



## Research Article

### Preliminary Analysis and Biochemical Characterization Related to Health Implications for African Populations in Some Maize Cultivars. A Special Look at the South African Environment

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#### Abstract

After its introduction to Africa, maize was quickly adopted as the cornerstone of local cuisine, especially in sub-Saharan countries. Although nutrient deficiencies constitute a heavy disease burden in the most vulnerable countries, white maize varieties, lacking provitamin A, carotenoids and vitamin A, were widely preferred in Africa. In this work we characterized two black-colored landraces, the Millo Corvo and Spinato cultivars, as well as three non-colored maize genotypes (B73, Argentina and Haiti). We examined the content of carotenoids and anthocyanins for their strong nutritional power, and also the phosphate content, polyphenols and protein levels. The two colored cultivars resulted particularly rich in antioxidant and nutraceuticals factors as carotenoids and anthocyanins pigments, while the white cultivar Argentina resulted particularly interesting for the high and unexpected content of free phosphate. The aim of the project is to cultivate the analyzed maize cultivars, tested to confirm the same nutritional profile observed in Italy, and verify whether they grow productively in South African soil;

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the subject cultivars will be used for breeding programs and then selected for use as functional food in poorer communities suffering of nutritional deficiencies.

**Keywords:** Africa; Anti-nutritional factors; Antioxidant metabolites; Healthy food; Maize

#### Introduction

Maize (*Zea mays*) is of paramount importance in the diets of many African populations: of the 22 countries in the world where maize forms the highest percentage of energy in the national diet, 16 are in Africa [1,2]. After its introduction to Africa by new world explorers in the 16<sup>th</sup> century [3], maize quickly established itself as a main ingredient in the local cuisine because of its relatively high grain yield, low labor requirements and favorable storage characteristics [4]. Lesotho, Malawi and Zambia rank as the world's top three countries subsisting on maize, surpassing the Mesoamerican countries where the crop originated [3]. The use of the maize in African countries is now comparable to that of rice in Asia [2]. In particular, in South Africa, estimated values calculated from FAO food balance sheets in 2007 showed a maize intake of 288.3g/capita per day, corresponding to 30% of daily energy intake; the same percentage is observed for the daily protein intake. According to the South African Department of Agriculture, Forestry and Fisheries (DAFF), the total amount of commercial maize expected for 2015 is close to 10 million tons, which more or less meets the national demand of around 195kg/capita [5]. African food preparation differs from that of other maize-growing regions of the world in that maize-based dishes are most often boiled or cooked rather than fried or baked [2]. In particular, in rural areas, maize flour serves as the raw material for fermented or boiled beverages and thick porridges, traditionally eaten twice daily; Africans often take pride in this as their own distinctive dish [6,7].

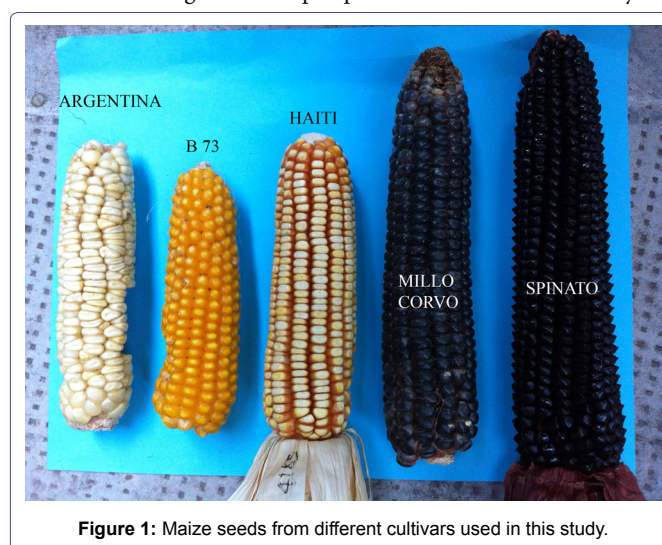
Micronutrient deficiencies constitute a heavy disease burden that is shouldered disproportionately by a highly vulnerable group in the most vulnerable countries in the world: children Under 5 years of age (U5) in Sub-Saharan Africa (SSA) and South Asia. SSA, with 11% of the world's population, accounts for more than half of the deaths and half of U5 Disability-Adjusted Life Years (DALYs) lost to deficiencies of vitamin A, iron, zinc, and iodine [8,9]. Maize supplies many micronutrients and macronutrients necessary for human nutritional needs; however, it lacks B vitamins and the essential amino acids, Lysine (Lys) and Tryptophan (Trp). White maize varieties, which are widely preferred in much of Africa, lack provitamin A, carotenoids and vitamin A, essential for immunity, growth and eyesight. In addition, some minerals in the maize grain have low bioavailability owing to the presence of a high content of phytic acid [2]. South Africa is one of few countries that meet all of the World Bank's three priority criteria for urgent nutrition action: (1) stunting/underweight greater than 20 percent; (2) vitamin A deficiency greater than 10 percent or iron-deficiency anemia greater than 10 percent and (3) an emerging overweight problem (Global Alliance for Improved Nutrition). At national level 24.8% and 39.2% of South African women are overweight or obese compared to 20.1% and 10.6% in men respectively, while children generally

suffered from both under nutrition (19.2% stunting, 43.6% vitamin A deficiency and for U5 and 10.7% anemia) and over nutrition (prevalence of 16.5% overweight and 7.1% obesity in girls and 11.5% overweight and 4.7% obesity in boys aged 2-14 years) [10,11]. Large-scale fortification is a system used to reduce the burden of malnutrition, and in 2003 the South African government passed legislation requiring all bread (wheat) flour and maize meal to be fortified. Maize biofortification has recently been introduced as a strategy to relieve vitamin A deficiency [12], which can lead to blindness, anemia, weakened resistance to infection and increased risk of death. Moreover, a nutritionally superior maize cultivar, referred to as Quality Protein Maize (QPM), was developed, containing twice the amount of lysine and tryptophan compared with the traditional maize type [2]. There are also several projects for improving the mineral content (mostly Zn and Fe) in crops like maize [13]. In this study we describe some nutritional parameters related to the antioxidant power of certain maize cultivars; in particular, we characterized an ancient colored landrace, the Millo Corvo, cultivated in the Spanish region of Galicia and used to produce different varieties of food: the peculiarity of Millo Corvo is the distinctive dark blue/black coloration of the kernels that imparts a typical blue coloration to the bread cooked using the flour. It is known that maize is able to accumulate pigments in the seeds: carotenoids impart a yellow-orange color and anthocyanins a red, purple, blue and black coloration, which confers antioxidant power, thought to be highly beneficial for human health. Moreover, the carotenoid zeaxanthin and its close relative lutein play a critical role in the prevention of Age-related Macular Degeneration (AMD), the leading cause of blindness [14]. Another parameter examined in this study was the phytic acid content. The myo-inositol 1, 2, 3, 4, 5, 6-hexakisphosphate, commonly called phytic acid, is the primary storage form of phosphorus in the seeds: it represents from 50% to over 80% of total phosphorus in mature seeds and accounts for one to several percent of the dry weight. Phosphorus and mineral cations reserves deposited in the phytate molecule are essential for germination and for the growth and development of seedlings. Moreover, phytic acid, because of its ability to chelate metal cations and, therefore, to reduce their bioavailability in the digestive apparatus, has long been regarded as an anti-nutrient for monogastric animals; on the other hand, phytic acid, by virtue of its ability to chelate iron, is a potent inhibitor of the iron-driven formation of reactive oxygen species [15]. The aim of this preliminary study is to analyze the cited maize cultivars for their nutritional profile in terms of antioxidant activity (especially for the carotenoids and anthocyanins content) and phytic acid content. This work must be considered as part of a multi-step project involving, primarily, the cultivation of these maize cultivars in order to verify whether they grow productively in South African soil; secondly, the subject cultivars will be tested to confirm the same nutritional profile as observed in Italy and then one or more candidates will be selected for breeding programs in poorer communities with nutritional deficiencies related to dietary intakes. In particular, cultivars enriched with antioxidant compounds could play a vital role in the reduction of blindness attributed to traditional maize consumption in SSA. Even the maize cultivar with high percentage of free phosphate and minerals (with relative low amount of phytic acid) will be used to improve the nutritional quality of this staple food.

## Materials and Methods

### Plant material

The maize seeds used in this study were collected in Italy from plants cultivated in the experimental field of the University of Milano located in Landriano (PV, Italy). To develop a new colored maize, a typical commercial yellow popcorn line was crossed with a source of tropical anthocyanin biosynthesis regulatory genes. This fieldwork was performed by the team of Professor Roberto Pilu of the University of Milano. Data about the Millo Corvo and Spinato maize cultivars, characterized by a dark coat, were compared with those obtained from the control lines B73, Argentina and Haiti (Figure 1), characterized by a yellow and white color. The Millo Corvo synthetic population, used in this work, was obtained by crossing selected individuals starting from the open pollinated Millo Corvo variety.



**Figure 1:** Maize seeds from different cultivars used in this study.

Flour samples were obtained using a coffee grinder and the seeds were ground for 3min before being further powdered using liquid nitrogen.

### Chemicals and solvents

Reagents and standard chemicals for HPLC analysis and the other assays were purchased from Sigma Aldrich.

### Protein analysis

The Bradford' method was used in the analysis of protein content. Flour prepared from each maize sample (three replicates) was suspended at 100 mg/ml in an extraction buffer (0.1M Tris-HCl pH 7.8, 1m MEDTA, 0.2M NaCl, and 0.2% Triton X-100) and gently mixed at room temperature for 30min. Appropriate dilutions were then prepared in PBS (phosphate buffered saline) and absorbance at 595nm was determined by spectrophotometry. The protein content was extrapolated from a calibration curve created using a series of solutions from 0.01 to 1.0mg/ml (concentrations within the linear range) of pure bovine serum albumin.

### DPPH test

By means of the widely used 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) test, following the procedure described by Brand-Williams et al., [16] it is possible to measure the ARP (Anti-Radical Power) of extracts prepared from any biological material. Two hundred mg of the flours prepared from the seeds of each maize genotype (four

replicates for each sample) were extracted with 1ml of a water/methanol 1:2 (v/v) solution by shaking vigorously for 1h at 4°C. Upon centrifugation at 8000g for 5min, different amounts (10-100ml) of the supernatants were added (except in the control samples) to 900ml of a water/methanol 1:1 (v/v) deep purple solution of DPPH (initial absorbance at 515nm between 0.8 and 1.0). This stable free radical reacts with antioxidants, and its consequent color loss, measurable at 515nm, correlates with antioxidant content. Each reaction mixture was incubated overnight at room temperature in the dark to allow it to reach a steady state before the final absorbance was read and the residual concentration of DPPH calculated by making reference to a calibration line (slope: 0.029) obtained by measuring absorbance at 515nm of a series of dilutions in methanol:water (2:1) of a freshly prepared 1mg/ml DPPH methanolic solution, so as to span the range 0.1-100mg/ml. These values were plotted against those of the volumes (ml) of extract added at time zero to the DPPH solution in order to calculate by interpolation the volume of extract required to consume 50% of the initial amount of DPPH. The reciprocal of this figure corresponds to the ARP value [17].

### Determination of total polyphenolic content

Total phenolic compounds were determined by the Swain and Hillis [18] method, using Folin-Ciocalteu reagent. An amount of 0.5g of seed flour was homogenized with 2.5ml of 50% methanol solution (pH 2.0) for 30' at room temperature. After centrifuging the samples for 10' at 2500g, the supernatant was collected and the pellet suspended with 2.5ml of acetone 70%; after a new centrifuging, the supernatants were unified and stored at -20°C before the assay.

In a test tube, 150µl of the methanol-acetone extract, 2400µl of nanopure water and 150µl of 0.25N Folin-Ciocalteu reagent were combined and then mixed well, using a vortex. The mixture was allowed to react for 3min, after which 300µl of 1N Na<sub>2</sub>CO<sub>3</sub> solution was added and mixed well. The solution was incubated at room temperature for 2h and absorbance was measured at 725nm against a blank. Results were expressed in Gallic Acid Equivalents (GAE; mg/100 g of fresh matter), using a gallic acid (0-0.1mg/ml) standard curve [19].

### Extraction and quantification of carotenoids

One gram of dry weight material was incubated for 1 hour at 4°C with hexane/acetone (1:1, v/v) containing 100mg/l Butylated Hydroxytoluene (BHT). Solutions were filtered on cellulose membrane to remove particles and the organo solvent extract was evaporated under vacuum (30°C). The residue was dissolved in hexane (3ml) and washed three times with 9ml of deionized-distilled water to remove hydrophilic compounds. Sample extracts were concentrated by a rota vapor centrifuge.

Carotenoid content was determined in the organo solvent extracts solubilized in hexane through UV-visible spectrophotometry by measuring the absorbance at 450nm (3 replicates/samples). For the purposes of total carotenoid content calculations, the mean absorption coefficient ( $\epsilon=2,300\text{L m}^{-1}\text{mol}^{-1}$  for hexane) was used.

The HPLC analysis of the carotenoid content was performed according to a modified protocol of Tukaj [20]. The extracted material was dissolved in acetone before being injected in HPLC (Perkin Elmer series 200 equipped with diode array detector UV/Vis and a C18 column, Mediterranean 5µm 25x0.46). The solvents were (A) Methanol: 1M Ammonium Acetate 8:2 and (B) Methanol:Acetone 8:2.

The injection volume was 20µl and the flow rate 1ml/min at 450nm of UV absorbance. The gradient for elution was linear from 0 to 100% B in 20min, and, after 5min, 100% of A in 5min. Finally, a linear flow of 100% A for 5min was used to equilibrate the column.

### Anthocyanin analysis

Total anthocyanin quantification was performed using the protocol adopted from Lago et al., [21] slightly modified. One hundred mg of the sample powder was boiled with 1ml of distilled water for 30min; 5ml of the extraction buffer (1% HCl, 95% ethanol) were added to the samples and left overnight in agitation. After centrifugation at 12000g for 10min, the supernatants were collected and the pellets were again shaken for 2h in 1ml of extraction buffer. Finally, the collected supernatants, unified, were centrifuged at 12000g for 30min and their absorbance was determined spectrophotometrically at 530nm. The amount of anthocyanin was calculated as cyanidin-3-glucoside equivalent (molar extinction coefficient ( $\epsilon$ )=26,900 Lm<sup>-1</sup>mol<sup>-1</sup>; MW 449.2). For the HPLC analysis, 200mg of maize flour from each sample was boiled for 40min with 1.5ml of 2M HCl. After freezing and centrifuging, 1ml of isoamyl alcohol was added to the samples. The upper phase was dried and suspended in methanol 100%; an aliquot of 20µl was finally injected into the HPLC system described above.

### Determination of free and phytic phosphate in seeds

In order to measure the free phosphate content, a sample of 50mg flour of each maize landrace (3 replicates) was extracted with 1ml of 12.5% TCA, 25mM MgCl<sub>2</sub> solution for 20min at room temperature and then left stirring overnight at 4°C. After centrifugation, 100ml of the supernatant were added to 900ml of a freshly prepared Chen's reagent (6N H<sub>2</sub>SO<sub>4</sub>:2.5% ammonium molybdate: 10% ascorbic acid:H<sub>2</sub>O (1:1:1:2, v/v/v/v)) and incubated at 50°C for 1h before reading the absorbance at 650nm of the blue reaction mixture [22]. A reference standard curve was routinely prepared using a series of Na<sub>2</sub>HPO<sub>4</sub> solutions within the linearity range (from 10 to 60N mol phosphate). Phytic acid content was determined in a similar way after samples were subjected to a ferric precipitation method, as described by Pilu et al., [23], and expressed as mg/g.

### Statistical analysis

Comparative statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS v22) for both inferential and descriptive statistics. The results indicate that there is a significant mean difference between pooled black-colored maize and non-black colored maize. Another separate test between individual black maize and non-black maize reconfirmed that there is a significant mean difference, with the black maize having more ARP than the non-black maize (p=0.000). With regard to correlation tests between all DPPH components (ARP, Polyphenols, Anthocyanin, Carotenoids), ARP was positively and significantly correlated with anthocyanin (r=0.969, p=0.006) and polyphenol (r=0.953, p=0.012) respectively. In this study, there was no significant correlation between ARP and carotenoids. T-tests analysis were used to compare means of two different cultivars pairs at a time in terms of ARPs. ANOVA analysis could not be used as it only deals with the overall mean differences between the cultivars and not as specific as T-tests. A further bivariate correlation test revealed that most of the cultivars were negatively corrected although not significant (p<0.05).

## Results

### Protein content

Results about the protein content are shown in table 1; the highest value was recorded for the cultivar Haiti, while for the other cultivars, the values were very homogeneous (CV=9%).

### Antioxidants and polyphenols

In order to collect information about the level of overall antioxidant activity shown by the maize populations, we used the DPPH test. Higher ARP values were indeed measured in the Spinato variety, almost five times higher than the average of the values of the uncolored ones (Figure 2). The MilloCorvo variety, also dark-colored, presents a level of antioxidant capacity that is significantly higher than that of the yellow and white varieties ( $p < 0.05$ ). The Spinato maize cultivar presents the highest polyphenol

	mg/g protein
B73	52.163 ± 0.009
MilloCorvo	51.786 ± 0.055
Argentina	50.957 ± 0.011
Spinato	50.958 ± 0.039
Haiti	62.038 ± 0.072

**Table 1:** Total protein content spectrophotometrically analyzed by using Bradford reagent. Values are the mean of three replicates.

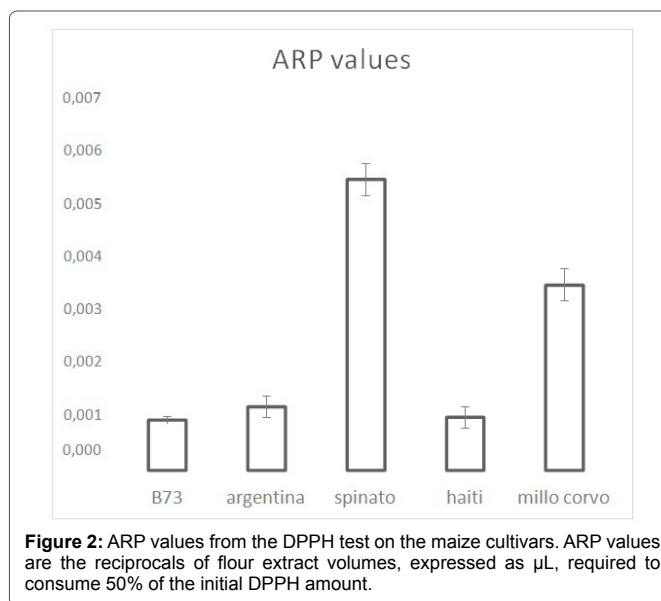
concentration (0.55mg/g), according to the results obtained from the antioxidant DPPH test (Figure 3); the Argentina cultivar, presenting a white coat, showed a polyphenol concentration less than half compared to Spinato (0.23mg/g) but equal to that found in the B73 cultivar (0.22mg/g). MilloCorvo also presents a relevant amount of polyphenols in the seed flour (0.43mg/g), reflecting the ARP values obtained by the DPPH test. The Haiti cultivar showed the lowest ARP value, although the polyphenol content was relevant and higher (around 30%) than in B73 and Argentina.

### Carotenoid analysis

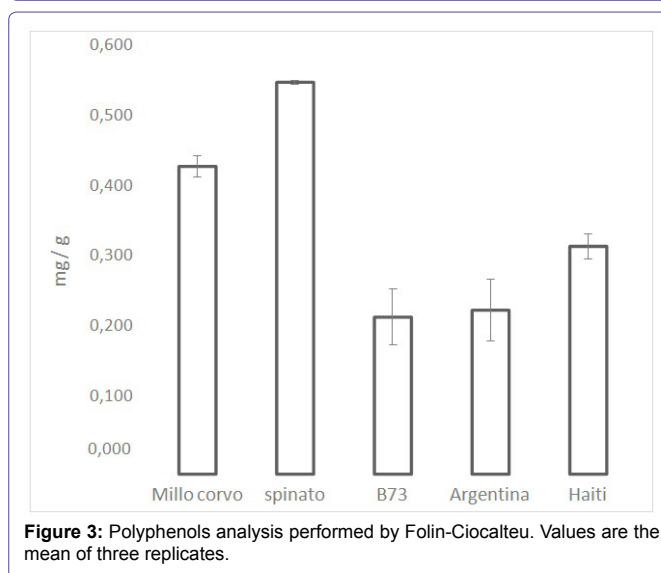
The analysis of total content of carotenoids, shown in figure 4, revealed that the highest level of the pigments occurred in the yellow/orange variety B73 (12.6µg/g), as expected. The two colored cultivars, Spinato and Millo Corvo, presented a relatively high concentration of carotenoids, especially the first one, which contain almost 30% more carotenoids than the second one and around 60% more than the other uncolored cultivar, Argentina; finally, the levels of carotenoids in the black Millo Corvo and the yellow Haiti are not significantly different. HPLC analysis of the main two carotenoids, lutein and zeaxanthin, showed high variability in all the maize genotypes (Table 2). B73 reported the highest values for both the analyzed carotenoids and, with the exception of Haiti, zeaxanthin was more represented respect to lutein in all the genotypes.

### Anthocyanin analysis

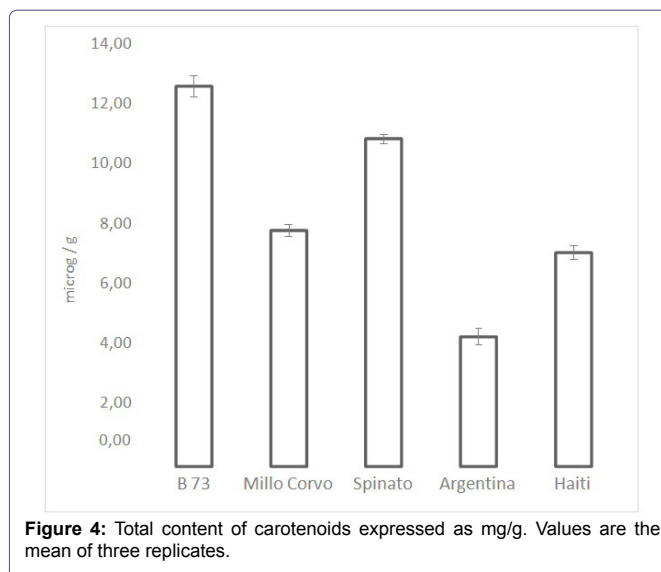
Results for the total amount of anthocyanin present in the maize varieties are shown in figure 5. As expected, the two black-colored cultivars. MilloCorvo and Spinato, present a very high concentration of the pigments responsible for the dark color. Only a few traces of anthocyanin were found in the other genotypes. The dark cultivars in fact, present a level of pigments more than 40 times higher than in B73 cv. In MilloCorvo cv the level of anthocyanin is very high (0.72mg/g),



**Figure 2:** ARP values from the DPPH test on the maize cultivars. ARP values are the reciprocals of flour extract volumes, expressed as µL, required to consume 50% of the initial DPPH amount.



**Figure 3:** Polyphenols analysis performed by Folin-Ciocalteu. Values are the mean of three replicates.



**Figure 4:** Total content of carotenoids expressed as mg/g. Values are the mean of three replicates.

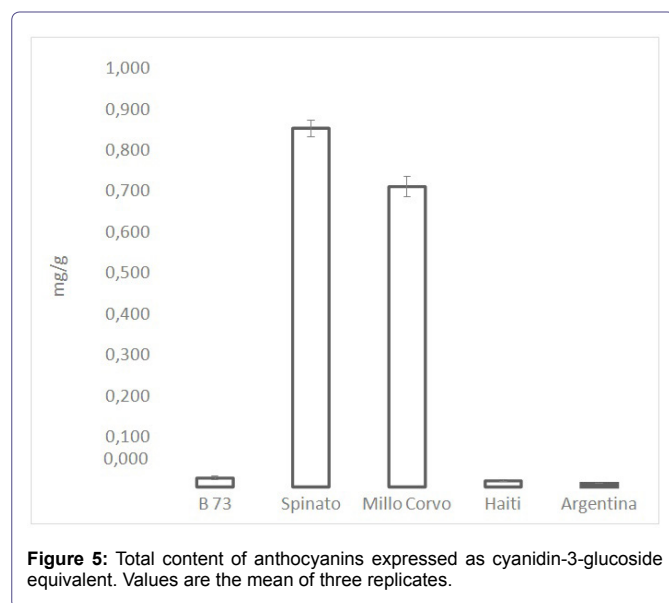
	µg/g	
	Lutein	Zea xanthin
B 73	4.902 ± 0.04	6.413 ± 0.09
Spinato	3.824 ± 0.05	4.598 ± 0.06
MilloCorvo	1.711 ± 0.03	5.445 ± 0.09
Argentina	1.583 ± 0.03	2.807 ± 0.05
Haiti	2.712 ± 0.04	2.577 ± 0.06

**Table 2:** Results of HPLC analysis of the two main carotenoids present in the examined maize cultivars. Values are the mean of three extraction replicates.

confirming the strong antioxidant power shown by the DPPH test. In order to characterize the anthocyanin profile of the two dark cultivars, a HPLC analysis was performed in the plant biochemistry laboratory at the University of Pavia in Italy and results were compared with those obtained from the B73 cultivar. Results, presented in table 3, as percentage of each anthocyanin and which will be published in more detail in the article by Lago et al., [21] (in press), showed a high percentage of cyanidin in MilloCorvo cv (65% of the total amount) and a relevant amount of peonidin in Spinato cv.

### Free phosphate and phytic acid analysis

The Phytic Acid Phosphate (PAP) content in the maize cultivars showed good variability with an average of 1.465mg of phosphate per g of seed flour; this variation ranged from 1.856mg/g (Spinato) to 1.086mg/g (Argentina). Since 1mg PAP=3.55mg phytic acid, by multiplying these figures for this conversion factor it was possible to find the corresponding phytic acid levels, shown in table 4,



**Figure 5:** Total content of anthocyanins expressed as cyanidin-3-glucoside equivalent. Values are the mean of three replicates.

Anthocyanidin	%	
	MilloCorvo	Spinato
Cyanidin	65.90	48.50
Pelargonidin	31.40	7.40
Peonidin	1.90	42.10
Delphinidin	0.75	0
Malvinidin	0	1.90

**Table 3:** HPLC analysis of the anthocyanidin content of the two black colored maize cultivars. Results are expressed as percentage of the content of each pigment present in the cultivar.

together with data about the free phosphate content. The most relevant results regard Argentina and MilloCorvo cv which presents a relevant amount of free phosphate (1.259mg/g and 0.656mg/g respectively), around 5.6 and 2.5 times higher than the level found in B73.

### Discussion

In this study we focused our attention on the positive effects of some compounds present in the dark-colored maize (Spinato & MilloCorvo) which can have an antioxidant effect. In particular, the relevant presence of polyphenols and anthocyanins in the colored

Cultivar	Phytic acid	Free phosphate
	mgP/g	mgP/g
B 73	6.063 ± 0.213	0.222 ± 0.050
Spinato	6.590 ± 0.284	0.418 ± 0.050
Argentina	3.866 ± 0.170	1.259 ± 0.080
Haiti	5.360 ± 0.121	0.314 ± 0.040
MilloCorvo	4.129 ± 0.128	0.656 ± 0.040

**Table 4:** Phosphorous level (atomic weight=31), expressed as mg of phosphate per g of seed flour, in the analyzed cultivars. Values are the mean of three replicates.

maize varieties, compared to the yellow (B73, Haiti) and white maize (Argentina), confer a relevant antioxidant capacity.

The protein content of all 5 maize cultivars, as expected, did not appear very variable; the average of the protein content was around 5%, in line with the values reported in literature, which are between 4 and 10% [24]; FAO corporate document repository (see the link <http://www.fao.org/docrep/t0395e/t0395e03.htm>). The presence of increasing amounts of pigments and polyphenols in dark varieties does not affect the protein level.

Even not a biological radical, DPPH test provides a good estimation of the total antioxidant activity in the examined maize samples. Results reveal a marked positive influence of the grain color on the measured ARP value. Haiti represents an interesting case in which the lowest ARP value corresponds to a relevant amount of polyphenols (30% higher than in B73 and Argentina) showing the presence, in the other cultivars, of additional factors with antioxidant effects.

Regarding the carotenoids level, the obtained results showed an important variability in the content of pigments with antioxidant activity. In particular the two dark-colored cultivars presented a concentration up to 60% higher than the white coat cultivars. Since the most represented carotenoids in maize are lutein and zeaxanthin, the content of each of these pigments was determined by HPLC. As expected, the sum of the two pigments corresponds to almost the total content of carotenoids; lutein and zeaxanthin together represent, in almost all cases, more than 90% of the total content of carotenoids, bearing in mind that the assay for the total carotenoid content is rather rough. Kurilich et al., [25] analyzed several different corn genotypes for the pigment content and found a level of lutein between 0.1 and 20µg/g while the level of zeaxanthin was between 0.01 and 8µg/g.

Kuhn et al., [26], analyzing a maize variety cultivated in southern Brazil, found a zeaxanthin content of 7µg/g and a lutein content of 3.7µg/g, a result comparable with the one obtained in this study. From HPLC analysis of the maize cultivars, a higher level of zeaxanthin than lutein was found in all the varieties examined except

for the Haiti, where the level of the two carotenoids was almost the same. In particular, in the Millo Corvo cultivar, the content of zeaxanthin was threefold higher than the content of lutein, while in the other dark-colored cultivar, Spinato, the zeaxanthin level was found to be only 20% higher than lutein. Comparing these results with the DPPH values, it is possible to notice that the orange-colored B73 cultivar, even presenting the highest carotenoid level (17% higher than Spinato, second highest in content), shows a low ARP value compared with that observed in the two dark maize varieties. Specifically, 5 times lower than that measured in Spinato cv.

Regarding the anthocyanins content, data about the two black-colored cultivars confirmed the high presence of the pigments, even the data regarding Spinato cv could be overestimated due to the presence of phlobaphene in the pericarp layer. Moreover, the obtained results showed as reported also in several publications [27-29], that cyanidin, pelargonidin, and peonidin glycosides are the main anthocyanins present in maize kernels, among which cyanidin 3-glucoside is the most abundant in the dark maize kernels. As a result of these findings, the two coloured examined cultivars therefore should represent an important food in the diet for the prevention of chronic diseases such as cardiovascular disease [30], cancers, respiratory diseases, diabetes and obesity.

Another important studied parameter regards the phosphate content in the examined maize seeds, both free or phytic. Obtained results are in line with those reported by Pilu et al., [23] where the phosphate content of the B73 cultivar was 0.29mg/g and 3.52mg/g for the free P and phytic P respectively. In particular, the most interesting data refer to the amount of free phosphate present in Argentina cv - more than 3 times higher than the average of the other cultivars. The PAP level in the examined cultivars is more homogeneous with respect to the free P level, with only Argentina and MilloCorvo cv presenting a significantly lower level of the PAP. Since this relevant reduction is usually accompanied by an increase of important minerals as iron and magnesium, these cultivars will be taken into account for the nutritional improvement programs. Perhaps the different mineral content of the soil where each cultivar was growing influenced the total amount of phosphate present in the seed.

## Conclusion

The analyses carried out in this study allowed us to evaluate some nutrient and bioactive content with antioxidant activity in certain maize cultivars. In particular, our attention was focused on some nutritional parameters of two black-colored maize genotypes. MilloCorvo and Spinato; we compared the results obtained for these two cultivars to those obtained for yellow (B73 and Haiti) and white (Argentina) genotypes. The analysis performed showed a significant difference in the total antioxidant power between the black-colored and the other maize cultivars, confirming that the high presence of anthocyanin pigments confers a relevant antioxidant capacity. Regarding the carotenoid analysis, the B73 cv presents the highest level of pigments but the two black cultivars also showed an important level of carotenoids, especially the Spinato cv. It can be concluded. Therefore, that the presence of anthocyanins, together with polyphenols, is chiefly affecting the antioxidant power in the maize seeds examined. Moreover, the different amounts of pigment in all the cultivars did not affect the total protein level, and the same was true for all the seeds analyzed. The black maize genotypes and presumably also the B73 and Argentina cv will therefore be utilized to feed the

local South African populations and will be taken into consideration for future breeding programs in order to improve the nutritional quality of the food made from corn.

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