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Impact of Provenance on Phytochemical Attributes of Pigmented Landrace Maize Varieties

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Abstract: Maize is one of the most diverse cereal crops that contribute highly in calorie supply to human diet. Pigmented maize secondary metabolites which are phytochemicals of healthy importance include carotenoids and phenolics which act as precursors of vitamin A and antioxidants respectively. Pigmented landrace maize is cultivated by smallholder farmers in their localities. Three different pigmented maize varieties from different growing districts were analysed for total carotenoids content (TCC) and total phenolic content (TPC), using spectrophotometer. The districts experience a warm temperate climate and higher rainfall in summer than in winter but they differ in that Ntcheu has highest average annual temperature of 20.3°C while Dedza receives the highest annual precipitation of about 1010 mm. Mzimba has an average annual temperature of 20.1°C and receives about 915 mm of precipitation annually. Individual carotenoids were analysed using high performance liquid chromatography (HPLC). TCC were significantly higher ($p < 0.05$) in Ntcheu and Mzimba provenances compared to Dedza. Lutein (22.84 ± 1.25 µg/g) and Zeaxanthin (23.16 ± 2.44 µg/g) were highest in landrace orange maize from Ntcheu. Mzimba provenance showed highest beta-cryptoxanthin (8.60 ± 2.15 µg/g). TPC were not significantly different between Dedza (204.29 ± 0.35 mg/Kg) and Mzimba (207.65 ± 0.22 mg /kg) red maize. Ntcheu provenance showed the least TPC (184.06 ± 0.61 mg/Kg). It seems provenance influenced phytochemical attributes of pigmented landrace maize varieties in some instances. This study also revealed that post-harvest handling of pigmented landrace maize affects phytochemical content. Care should, therefore, be taken in handling pigmented landrace maize to avoid phytochemical loses. Farmers and all other stakeholders in Malawi are encouraged to produce and include pigmented landrace maize varieties in their diets in order to gain the associated health benefits. Further studies are needed for complete characterisation of phenolic compounds of pigmented landrace maize of Malawi.

Keywords: Cereal crops, lutein, carotenoids, phenolic, vitamin A, antioxidants

1. Introduction

Maize (Zea mays L.) is amongst the three main cereal crops that are used in the diet globally, apart from rice and wheat [1]. Maize is one of the most important staple food crop in Sub-Saharan African countries and is a source of income to millions of subsistence smallholder farmers [2]. Continued production of maize is therefore of great importance to the wellbeing of the people and economy of many countries globally. Maize is among the main cereal crops that possess a

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wide diversity within and between crops [2]. Among the diverse maize varieties, pigmented landrace maize varieties are associated with many health benefits such as growth, reproduction, vision and immunity [3; 4] Whole grain foods have gained much attention in recent years as their consumption has been associated with reduced total mortality [5]. Phytochemicals such as carotenoids and phenolic compounds in pigmented crops help in reducing chronic diseases such as diabetes, cancer and cardiovascular diseases [6]. Phytochemicals have been reported to prevent degenerative diseases due to their antioxidant activities and their increased affinity for reactive oxygen species (ROS) [4]. Nevertheless, limited information is available on the effect of provenance on the phytochemical content of pigmented landrace maize varieties grown in Malawi [3]. This information could help understand the associated health benefits and also augment its utilization as a source of functional food material in the diet [6]. Hence the present study was carried to fill the gap.

Years of exclusive promotion of white hybrid maize has decreased the production and access to pigmented landrace maize varieties in Malawi [7]. Increased specialization, intensification of production systems and government agricultural policies have led to a rapid reduction in crop biodiversity [8]. The present scenario which seeks to promote only hybrid varieties of crops offers a big threat to biodiversity conservation [9]. An understanding of the impact of provenance on important health contributing elements in pigmented landrace maize has the potential to stimulate increased production of such varieties. Determination of phytochemical content of pigmented landrace maize varieties would be very useful in understanding the overall characteristics of diversified maize cultivars [10].

The potential increases in white maize yield achieved in recent years are far from solving the problem of malnutrition in most countries despite the availability of knowledge and technologies provided by science about diversification [7]. It is believed that the consumption of phytochemical-rich indigenous crops can assist in reducing and preventing non-communicable nutrition-related disorders among the vulnerable resource-poor populations in developing countries like Malawi [11]. Pigmented landrace maize varieties greatly produce the primary and secondary metabolites in the areas they are best suited to grow [7]. They are highly adapted to their environment and have developed great resistance to a wide variety of diseases due to co-evolution with their pests and disease-causing agents [12]. Maximum utilization of a wide diversity of food crops that are resilient and possess a high potential of yield stability would solve the problem of food and nutrition insecurity. But for this to be achieved there is need to understand the potential productivity of other contents that influences the complex dimensions of the crop such as phytochemicals.

Yield stability is one of the important objectives for farmers producing near the subsistence level. Pigmented landrace maize varieties are associated with diversity within and between crops [9], through buffering of stresses at the field and farm levels in different areas of production. Such crops are known for high yield stability under low input agricultural systems [7]. Yield stability is linked to a range of plant characteristics such as the plant architecture, phytochemical content, high germination rate, adaptation to local conditions but also pest and disease tolerance [3]. The stable yield of pigmented landrace maize varieties is achieved under low input conditions with minimal use of organic or mineral soil amendments [13], hence pigmented maize varieties are the best option for resource poor smallholder farmers. Higher and stable yields means more food and income for farmers. Disposable income could enable smallholder farmers to have a wide choice of diversified food to solve the problem of food and nutrition insecurity.

Different locations experience different climatic conditions; and have different soil characteristics [14]. Nutrients availability in the food crops is affected by different soil factors such as soil pH, mineral composition of the parent rock from which the soil was formed, nutrient solubility and the nutrient absorption capacity of the different crop varieties [6] but also on environmental factors including climatic conditions [14]. For instance the present study was conducted in districts having warm temperate climate and higher rainfall in summer than in winter but they differ in that Ntcheu (central region) has an average annual temperature of 20.3°C and receives about 986 mm of precipitation annually, it is located between latitude and longitude coordinates of 14°49′12.97′′S, 34°39′9.1′′E while Dedza (central region) has an average annual temperature of 18.2°C and receives about 1010 mm of precipitation annually and is located between latitude and longitude coordinates of 14°22′40.44′′S, 34°19′59.59′′E. Mzimba (northern region) on the other hand has an average annual temperature of 20.1°C and receives about 915 mm of precipitation annually and is located between latitude and longitude coordinates of 11°54′0′′S, 33°36′0′′E. These different climatic factors may influence nutritional and phytochemical attributes of landrace pigmented maize varieties grown in Malawi. This study was therefore conducted to assess the impact of provenance on total carotenoids content (TCC) and total phenolic content (TPC) in pigmented landrace maize varieties grown in Malawi. Selected carotenoids including lutein, beta-cryptoxanthin, beta-carotene and zeaxanthin were also analysed.

2. Materials and Methods

2.1 Pigmented Landrace Maize Samples

Three different pigmented landrace maize varieties (orange, red and purple) were collected from fifty-four different smallholder farmers. Samples were collected and carried in ziplock plastic bags from Mzimba, Ntcheu and Dedza districts of Malawi. Sampling were as follows: orange, red and purple maize samples were collected from six different farmers from each study area. Samples were cleaned and ground using wooden mortar and pestle to uniform size (0.5 mm). The ground samples were kept at -18°C until extraction.

2.2 Extraction and Determination of Total Carotenoids Content

Extraction of carotenoids from landrace pigmented maize powder was undertaken as described by Ndolo and Beta [15] with modifications. Briefly, 0.5 g of pigmented landrace maize powder was mixed with water-saturated butan-1-ol (10 mL) in plastic tubes covered with cup of aluminium foil in a fume hood. Each mixture was homogenized (Labora Vortex Genie 2, New York, USA) for 5 min at high speed (5,000 rpm) and left to stand for 1 h at 25°C in the fume hood. After 1 h, the sample was vortexed (45 s) and left to stand a further 1 h. Then, the extract (10 mL) was centrifuged (5,000 rpm, 25°C) (Labtech, Mumbai, India) for 5 min. Supernatants of extracted sample was transferred from centrifuge tubes into grass semi micro-quartz cuvette and absorbance of the samples was read at 450 nm, using UV/Visible spectrophotometer (PG Instrument T90+, Alma park wibtoft Leicestershire, England). All the procedures were carried out in the dark.

Lutein standard solutions (0.5 to 5.0 µg/mL) were used to construct a calibration curve for quantification (Table 1). Total carotenoid content (TCC) was calculated using the following equation and expressed as µg lutein equivalent per gram sample (µg LE/g). Total carotenoids (µg/g) = (A/R) (1/W) ×10; 10 = Total volume of the extract (mL), A=Absorbance, R= Gradient coefficient of regression (slope of the relationship between the concentration of lutein µg/mL and absorbance reading), W = Sample weight in grams [15].

2.3 Identification and Quantification of Carotenoids Using HPLC

Identification and quantification of carotenoids in pigmented landrace maize powder was done according to the method described by Ndolo and Beta [15] with modifications. Briefly, supernatants from centrifugation step were filtered using 0.45 µm nylon disc filter (VWR International LLC, Radnor, PA) into brown HPLC vials ready for analysis. The HPLC system was Agilent HPLC 1200 infinity series equipped with diode array detector (DAD, Mumbai, India) and autosampler (Waters 717 plus, Waters, Milford, MA). The column was a 4.6x100 mm YMC^{TM} carotenoids S-3 with 3 µm packing (Waters, Milford, MA) operated at 35 °C. The sample injection volume was 20 µL. Elution was performed using binary mobile phase system of solvent (A) Methanol/methyl tert-butyl ether/Milli-Q water (81:15:4, v/v/v/) and (B) methyl tert-butyl ether (MtBE) /methanol (90:10, v/v). Run time for 20 min as follows: The initial mobile phase gradient was 100% of A, to 51% of A and 49% of B in 16min., followed by 100% B within 4 min. The flow rate was 1 mL/min. Detection wavelength was 450 nm. Carotenoids were identified and quantified based on similar retention times and UV spectra with their corresponding standards as well as those in literature [15] using lutein, zeaxathin, β-cryptoxanthin and β-carotene standards. Standard calibration curves (peak area against concentration) were plotted for quantification. Results were expressed as microgram per gram of sample dry weight (µg g-1 DW).

2.4 Extraction and Determination of Total Phenolic Content

Extraction and determination of total phenolic content (TPC) were undertaken as described by Tembo and co-researchers [16] with modifications. Briefly, pigmented landrace maize powder (1 g) was mixed with acidified methanol (10 mL) in plastic tubes. The mixture was sonicated in a water bath (60°C) for 30 min and cooled in a desiccator. Thereafter, the mixture was centrifuged (Latch centrifuge, 3500 rpm, 5 min). Within 3-8 min, folin-ciocalteau reagent (5 mL) and sodium carbonate solution (20%, 15 mL) were added to sample extract/standards (1 mL), vortexed and left to stand for 2 h at 26 °C for incubation. Absorbance of the samples, standards and blank were measured at 320 nm using UV/Visible Spectrophotometer (PG Instrument T90+, Leicestershire, England). External gallic acid standards (0 to 500 mg/L) were used to construct calibration curve for quantification. Total phenolic content was expressed as milligrams gallic acid equivalent per Kilogram dry weight (mg GAE kg^{-1} DW).

2.5 Statistical Analysis

Results are presented as mean ± standards deviation (SD) of triplicate analysis. Analysis of variance (ANOVA) by Turkey's Honestly Significant Differences (HSD), method was used to compare the means of composition attributes in the three production locations. All analyses were performed at 95% confidence level ($p < 0.05$) using IBM SPSS version 26.

3. Results and Discussions

Different calibration curves for the standards were plotted and used to determine deferent phytochemical in pigmented landrace maize varieties table 1.

Table 1 - Calibration equations and linearity (R^2) for lutein, gallic acid, beta-carotene, beta-cryptoxanthin and zeaxanthin standards

Standard	Regression equation	R^2
Lutein (TCC)	$y = 0.1319x + 0.0058$	0.9968

Gallic acid (TPC)	$y = 0.091x + 0.2873$	0.9672
Lutein	$y = 24.979x + 11.135$	0.9682
Beta-Carotene	$y = 39.24x + 13.424$	0.9772
Beta-Cryptoxanthin	$y = 7.0794x - 0.7139$	0.9890
Zeaxanthin	$y = 3.0607x - 0.139$	0.9992

3.1 Total Carotenoids Content

Orange maize from Ntcheu (58.90±0.37) and Mzimba (58.38±0.38 μg/g) showed significantly higher TCC than orange maize from Dedza (55.35±0.43 μg/g) (Table 2). There was a decreasing trend in carotenoids content in orange, red and purple maize due to their variety differences [6; 14; 16]. TCC widely vary in many cereal grains and other plants [17; 15]. The decreased trend of TCC in orange maize from Ntcheu, Mzimba and Dedza positively correlated with the decreasing trend in average annual temperatures received by these different production locations. Temperature might have influenced better growth and development by affecting enzyme activities in TCC metabolism. High annual precipitation received by Dedza would have affected TCC content due to mineral leaching, hence plants would lack essential ingredients for the production of these secondary metabolites [3; 6]. Some grains, as observed in present study, contain higher carotenoids content compared to others as well as fruits and vegetables due to their differences in genetic make-up [4]. Low TCC levels have been reported in pigmented landraces and inbred maize [18]. Hwang et al. [3] reported higher TCC levels in orange maize than in white maize. Lower levels in TCC than in present study have also been reported by Ndolo and Beta [15]. These variations could highly be attributed to the interaction of environmental factors and genetic make-up of the crops because they influence physical and chemical processes which are very essential in pigment production in different crops [19; 14]. Thus the trend of results in present study are in line with the published research findings. Differences in quantities might also be due to differences in soil mineral composition which are made available to plants differently and play a greater role in pigment metabolism. Minerals act as activators of nitrogen metabolism in plants, they help in protein synthesis of essential proteins such as tryptophan, and they also act as coenzymes of different metabolic processes which would influence TCC production in pigmented landrace maize varieties. Differences in soil minerals and other climatic factors [14] such as precipitation and annual temperatures received in different geographical areas are therefore important elements influencing phytochemical composition of plants. Knowledge of total phytochemical content of pigmented landrace maize varieties could be utilised to improve health benefits [20].

Table 2 - Total carotenoids content (μg g^{-1} DW basis) in orange pigmented landrace maize whole grains

Maize type	District	Total Carotenoids Content (μg g^{-1})
Orange	Ntcheu	58.90 ± 0.37^{a}
	Dedza	55.35 ± 0.43^{ab}
	Mzimba	58.38 ± 0.38^{a}
Red	Ntcheu	50.25 ± 0.60^{a}
	Dedza	50.34 ± 0.64^{a}
	Mzimba	42.13 ± 0.45^{b}
Purple	Ntcheu	35.18 ± 1.33^{b}
	Dedza	37.42 ± 1.36^{a}
	Mzimba	36.60 ± 1.34^{a}

Values with different subscript letters are significantly different ($p < 0.05$). Values are the means of three triplicates.

Studies on carotenoids composition in different crops such as white and orange maize, wheat, oat, barley, orange fresh sweet potatoes and Paw-paw have been reported in Malawi, Brazil and France [21; 3; 4; 15]. Lutein and zeaxanthin have been found to be the main carotenoids in maize [22; 4]. Maize relatively contain high amounts of lutein and zeaxanthin than the amounts of beta-carotene and β-cryptoxanthin which are considered provitamin A carotenoids [3]. Abundant [23] and high levels of lutein have also been reported in wheat [24]. Lutein was found to be the most abundant carotenoid than zeaxanthin and was significantly higher in orange maize than in white maize [3]. Zeaxanthin has been reported to be the dominant carotenoid in maize while lutein is the main component in non-pigmented cereals of oat, barley, spelt and durum wheat [22]. Another study reported higher zeaxathin than lutein content in maize corn which is in line with the results of the present study [25] (Table 3). These observed differences might be due to environmental factors and genetic differences of the maize varieties [6; 14].

Table 3 - Carotenoids concentrations (µg g^{-1} DW basis) in orange maize whole grains

Carotenoids	District	Mean (µg g^{-1})
Lutein	Ntcheu	22.84±1.25^{a}
	Dedza	20.17±2.36^{b}
	Mzimba	20.67±2.31^{b}
Zeaxanthin	Ntcheu	23.16± 2.44^{a}
	Dedza	22.14±2.56^{b}
	Mzimba	22.43±2.49^{b}
Beta- cryptoxanthin	Ntcheu	7.34 ± 1.15^{b}
	Dedza	7.40 ± 1.17^{b}
	Mzimba	8.60 ± 2.15^{a}

Values with different subscript letters are significant ($p < 0.05$). The values are the means of three triplicates.

The differences might also be directly attributed to mineral composition which affect carotenoid synthesis [6]. Mineral compositions in plants are influenced by a number of factors mainly types of rocks as well as rainfall and annual temperature received by an area [14]. Differences in annual temperature and precipitation observed in the production locations of present study would have influenced the soil fertility of the areas hence differences in mineral nutrient availability to pigmented maize. These would have resulted in the differences of the individual carotenoids in orange pigmented maize. Apart from the influence of environmental conditions, post-harvest handling of pigmented landrace maize varieties also affect phytochemical availability in the maize. This study revealed the loss of carotenoids in some of the pigmented maize due to length of storage. After storing orange, red and purple maize for one year, it was observed that some of the carotenoids evolved. For example in red and purple maize stable trans-lutein evolved to ci-isomers (Fig.2.).

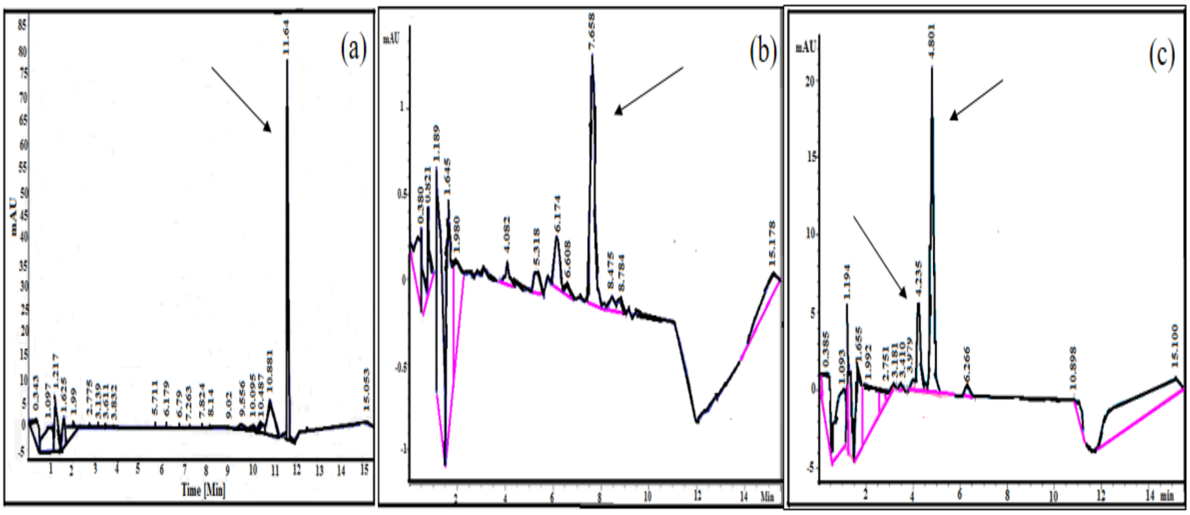

Fig. 1: Chromatograms of standards with their retention times and peak areas. Chromatogram (a) β-Carotene standard, retention time (t_r) 11.64 min; (b) β-cryptoxanthin retention time (t_r) 7.656 min; (c) lutein and zeaxanthin retention times (t_r) 4.235 min and 4.801 min respectively

The standards chromatograms were used to determine the presence or absence of individual carotenoids in pigmented landrace maize varieties Fig. 1. Chromatograms for samples in Fig. 2 were compared with those of the standards. Orange maize samples represented by chromatogram (d) had similar retention times as standards chromatograms for β-Carotene (a), β-Cryptoxanthin (b) but also for lutein and zeaxathin (c). Purple (e) and red maize (f) showed retention times of 13-cis lutein which is an isomer of stable trans-lutein [15].

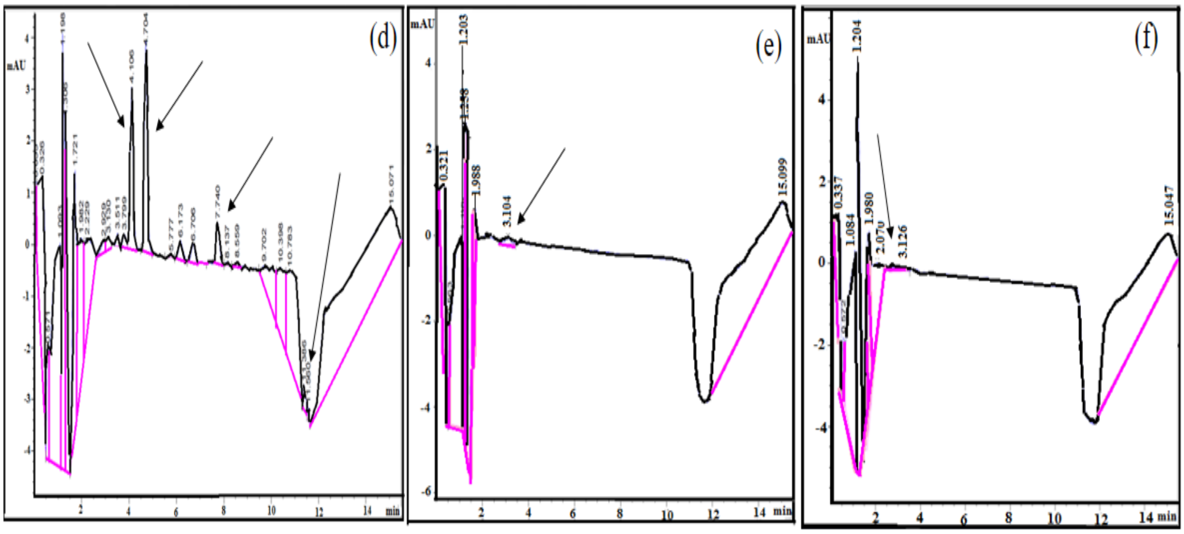

Fig. 2 - Chromatograms (d), (e) and (f) are for pigmented maize samples. In orange maize samples, shown by Chromatogram (d) lutein, zeaxanthin, β-cryptoxanthin and β-carotene eluted at 4.106 min, 4.704 min, 7.740 min, and 11.560 min respectively. Chromatograms (e) and (f) shows the retention times of the isomers of lutein which are 13-cis lutein in purple and red maize samples at 3.104 min. and 3.125 min. respectively

Isomerization and oxidation of the stable trans-carotenoids to cis-isomers during storage time and due to heat effects may have altered the carotenoids [21]. Enzyme activity within the plants may also have caused degradation and loss of carotenoids [4; 21]. Isomerization might have occurred due to high temperature, light and increased storage time [4] which might have resulted in loss of minimal contents of carotenoids which was available in red and purple maize varieties as illustrated in (Fig. 3.).

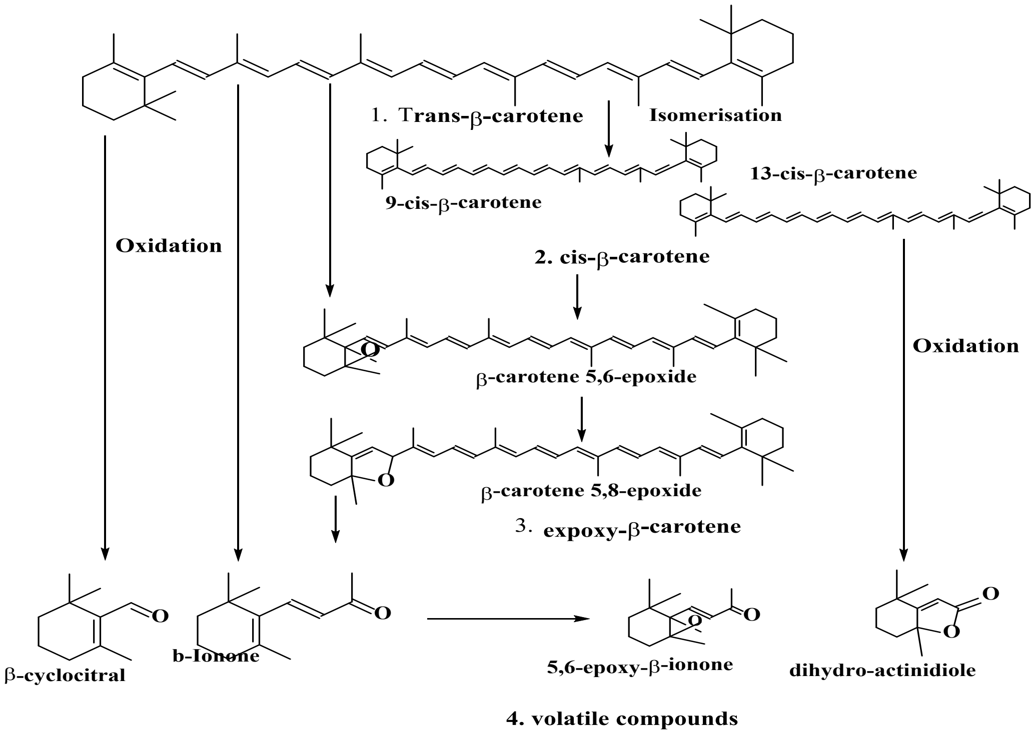

Fig. 3 - Schematic illustration of carotenoids evolution

Although cereals are one of the outstanding sources of macronutrient, micronutrient and phytochemicals, these bioactive compounds are heavily affected by post-harvest processes [26; 27]. Isomerization and oxidation of carotenoids may lead to vitamin A deficiency and its associated disorders [4; 21]. Whole-grain consumption with minimal exposure to heat and reduced storage period of pigmented landrace maize varieties can increase the uptake of carotenoids in diets before they are lost and help reduce malnutrition and mortality that comes due to lack of benefits that are gained from stable carotenoids [5]. In Malawi, problems of lack of carotenoids can also be attributed to how smallholder farmers handle their crop produce after harvest. Farmers should avoid long time storage and high exposure of pigmented landrace maize varieties to sunlight energies in order to minimise carotenoids loss. Of course this is a challenge because smallholder farmers who are poor and rely on sunlight energy cannot afford new technologies which are expensive. Traditional methods which do not require direct sunlight contact may be effective to poverty stricken smallholder farmers. These methods would ensure maximum utilisation of important pigmentations such as carotenoids before getting degraded.

3.2 Total Phenolic Content

Phenolic compounds are the major components among the phytochemicals in cereals but levels differ in different grain varieties [15; 23]. High and low ranges of TPC have been reported in cereal grains [23; 27]. Research suggest that different cereal grains have different composition and distribution of phytochemicals [28]. Variations in levels of phenolic compounds have been reported across pigmented and non-pigmented cereals from different genotypes and growing regions [5]. It has been established that nutrient and phytochemical composition of cereal grains both in quantity and quality depend much on genotype and environmental factors [14; 20]. The highest concentration of phenolic compounds in the present study in Table 4 was lower than that reported in Durum wheat species [24].

Table 4 - Total Phenolic content (mg GAE Kg^{-1} DW basis) in pigmented maize whole grains

Maize type	District	Total Phenolic content (mg Kg^{-1})
Orange	Ntcheu	216.02 ± 0.47^{b}
	Dedza	211.80 ± 0.71^{b}
	Mzimba	230.39 ± 0.30^{a}
Red	Ntcheu	184.06 ± 0.61^{b}
	Dedza	204.29 ± 0.35^{a}
	Mzimba	207.65 ± 0.22^{a}
Purple	Ntcheu	205.44 ± 0.29^{b}
	Dedza	235.16 ± 0.27^{a}
	Mzimba	130.25 ± 0.30^{c}

Values with different subscript letters are significantly different ($p < 0.05$). Values are means of three triplicates. Areas of production plays a greater role in influencing the interactions between genetic make-up of different cultivars and biochemical synthesis [6]. Plants receive different amounts of heat due to differing temperatures in different production locations. Apart from temperature, differences in annual precipitations influences mineral nutrient availability in soil. These differing weathering agents coupled with different absorption capabilities of different crop varieties affects mineral availability in the food crops. These generally affects metabolism of TPC in pigmented landrace maize because low ingredients for production will also result in low TPC availability in the crop. This is so because minerals are crucial in acting as coenzymes in different metabolic processes for TPC production. It is therefore important to have increased production of pigmented landrace maize varieties in areas where they are best suited to realize increased TPC and phytochemicals in general. And increased consumption of pigmented maize and other crops rich in TPC would help lower the risk of chronic diseases and promote human health [28].

Conclusion

There are significant differences in the phytochemical content in pigmented landrace maize varieties in their areas of production. Knowledge of differences in carotenoids and phenolic content in pigmented landrace maize varieties would be very important to all stakeholders who would wish to include these varieties in their diets. This information would also be of great benefit to subsistence and commercial farmers in choosing the verities to produce in their different areas of production. Apart from Orange maize, red and purple maize would be utilised in development of functional foods for individuals that are healthy conscience. There is need for proper handling of pigmented landrace maize varieties in order to maximise the intake of phytochemicals before they are degraded.

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