and zinc contents ranging from 1.1 to 3.9 mg/100 g (mean = 2.0 mg/100 g) and 1.5 to 4.7 mg/100 g (mean = 2.5 mg/100 g), respectively (Johnson, 2000).

The phosphorus levels in the biofortified maize studied varied widely across varieties (296.44 to 409.57 mg/100 g; mean = 363.28 mg/100 g). The mean iron values of the provitamin A-biofortified maize varieties of this study were significantly lower than that of the white maize variety (CC-37) ($p<0.05$). In contrast, the zinc values of the biofortified maize varieties compared well with that of the reference white variety. The phosphorus values (296.44 to 409.57 mg/100 g) of the biofortified maize varieties were comparable with that of the white maize variety (393.97 mg/100 g). Although, lower when compared to white maize, the mean iron values reported in this study were higher than the values reported in other studies

Table 2a. Amino acid composition of provitamin A-biofortified maize varieties (dry weight).

Variety	Protein (g/100 g)	Essential amino acid							Non-essential amino acid							
		His	Thr	Val	Isoleu	Leu	Phe	Lys	Asp	Glu	Ser	Gly	Arg	Ala	Pro	Tyr
CC -37$^{\Psi}$	10.7[a] (0.1)[b]	0.44 (0.02)	0.44 (0.01)	0.56 (0.02)	0.40 (0.00)	1.41 (1.11)	0.60 (0.00)	0.39 (0.00)	0.67 (0.06)	2.18 (0.16)	0.65 (0.04)	0.49 (0.05)	0.61 (0.00)	0.87 (0.01)	1.30 (0.19)	0.40 (0.01)
10 MAK 7-1	13.6 (0.5)	0.43 (0.01)	**0.52** (0.01)	0.66 (0.02)	**0.49** (0.00)	**1.79** (1.14)	**0.73** (0.02)	**0.29** (0.03)	0.77 (0.06)	**2.68** (0.18)	**0.75** (0.02)	0.47 (0.04)	0.54 (0.01)	**1.15** (0.10)	1.40 (0.03)	**0.48** (0.04)
10 MAK 7-2	13.4 (0.1)	0.42 (0.04)	**0.54** (0.00)	**0.68** (0.00)	**0.48** (0.01)	**1.80** (0.04)	**0.70** (0.02)	**0.28** (0.00)	0.77 (0.04)	**2.69** (0.08)	**0.74** (0.04)	0.51 (0.06)	0.59 (0.01)	**1.16** (0.11)	1.38 (0.12)	0.45 (0.02)
10 MAK 7-3	11.9 (0.1)	0.38 (0.00)	0.45 (0.00)	0.62 (0.07)	**0.45** (0.02)	1.61 (0.10)	**0.66** (0.04)	**0.30** (0.01)	0.75 (0.06)	2.45 (0.22)	0.67 (0.00)	0.43 (0.02)	0.55 (0.01)	**1.04** (0.01)	1.22 (0.03)	0.36 (0.00)
10 MAK 7-5	12.4 (0.1)	0.39 (0.00)	**0.50** (0.00)	0.64 (0.05)	**0.47** (0.01)	**1.67** (0.06)	**0.70** (0.02)	**0.30** (0.02)	0.78 (0.02)	2.52 (0.18)	0.70 (0.01)	0.44 (0.02)	0.57 (0.01)	**1.04** (0.04)	1.25 (0.06)	0.41 (0.05)
10 MAK 7-7	13.5 (0.1)	0.42 (0.03)	**0.54** (0.02)	**0.69** (0.01)	**0.51** (0.01)	**1.96** (0.01)	**0.75** (0.00)	**0.32** (0.00)	**0.87** (0.00)	**3.03** (0.03)	**0.74** (0.00)	0.46 (0.00)	0.61 (0.02)	**1.15** (0.01)	1.40 (0.02)	0.46 (0.00)
10 MAK 7-8	13.2 (0.1)	0.40 (0.00)	**0.52** (0.01)	0.63 (0.02)	**0.51** (0.00)	**2.01** (0.03)	**0.79** (0.01)	**0.29** (0.01)	**0.86** (0.00)	**2.95** (0.04)	**0.75** (0.00)	0.43 (0.00)	**0.53** (0.00)	**1.14** (0.01)	1.31 (0.01)	**0.51** (0.03)
10 MAK 7-9	11.2 (0.0)	**0.38** (0.00)	0.46 (0.01)	0.57 (0.03)	**0.44** (0.02)	**1.68** (0.02)	**0.66** (0.01)	**0.32** (0.00)	0.77 (0.02)	2.60 (0.00)	0.67 (0.00)	0.44 (0.00)	0.57 (0.02)	1.00 (0.02)	1.19 (0.03)	0.42 (0.01)
10 MAK 7-10*	12.1 (0.1)	0.46 (0.01)	0.47 (0.02)	0.56 (0.01)	0.42 (0.01)	1.41 (0.01)	0.59 (0.01)	**0.28** (0.02)	0.67 (0.02)	2.32 (0.09)	0.65 (0.03)	0.45 (0.02)	0.67 (0.05)	0.94 (0.02)	1.28 (1.00)	0.37 (0.00)

[a] Mean; [b] Standard deviation; figures in bold are significantly different from CC-37 (white maize) for that amino acid (Dunnett test, p significant at < 0.05); $^{\Psi}$ White maize variety (reference); * Reference yellow/orange maize variety.

(Oikeh et al., 2003a, 2003b, 2004; Šimić et al., 2009). The zinc values of the biofortified maize varieties of this study were also higher than the mean zinc values reported by other researchers in white maize varieties (Oikeh et al., 2003a, 2003b, 2004; Šimić et al., 2009).

The phosphorus content of the biofortified maize in this study was higher than that reported by researchers in normal white maize varieties (Bressani et al., 1989). The iron and zinc content of the biofortified maize varieties used in this study and those of the CIMMYT maize samples remained lower than the HarvestPlus target values of > 6.0 mg/100 g for both iron and zinc in biofortified maize (Ortiz-Monasterio et al., 2007). This identifies an opportunity to improve the content of other nutrients such as zinc and iron through breeding. The fact that phosphorus was found in

Table 2b. Essential amino acid composition of maize grain (g/100 g, DW) and comparison of essential amino acid concentration with the pattern of essential amino acid requirements.

Varieties	Protein (g/100g)	Essential amino acid						
		His	Thr	Val	Isoleu	Leu	Phe	Lys
CC 37[Ψ]	10.7[a]	0.44[b] 41[c]	0.44 41	0.56 52	0.40 37	1.41 132	0.60 56	0.39 36
10 MAK 7-1	13.6	0.43 32	0.52 38	0.66 49	0.49 36	1.79 132	0.73 54	0.29 21
10 MAK 7-2	13.4	0.42 31	0.54 40	0.68 51	0.48 36	1.80 134	0.70 52	0.28 21
10 MAK 7-3	11.9	0.38 32	0.45 38	0.62 52	0.45 38	1.61 135	0.66 55	0.30 25
10 MAK 7-5	12.4	0.39 31	0.50 40	0.64 52	0.47 38	1.67 135	0.70 56	0.30 24
10 MAK 7-7	13.5	0.42 31	0.54 40	0.69 51	0.51 38	1.96 145	0.75 56	0.32 24
10 MAK 7-8	13.2	0.40 30	0.52 39	0.63 48	0.51 39	2.01 152	0.79 60	0.29 22
10 MAK 7-9	11.2	0.38 34	0.46 41	0.57 51	0.44 39	1.68 150	0.66 59	0.32 29
10 MAK 7-10[*]	12.1	0.46 38	0.47 39	0.56 46	0.42 35	1.41 117	0.59 49	0.28 23
Pattern of amino acid requirements (mg/g protein requirement)[d]								
Year								
0.5		20	31	43	32	66	N/A	57
1-2		18	27	42	31	63	N/A	52
3-10		16	25	40	31	61	N/A	48
11-14		16	25	40	30	60	N/A	48
15-18		16	24	40	30	60	N/A	47
> 18		15	23	39	30	59	N/A	45

[a] g/100 g, dry weight; [b] Amino acid content (g/100 g, db); [c] Amino acid concentration (mg/g protein; rounded off to a whole number; [d] WHO (2002a); N/A = Not available; [Ψ] White maize variety (control); [*] Reference yellow/orange maize variety.

Table 3. Effect of variety on the nutritional composition of provitamin A- biofortified maize grain varieties.

Nutrient	df	F	P value[a]
Starch	32	102.43	0.000
Fat	32	70.06	0.000
Protein	32	91.28	0.000
Iron	32	188.55	0.000
Zinc	32	13.10	0.000
Phosphorus	32	8.130	0.000

[a] Univariate analysis of variance, p significant at < 0.05; F = The mean square for each main effect or interaction divided by the residual mean square; df = Degrees of freedom.

Table 4. Maize grain quality attributes.

Maize variety	1000 kernel weight (g)	Hectolitre mass (kg/hl)	Stress cracks (%)	Milling index	Fungal infection (% maize grains infected)		
					Penicillium spp.	*Fusarium* spp.	*Fusarium oxysporum*
CC-37	507.4	88.2[a] (0.5)[b]	23	77.7 (0.9)	48	28	56
10 MAK 7-1	429.1	93.6 (0.1)	0	96.6 (1.3)	66	14	24
10 MAK 7-2	410.8	93.5 (0.3)	0	101.9 (1.3)	40	32	30
10 MAK 7-3	355.5	91.7 (0.2)	21	104.6 (1.9)	68	18	18
10 MAK 7-5	328.8	94.6 (0.0)	0	104.0 (3.5)	48	6	8
10 MAK 7-7	444.1	93.1 (0.2)	1	101.8 (3.1)	72	50	56
10 MAK 7-8	376.7	94.9 (0.3)	1	100.9 (2.1)	62	20	22
10 MAK 7-9	345.1	96.3 (0.2)	13	98.6 (1.6)	88	60	56
10 MAK 7-10	677.9	88.2 (0.6)	17	70.5 (4.7)	90	58	80

[a] Mean; [b] Standard deviation.

much higher levels compared to iron and zinc in this study supports the finding that phosphorus is the most abundant mineral in maize (FAO, 1992). Table 3 shows that the nutritional composition of the provitamin A-biofortified maize varieties was very highly influenced by maize genotypes (variety). Therefore, the differences in the nutritional composition, including fat and protein content, of the provitamin A-biofortified maize varieties of this study is attributed to genetic differences of the maize varieties. However, the levels of nutrients, including protein and minerals (for example, zinc and iron), in the maize grain have been found to be affected by several complex factors, including genotype, soil properties, environmental conditions and nutrient interactions (Bänziger and Long, 2000).

Our work is only a baseline study that did not investigate the genotype × environment effects (G×E) due to the resource constraints. We therefore recommend further studies to investigate the effects of GxE on conditioning nutritional composition in provitamin A-biofortified maize. However, a survey of the literature does not show any data regarding the role of G×E on conditioning nutrient levels in provitamin A-biofortified maize grain. Although, similar work has been done for golden rice, the data for both G×E and levels of nutrients other than provitamin A-biofortified has not yet been released in the public domain.

Results of maize grain quality are shown in Table 4. The provitamin A-biofortified varieties had higher hectorlitre mass and milling index values relative to the white maize (CC-37) and the reference yellow/orange maize (10 MAK 7-10). This indicates that the provitamin A-biofortified varieties have a better milling quality compared to the white maize variety (CC-37) and the reference yellow/orange maize variety (10 MAK 7-10). The maize varieties CC-37 (control) and 10 MAK 7-10 (reference) had a higher percentage of kernels with stress cracks compared to the provitamin A-biofortified maize varieties, which indicates that their grains were of inferior quality.

Table 4 shows that the maize grains were infected by the following fungal genera: *Penicillium*, *Fusarium*, specific species of *Fusarium* and *Fusarium oxysporum*. The provitamin A-biofortified maize varieties 10 MAK 7-7 (50 to 72% infected), 10 MAK 7-9 (56 to88% infected) and 10 MAK 7-10 (58 to 90% infected) had higher fungal infection levels than the white variety CC-37 (reference) (28 to 56% infected). *Penicillium* spp. was the most predominant fungus infecting the maize grain (40 to 90% grains infected), followed by *F. oxysporum* (8 to 80% grains infected) and *Fusarium* spp. (6 to 60% grains infected). *Fusarium* spp. is the main pathogenic fungus causing spoilage of maize in the ear while *Penicillium* spp. can be found invading maize preharvest (Pitt and Hocking, 1999). These fungi may cause grain discolouration, reduced germinability and overall grain deterioration as well as heating, mustiness, shriveling and rotting (Agarwal and Sinclair, 1987; Christensen and Kaufmann, 1974). This reduces the nutritional value of the maize and makes it unfit for human consumption (Fandohan et al., 2003). Both *Fusarium* and *Penicillium* genera contain species that produce mycotoxins; some of which can be carcinogenic, mutagenic or teratogenic (Bennett and Klich, 2003; Bauduret, 1990; Abramson et al., 1983). Mycotoxins can also cause large economic losses for many commercial sectors including crop producers, animal breeders and food and feed processors (Jestoi et al., 2004; Miller, 1999). Therefore, biofortified maize varieties whose grains have low resistance to fungal invasion should be further worked on in breeding programmes to improve their resistance to fungal infection.

Although, it has been often observed in cereals that hard, high-protein grains are more resistant to fungal infection than low-protein, soft grains (Audilakshmi et al., 1999; Jambunathan et al., 1992; Bueso et al., 2000; Rodríguez-Herrera et al., 1999; Kumari et al., 1994, 1992) that was not the case in this study. Other factors could have caused the lower resistance of the biofortified maize varieties to fungal infection. The biofortified maize varieties had higher grain fat content than the white variety.

The fat content of the biofortified maize varieties could have contributed to their lower resistance to fungi similar to the findings of Ratnavathi and Sashidhar (2003), working with sorghum grain. Similar to their nutri-tional composition, the quality of the grains of the pro-vitamin A-biofortified maize varieties of this study would be likely influenced by environmental factors, therefore we recommend further studies to investigate the G×E effects on grain quality of provitamin A-biofortified maize, including resistance to fungal infection.

Conclusions

The study findings indicate that the nutritional composition of provitamin A-biofortified maize is influenced by genetic factors. These findings also support the feasibility of enhancing the nutritional composition of the biofortified varieties through selection in a conventional breeding programme. The provitamin A-biofortified varieties exhibited superior levels of starch, fat and protein relative to the white variety, but were lower in minerals. Investigations on the influence of environmental factors and the interaction of the environmental factors with genetic factors on the nutritional composition of the provitamin A-biofortified maize varieties should be conducted. Grains of the provitamin A-biofortified maize varieties were found to have a lower resistance to fungal invasion than the reference white maize variety, which highlights a need to combine the superior nutritional traits of provitamin A-biofortified maize with desirable grain quality, especially resistance to fungal infection, in a breeding programme.

ACKNOWLEDGEMENTS

The authors thank the National Research Foundation (NRF) (South Africa) and HarvestPlus for funding this study.

REFERENCES

AACC International (2000). Approved Methods. 10th Edition. AACC Method 55-10 Test Weight per Bushel. AACC International, St Paul.

Abramson D, Mills JT, Boycott BR (1983). Mycotoxins and mycoflora in animal feedstuffs in western Canada. Can. J. Comp. Med. 47:23-26.

Agarwal VK, Sinclair JB (1987). Principles of Seed Pathology. Vol 1. CRC Press, Boca Raton. pp. 4-12.

AOAC International (2002). Official methods of analysis of AOAC international. 17th Edition. Volume I and II. AOAC International, Maryland.

Audilakshmi S, Stenhouse JW, Reddy TP, Prasad MVR (1999). Grain mould resistance and associated characters of sorghum genotypes. Euphytica 107:91-103.

Bänziger M, Long J (2000). The potential for increasing the iron and zinc density of maizethrough plant breeding. Food Nutr. Bull. 20:397-400.

Bauduret P (1990). A mycological and bacteriological survey on feed ingredients and mixed poultry feeds in Reunion Island. Mycopathologia 109:157-164.

Bennett JW, Klich M (2003). Mycotoxins. Clin. Microbiol. Rev. 16:497-516.

Bidlingmeyer BA, Cohen SA, Tarvin TL (1984). Rapid analysis of amino acids using pre-column derivatization. J. Chromatogr. A 336:93-104.

Bouis H (1996). Enrichment of Food staples through plant breeding: a new strategy for fighting micronutrient malnutrition. Nutr. Rev. 54:131-137.

Bressani R, Breuner M, Ortiz MA (1989). Contenidodefibraacidoy neutron-detergente y de minerals menores en maiz y su tortilla. Arch. Latinoam. Nutr. 39:382-391.

Bueso FJ, Waniska RD, Rooney LW, Bejosano FP (2000). Activity of antifungal proteins against mold in sorghum caryopses in the field. J. Agric. Food Chem. 48:810-816.

Christensen CM, Kaufmann HH (1974). Microflora. In: Christensen CM (Ed) Storage of Cereal Grains and their products. AACC International, St. Paul.

De Onis M, Blössner M (2003). The World Health Organization Global Database on Child Growth and Malnutrition: methodology and application. Int. J. Epidemiol. 32:518-526.

Dugan FM (2006). The Identification of Fungi – an illustrated introduction with keys, glossary, and guide to literature. American Phytopathological Society, St Paul.

Fandohan P, Hell K, Marasas WFO, Wingfield MJ (2003). Infection of maize by *Fusarium* species and contamination with fumonisin in Africa. Afr. J. Biotechnol. 2:570-579.

Food and Agriculture Organization of the United Nations (FAO) (1992). Maize in Human Nutrition. Rome, Italy.

Giron HC (1973). Atomic Absorption Newsletter 12, 28. Perkin Elmer Atomic Spectrophotometer.

HarvestPlus Brief (2006). HarvestPlus: breeding crops for better nutrition. Washington DC, USA.

International Institute of Tropical Agriculture (IITA) (2010). New varieties to boost maize output in West and Central Africa http://www.iita.org/maize/-/asset_publisher/jeR0/content/new-varieties-to-boost-maize. Accessed 22 November 2011

Jambunathan R, Kherdekar MS, Stenhouse JW (1992). Sorghum grain hardness and its relationship to mold susceptibility and mold resistance. J. Agric. Food Chem. 40:1403-1408.

Jestoi M. Ritieni A, Rizzo A (2004). Analysis of the Fusarium mycotoxins fusaproliferin and trichothecenes in grains using gas chromatography mass spectrometry. J. Agric. Food Chem. 52:1464-1469.

Johnson LA (2000). Corn: the major cereals of the Americas. In: Kulp K, Ponte JG (eds) Handbook of cereal science and technology, 2nd edn. Dekker Inc, New York. pp. 38.

Kumari RS, Chandrashekar A, Shetty HS (1992). Proteins in developing sorghum endosperm that may be involved in resistance to grain moulds. J. Sci. Food. Agric. 60:275-282.

Kumari RS, Chandrashekar A, Shetty HS (1994). Antifungal proteins from sorghum endosperm and their effects on fungal mycelium. J. Sci. Food. Agric. 66:121-127.

Li S, Tayie FAK, Young MF, Rocheford T, White WS (2007). Retention of Provitamin A Carotenoids in High β-Carotene Maize (*Zea mays*) During Traditional African Household Processing. J. Agric. Food Chem. 55:10744-10750.

Machida L, Derera J, Tongoona P, MacRobert J (2010). Combining Ability and Reciprocal Cross Effects of Elite Quality Protein Maize Inbred Lines in Subtropical Environments. Crop Sci. 50:1708-1717.

Menkir A, Liu W, White WS, Maziya-Dixon B, Rocheford T (2008). Carotenoid diversity in tropical-adapted yellow maize inbred lines. Food Chem. 109:521-529.

Miller JD (1999). Mycotoxins. In: Francis FJ (ed) Encyclopedia of Food Science and Technology. John Wiley & Sons, New York. pp. 1698-1706.

Oikeh SO, Menkir A, Maziya-Dixon B, Welch R, Glahn RP (2003a). Genotypic differences in concentration and bioavailability of kernel-iron in tropical maize varieties grown under field conditions. J. Plant Nutr. 26:2307-2319.

Oikeh SO, Menkir A, Maziya-Dixon B, Welch R, Glahn RP (2003b). Assessment of concentrations of iron and zinc and bioavailable irons in grains of early-maturing tropical maize varieties. J. Agric. Food Chem. 51:3688-3694.

Oikeh SO, Menkir A, Maziya-Dixon B, Welch R, Glahn RP, Gauch G

(2004). Environmental stability of iron and zinc concentrations in grain of elite early-maturing tropical maize genotypes grown under field conditions. J. Agric. Sci. 142:543-551.
Ortiz-Monasterio JI, Palacios-Rojas N, Meng E, Pixley K, Trethowan R, Pena RJ (2007). Enhancing the mineral and vitamin content of wheat and maize through plant breeding. J. Cereal Sci. 46:293-307.
Pitt JI, Hocking AD (1999). Fungi and Food Spoilage, 2nd edn. Aspen Publishers, Maryland. pp. 483-484.
Rabie CJ, Lübben A (1984). The mycoflora of sorghum salt. S. Afr. J. Bot. 4:251-255.
Rabie CJ, Lübben A, Marais GJ, Van Vuuren (1997). Enumeration of fungi in barley. Int. J. Food Microbiol. 35:117-127.
Raboy V, Below FE, Dickison DB (1989). Alteration of maize kernel phytic acid levels by recurrent selection for protein and oil. J. Hered. 80:311-315.
Ratnavathi CV, Sashidhar RB (2003). Substrate suitability of different genotypes of sorghum in relation to Aspergillus infection and aflatoxin production. J. Agric. Food Chem. 51:3482-3492.
Rodríguez-Herrera R, Waniska RD, Rooney WL (1999). Antifungal proteins and grain mold resistance in sorghum with nonpigmented testa. J. Agric. Food Chem. 47:4802-4806.
Šimić D, Sudar R, Ledenćan T, Jambrović A, Zdunić Z, Brkić I, Kovačević V (2009). Genetic variation of bioavailable iron and zinc in grain of a maize population. J. Cereal Sci. 50:392-397.
Southern African Grain Laboratory (SAGL) (2001). Industry Accepted Method for Stress Crack Analysis of Maize Kernels. Pretoria, South Africa.
Southern African Grain Laboratory (SAGL) (2007). Industry Accepted Method for estimating Milling Index using NIT. Pretoria, South Africa.
Tanumihardjo SA (2008). Food-based approaches for ensuring adequate vitamin A nutrition. Compr. Rev. Food. Sci. Food Saf. 7:373-381.
Taylor JRN, Duodu KG (2009). Applications for non-wheat testing methods. In: Cauvain SP, Young LS (eds) The ICC Handbook of Cereals, Flour, Dough and Product Testing: Methods and Applications. DEStech Publications, Pennsylvania. pp. 200-203.
Whitney E, Rolfes SR (2011). Understanding Nutrition, 12th edn. Wadsworth, Cengage Learning, Belmont. pp. 188.
World Health Organization (WHO) (2002a). Joint FAO/WHO/UNU Expert Consultation on Protein and Amino Acid requirements in Human Nutrition. Technical Report Series No 935. Geneva, Switzerland.
World Health Organization (WHO) (2002b). The World Health Report 2002: Reducing Risks, Promoting Healthy Life. Geneva, Switzerland.
World Health Organization (WHO) (2009). Global prevalence of vitamin A deficiency in populations at risk 1995-2005: WHO Global Database on Vitamin A Deficiency. http://whqlibdoc.who.int/publications/2009/9789241598019_eng.pdf Accessed 23 August 2011.