Variation in Leaf Macro-nutrient and Anti-nutrient Contents Associated with Leaf Maturity in Selected Roselle (*Hibiscus sabdariffa*) Genotypes

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ABSTRACT

Studies were conducted to determine variability in leaf nutritive contents associated with leaf phenological maturity and genotype among local Zambian roselle (*Hibiscus sabdariffa*) landraces. A split-plot design was used with leaf maturity as the main plot, genotype as the subplot and 4 replications. Four local landraces ZM 5729, ZM 5738, ZM 5748 and ZM 6839 from Western and Southern provinces of Zambia were compared. Leaves were harvested at three plant maturity stages: immature (7- 11 weeks old), mature (12- 15 weeks old) and senescence (more than 16 weeks old). Crude protein, carbohydrates, fat, fibre, oxalate, alkaloid and vitamin C contents were analysed. There were highly significant interactions among genotypes and maturity for macro-nutrient, anti-nutrient and vitamin C contents. Ash content increased with advance of maturity. Crude protein was highest in the immature phase and declined slightly in the mature phase. Crude fat was lowest in the immature stage and increased to a maximum in the mature stage. Crude fibre increased with maturity, by about 215 % from the immature to the senescence stage. For the two anti-nutrients, alkaloids increased with age from 1.58 % in immature to 4.17 % in senescent stage; and oxalates followed a reverse pattern, decreasing with age. Vitamin C followed a trend that showed moderate levels in the immature phase, which increased by about 20 % in the mature stage and thereafter declined by about 70 %. Vitamin C increased with increases in fat and carbohydrate contents but was reduced with fibre content. Ash increased with alkaloid contents but was reduced with oxalate content. The study demonstrated the variability in nutritional, anti-nutrient and vitamin C contents among local roselle genotypes as they mature. These changes have an important bearing on the nutritional quality of the vegetable related to when leaves are harvested.

INTRODUCTION

About 30 crop species provide 95% of the world's food, fibre and other needs whereas 7,000 species have been known to be used for food and are either partly or fully domesticated (Williams or Huq, 2002). This large array of plant species has over the decades have been largely ignored are largely considered as under-utilised minor crops. Unbalanced diets have been implicated worldwide in the cause and severity of many diseases, including cancer, heart disease and diabetes (Davies and Doyle, 2015; Pomerleau et al., 2004). Evidence indicates that consumption of more balanced diets can alleviate both the incidence and severity of not only these diseases, but also obesity, which is a causal factor for many chronic diseases (Sams, et al., 2011; Simon, 2014). With the rising incomes and resultant change in diets from vegetable based, it is anticipated that health challenges such as cardiovascular diseases, diabetes and cancer will continue to increase (Davies and Doyle, 2015; NFNC, 2009; Pomerleau et al., 2004). The decline in consumption of fresh fruits and vegetables is a worldwide phenomenon (Casagrande et al., 2007; Hampwaye et al., 2016; Pomerleau et al., 2004). This decline when coupled to a diet largely rich in calories and energy has been associated with malnourished or obese populations (Hoffman et al., 2017, 2018). An added complication is the declining nutritional content in new crop varieties that has been observed as plant breeders attempt to improve agronomic characteristics of crops such as yield (Simon, 2014).

Consumption of vegetables and fruits can be improved by introducing and popularising 'novel' commodities or under-utilised plants (Casagrande et al., 2007; Pomerleau, et al., 2004). Indigenous plants provide a cheaper and affordable source of nutrients and can play a critical role especially among the low-income brackets of the population (Williams and Huq, 2002; Davies, 2015; NFNC, 2009, Weller et al., 2015).

In Zambia, one of the important novel sources of nutrients and secondary metabolites is the under-utilised 'indigenous' vegetable, roselle (*Hibiscus sabdariffa* var. *sabdariffa*) (Mataa et al., 2018). The genus Hibiscus includes more than 300 species of annual or perennial shrubs or trees (Wang et al., 2012). Native distribution of the species is uncertain, but evidence shows that roselle was domesticated in western Sudan before 4000 BC (Ismail et al., 2008). In addition to high carbohydrates and fibre, other research has shown roselle to be rich in secondary metabolites (Obouayeba et al., 2014). These compounds play many roles in plant development including protecting plants from environmental hazards such as pollution, stress, drought, disease and damage (Larcher 1995; Man et al., 2011). Roselle extracts have shown antibacterial, antioxidant, nephron and hepato-protective, renal diuretic effects, effects on lipid metabolism (anti-cholesterol), anti-diabetic and anti-hypertensive effects (Da Costa-Rocha et al., 2014; Villani et al. 2013). The quality of the product is determined by seed stock, local growing conditions, time of harvest and post-harvest handling (Da Costa- Rocha et al., 2014). Generally, the leaves and calyces are important harvested products, with the former being the important part of the plant in Zambia. Roselle is said to grow relatively easily in most well drained soils and leaf yield is about 10 t/Ha. However, information is lacking regarding general nutritional content and influence of leaf ontogeny on the phytochemical and nutritive characteristics in different local accessions of roselle. In this study, four genotypes were collected from two contrasting agro-ecologies of Zambia; from agro ecological zone I - the low rainfall and hot Zambezi river valley (ZM 6839) and region II – medium rainfall and moderate temp (ZM 5729, ZM 5738, ZM 5748) (Bunyolo et al., 1995). The objective of the study was to determine the effects of genotype and maturity phases on the nutrient composition value of the leaves of different local genotypes of roselle in Zambia. In addition to desirable nutrition components (proteins, carbohydrates, vitamin C, fibre), the study also examined anti-nutritional properties (alkaloids and oxalates). Information generated will contribute harvesting methods to maximise nutritional benefits of the plant for consumption. Additionally, by demonstrating high nutritional quality may lead to increased demand and therefore lead to increased production of this underutilised crop.

MATERIALS AND METHODS

Field study location. A series of related field experiment were planted at Siziya farm in Ibex hill, Lusaka with a geographical location of latitude

15°25', longitude 28°21' and at an altitude of about 1,279 m (Mataa et al., 2018). According to the Zambian system the site was in Agro-ecological zone II, which is characterised by annual rainfall between 800 and 1000 mm, with mean minimum of 10 °C and maximum of 32 °C (Bunyolo et al., 1995).

Plant materials. Four local landraces of roselle (*Hibiscus sabdariffa)* derived from different Agro-ecological zones selected randomly were obtained from the Zambia Agricultural Research Institute gene bank (Table 1). Of the four landraces used in the study, ZM 5729, ZM 5738, ZM 5748 were collected from Sesheke- Agro-ecological zone II and ZM 6839 collected from Gweembe valley which is classified as Agro-ecology I. The Agro-ecological zone I climate is characterised by mean minimum temperature of 20 to 25 °C and maximum of 38 °C; annual rainfall not exceeding 800 mm. Agro ecological zone II is characterised by annual rainfall between 800 and 1000 mm, with mean minimum of 10 °C and maximum of 32 °C. For production, standard vegetable management was followed as recommended (Mataa, 2015).

Sample collection. Leaf harvesting was done at three stages of plant growth: pre-flowering/immature plants (7- 11 weeks after planting); flowering/mature plants (12 to 16 weeks after planting) and senescing/old plants (more than 16 weeks after planting). Three plants per replicate were sampled. Each sample consisted of about 3-5 leaves from the upper third of each plant and were collected very early in the morning. Immediately after harvest the leaves were placed under crushed ice in a domestic 10 L cooler box. Leaves were protected from direct contact with the crushed ice with a thick cheesecloth to prevent ‘ice scald’. In preparation for analyses, the leaves were cleaned and foreign materials like dirt and grass were removed by briefly washing with tap water. Leaves were then rinsed with distilled water.

*Chemical analysis***.** The following properties were determined in the Food Science laboratory; crude fibre, crude protein, crude fat, ash and total carbohydrates (macro-nutrients), as well as alkaloids, oxalate (anti-nutrients) and vitamin C. Proximate composition (moisture, ash, protein, fat, fibre) was determined according to the AOAC official methods (1995). Total carbohydrates were determined by calculation using the difference method (Mataa et al., 2018). Alkaloid determination was done using the alkaline precipitation gravimetric method described by Harborne (1973). Oxalate content was determined using method of Day and Underwood (1986). Vitamin C was determined by titrimetric method using AOAC Method 967.21 (AOAC, 2000). For each analysis, triplicate samples were analysed. Results were expressed on dry matter basis.

*Data analysis***.** A split-plot experimental design was used with the main plot as the maturity stage and subplot as the genotype (Sokal and Rolfe, 1981). Data was analysed using GenStat version 17 (VSN, 2009). Analysis of variance was done, and means were separated by least significant difference method. Differences were considered significant at $p < 0.05$ and highly significant at $p< 0.001$.

RESULTS AND DISCUSSION

There were highly significant differences ($p < 0.001$) in macronutrients, anti-nutrients and Vitamin C among the different genotypes determined at different maturity stages. Significant maturity by genotype interactions were also observed.

Macronutrients. Single factor effects of maturity and genotype on macronutrients are presented in Table 2. Ash content was highest in the mature stage and senescent stages (12.6 %) compared to 8.3 % in the senescing leaves, with an overall decrease of 25 % from mature to senescence stage. Among the genotypes, highly significant differences in ash content were observed ($p < 0.001$). ZM 5748 (12.0 %) and ZM 5738 (11.1 %) had the highest ash content, followed by ZM 6839 (10.3 %) and ZM 5729 (9.4 %). There was a nearly linear decrease in the crude protein content, which decreased by 55% from immature to senescent stage. Among the genotypes, crude protein content was significantly higher in ZM 5729, ZM 6839 and ZM 5748 (≈ 15.4 %) compared to ZM 5738 (13.2 %). The ash content, an index of the mineral content, is important in many biochemical reactions. The ash content increased from young plants to mature plants before decreasing to their lowest content in senescent plants. Major component of ash are the minerals that the plant takes up in process of development and therefore the level of ash increases with age. The genotype exerted different effects on the ash contents of leaves, responding differently with regards to their ash content.

Fat content was lowest in the immature stage (0.9 %) and increased seven-fold to 6.2 % in the mature stage before declining at the senescent stage to the same level of the immature stage which represented an overall decrease of 60 % between immature and senescent stages. ZM 5729 had the highest fat content among the genotypes. Crude fat showed a pattern of being low in the immature stage, increasing to reach a maximum in mature stage and then falling to lowest levels in the senescent stage. ZM 5738 had the lowest crude fat content in the immature and mature phase whereas ZM 5748 had the lowest crude fat in the senescent stage. Changes in carbohydrate content were similar to that of crude ash and fat, while its trend was opposite that of crude fibre. In human diets, lipids are a good source of energy and participate in the transportation of fat-soluble vitamins; maintenance of cell membrane integrity, insulation and where carbohydrates are deficient, fats can contribute to calorie intake (Pamela et al., 2005). There was a significant difference in the mean fat contents of the leaves at the three stages of maturity. The crude fat content of the leaves was highest in the mature plants and lowest in senescing plants. The fat content increased from the young to mature plants. Similar observations have been observed in *Moringa oleifera* leaves (Bamishaiye et al., 2011). Oulai et al., (2015) reported fat content in mature roselle as being 4.8 %. Generally, few studies include nutritional analysis in the senescence phase but leaves in this stage of development are also consumed especially where they are destined for drying and storage. Across maturity stages, the lowest fat content was found in the senescent ZM 5738 while the highest was in the senescent phase of ZM 6839.

Fibre content was lowest in leaves harvested in the immature phase and it increased from 23 % to 74 % in the senescent leaves, with an overall 215% increase between the immature and senescent stages. Among the landraces, fibre content was highest in ZM 5738 (41.5 %) followed by ZM 5748 and ZM 6839 (≈ 41%) ZM 5729 had the lowest crude fiber (38.8 %). Generally, fibre content increases with age as plant strengthens its structural components. In this study, mean fibre content increased with plant age. Fibre content was negatively correlated to carbohydrate content. Carbohydrates are an important energy source for metabolic reactions. There was an increase in the carbohydrate content as the young plants matured and then a decrease began during senescence. There was an increase in carbohydrates as the young plants matured, possibly due to increased synthesis in order to provide energy for the metabolic reactions and formation of plant components (such as pollen and flower development) that take place during mature phase. The decrease in their content as the plant begins the senescence period could have been due to their breakdown in the processes leading to the plant death. It was significantly higher than that of senescent plants. The lowest carbohydrate content was in the senescent leaves of the ZM 5738 accession.

Crude protein was highest in the immature phase and declined in the mature phase and lowest in the senescent stage. The decline was highest in ZM 6839 (28 %) compared to the other genotypes. In many plant species there is usually rapid remobilization of proteins from storage and non-reproductive structures to developing seeds as the plant matures and approaches reproduction (Tollenaar and Dwyer, 1999). Enzymatic and metabolic processes decline with age and this is particularly true of annual plants such as roselle. The protein content of the leaves decreased with maturity. There was genotypic variation, with ZM 5729 having the highest and ZM 5738 having the lowest crude protein content. Proteins contribute significantly to the calorie value of plants and as such, the young plants would be more suitable to include in human diets for the purpose of obtaining more protein or energy when consumed. There were highly significant interactions between genotypes and maturity for ash, crude protein, crude fat, crude fibre and total carbohydrates ($p \leq 0.001$, Fig. 1).

Generally, the ash content increased with advance of maturity; the immature stage had significantly lower ash content which increased by about one third in the mature phase and then declined slightly in the senescence phase. This was only significant in ZM 6839, ZM 5729 and ZM 5738 whereas ZM 5748 showed a consistent decline with age.

As plants age, they undergo metabolic and constituent changes, the reasons for these changes are varied and include normal physiological changes associated with development (Mohr and Schopfer, 1995) and adjustment to changes in the environment (Mataa et al., 1996). Roselle is an annual plant with typical monocarpic type of senescence, where the whole plant dies after formation of fruits and seeds. Endogenous factors control the aging process in leaves and this process is influenced by external factors such as light and temperature (Mataa and Tominaga, 1998; Mohr and Schopfer, 1995). The key processes involved in aging are changes in chlorophyll, proteins and consequently changes in photosynthesis and respiration rates.

Several physiological events occur in the plants as they develop. From the immature vegetative phase to the mature stage the plant is actively accumulating assimilates for its growth and full development. During senescence, drastic changes such as reduction in moisture as well as macronutrients, loss of membrane integrity, decline in mRNA and protein synthesis that eventually lead to death (Sexton and Woolhouse, 1984) occur. Changes in the macronutrient and phyto- chemical composition of the plants as they mature can largely be attributed to the functional and structural changes taking place (Sexton and Woolhouse, 1984). The basis of which are genetic differences.

Anti-nutrients and Vitamin C. Maturity stage and genotype impacted the accumulation of the anti-nutrients (alkaloids and oxalates), and vitamin C (Table 3). Alkaloids increased with age from immature to senescent stages, with the highest increase of 190% between immature and mature stages, minimal increase of 8 % between mature and senescent stages, and an overall increase of 198% between mature and senescent stages. Across maturity stages, ZM 5738 (4.14 mg/ kg) had the highest alkaloids, followed by ZM 5748 (3.75 mg/kg), ZM 6839 (3.75 mg/kg) and ZM 5729 had the least amount (3.54 mg/ kg). Oxalates followed a reverse pattern decreasing with age. The highest oxalate content was at immature stage (2.19 mg/g) decreasing by 39 % in mature leaves and finally declining to 1.20 mg/g in senescent leaves, with overall decrease of 45 % between immature and senescent stages. Among genotypes, they were highest in ZM 5729 (1.69 mg/g) and lowest in ZM 5738 and ZM 6839 (1.50 mg/g).

In contrast, vitamin C pattern was different to that observed with oxalates. The highest vitamin C content was in mature leaves, immature leaves were intermediate at 243.1 mg/kg and the lowest content was in senescing leaves (84.5 mg/kg). Vitamin C varied among genotypes (Table 3). ZM 5738 had the highest vitamin C (218 mg/kg), followed by ZM 5748 (209.4 mg/kg) and lowest in ZM 6839 (190 mg/kg).

Interactive effects between maturity and genotype. There was significant genotype by maturity interactions for alkaloids, oxalates and vitamin except for ZM 6839 where there was a decline as the plants entered senescence (Fig. 2A). In the immature stage ZM 6839 had significantly lower alkaloids compared to the other three genotypes. The highest alkaloid content was observed in ZM 5738 during senescence. For oxalates, the immature stage, ZM 6839 accumulated the highest (2.42 %), while in mature and senescence phases, ZM 5729 showed the highest oxalates (1.54 and 1.32 %, respectively). Highest oxalic content occurred in ZM 6839 in the immature phase. Changes in vitamin C followed a trend that showed moderate levels in the immature phase, which increased by about 20 % in the mature stage and thereafter declined by about 70 %, resulting in an overall 65% decrease between immature and senescent stages. In the immature and mature stages ZM 5738 had highest vitamin C (252.3 mg/kg). In the senescent stage the highest Vitamin C was ZM 5729 (96.2 mg/kg).

Alkaloids are typically found in the form of salts with organic acids and can be both beneficial and detrimental. Some alkaloids are known to cause neurological and gastrointestinal upsets and disorders at high doses (Osagie, 1998). The alkaloid content in the leaves increased with plant maturity and comparing the effects of the three stages of maturity, age had a relatively positive effect on the alkaloid content as it increased with maturity for all genotypes. Overall, there was a significant difference between the mean alkaloid contents of the three stages of maturity under the four genotypes. Alkaloids can be toxic and for the purpose of reducing the risk of toxicity, however, tender immature leaves are the ones that are consumed so the risk of alkaloid toxicity is minimal.

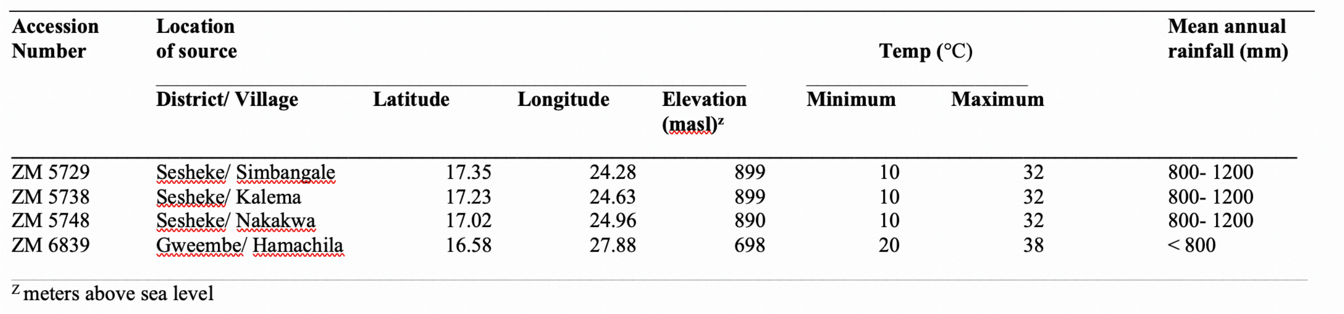
Oxalic acid is thought to be necessary in plant growth during active cell division and it is therefore high in actively growing plants or organs (Hirooka and Sugiyama, 1992). Oxalate content of the leaves decreased with maturity. Accumulation in plant tissue may be attributed to a shift in equilibrium towards biosynthesis rather than towards degradation (Hitomi et al., 1992). Results indicated that these genotypes of roselle accumulate low levels of total oxalates indicating there should be no risk of oxalosis relative to consumption.

Vitamin C, the most abundant of the antioxidants found in plants, is an essential nutrient important for growth and development (Larcher, 1995) as well as being associated with the reduction of the oxidative stress and providing overall health benefits (Ames et al., 1994). The vitamin C content of the leaves increased from young plants to mature plants and then decreased as they senesced.

Among the four genotypes evaluated, ZM 5729 had the lowest ash content and ZM 6839 highest protein (except in senescence). ZM 5748 had the highest carbohydrate and oxalate content and lowest in alkaloid content compared to the other three genotypes. ZM 5738 was highest in fibre, and vitamin C in addition to being highest in alkaloid content and lowest in oxalate content. ZM 6839 had least crude fibre, carbohydrate and vitamin C contents and the highest oxalic acid content. While this work focused on the nutritional benefits and phytochemistry of roselle leaves for health and food security given this plant is consumed in Zambia for this purpose, the calyx is more often the product known in international commerce. Besides its importance as a food or traditional medicine in countries of origin, the flower (calyx) is traded and widely used worldwide as an important ingredient in industrially produced teas and beverages (Juliani et al., 2009; Plotto, 2004) and thus also presents a potentially lucrative opportunity to reduce poverty of poor rural communities in countries such as Zambia.

The study demonstrated the variability in nutritional and anti-nutrient contents among local roselle genotypes over the course of growth and development. These changes have an important bearing on the nutritional quality of the vegetables. Of the three developmental stages, the mature stage appeared to provide the best nutritional content for the consumer. The study demonstrated significant compositional variation within the existing landraces. While only a limited number of genotypes were evaluated, results suggest that the locality where the genotypes were sourced can have an effect on the phytochemical profile. ZM 6839 acquired from Agro-ecological zone I (which was hotter and drier) differed slightly from other three genotypes (obtained from a cooler and wetter Agro-ecological zone II), and except for the crude protein, expressed slightly lower nutritional content.

Table 1. Geographical parameters of sources of roselle *(Hibiscus sabdariffa)* genotypes used in the study.



Accession Number	Location of source				Temp (°C)		Mean annual rainfall (mm)
	District/ Village	Latitude	Longitude	Elevation (masl)[z]	Minimum	Maximum	
ZM 5729	Sesheke/ Simbangale	17.35	24.28	899	10	32	800- 1200
ZM 5738	Sesheke/ Kalema	17.23	24.63	899	10	32	800- 1200
ZM 5748	Sesheke/ Nakakwa	17.02	24.96	890	10	32	800- 1200
ZM 6839	Gweembe/ Hamachila	16.58	27.88	698	20	38	< 800

[z] meters above sea level

Table 2. Single factor effects of maturity and genotypic on macronutrient contents of roselle *(Hibiscus sabdariffa)* leaves.

Factor	Proximate analysis (%)[z]				
	Ash	Protein	Fat	Fibre	Carbohydrates
Maturity					
Immature	1.80 (0.08)[y]	3.27 (0.09)	0.37 (0.02)	3.78 (0.11)	6.86 (019)
Mature	2.68 (0.06)	3.17 (0.06)	1.31 (0.01)	4.79 (0.14)	9.27 (0.32)
Senescent	2.53(0.06)	2.77 (0.05)	0.27 (0.02)	22.6 (0.21)	2.30 (0.30)
LSD	*0.18*	*0.15*	*0.04*	*0.17*	*0.63*
Genotype					
ZM 5729	2.12 (0.17)	3.21 (0.09)	0.71 (0.16)	10.53 (3.16)	6.21 (0.95)
ZM 5738	2.46 (0.15)	2.83 (0.33)	0.60 (0.17)	10.91 (3.08)	6.25 (0.97)
ZM 5748	2.54 (0.11)	3.08 (0.05)	0.61 (0.17)	10.13 (3.09)	5.79 (1.39)
ZM 6839	2.19 (0.16)	3.16 (0.17)	0.61 (0.16)	10.01 (2.91)	5.74 (0.86)
LSD	*0.14*	*0.10*	*0.03*	*0.24*	*0.50*
Factor significance					
Maturity	≤0.001	0.002	≤0.001	≤0.001	≤0.001
Genotype	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001

[z] Expressed as fresh weight basis

[y] Figures in parenthesis represent the standard error (n= 3)

Table 3. Effect of maturity and genotypic on anti-nutrients and Vitamin C in roselle *(Hibiscus sabdariffa)* leaves.

Factor	Anti-nutrient and Vitamin C contents (% FW)		
	Alkaloids (mg/Kg)	Oxalates (mg/g)	Vitamin C (mg/Kg)
Maturity (Main plot)			
Immature	1.58 (0.68)[y]	2.19 (0.52)	243.10 (2.09)
Mature	4.58 (0.19)	1.33 (0.04)	290.37 (6.84)
Senescent	4.71 (0.18)	1.20 (0.04)	84.47 (2.33)
LSD	*0.11*	*0.02*	*2.03*
Genotype (Split plot)			
ZM 5729	3.07 (0.33)	1.69 (0.13)	206.04 (28.3)
ZM 5738	4.14 (0.62)	1.50 (0.11)	218.22 (35.99)
ZM 5748	3.75 (0.52)	1.60 (0.15)	209.42 (31.68)
ZM 6839	3.54 (0.58)	1.51 (0.23)	190.22 (28.83)
LSD	*0.07*	*0.02*	*0.93*
Factor significance			
Maturity	≤0.001	≤0.001	≤0.001
Genotype	≤0.001	≤0.001	≤0.001

[y] Figures in parenthesis represent the standard error (n= 3).

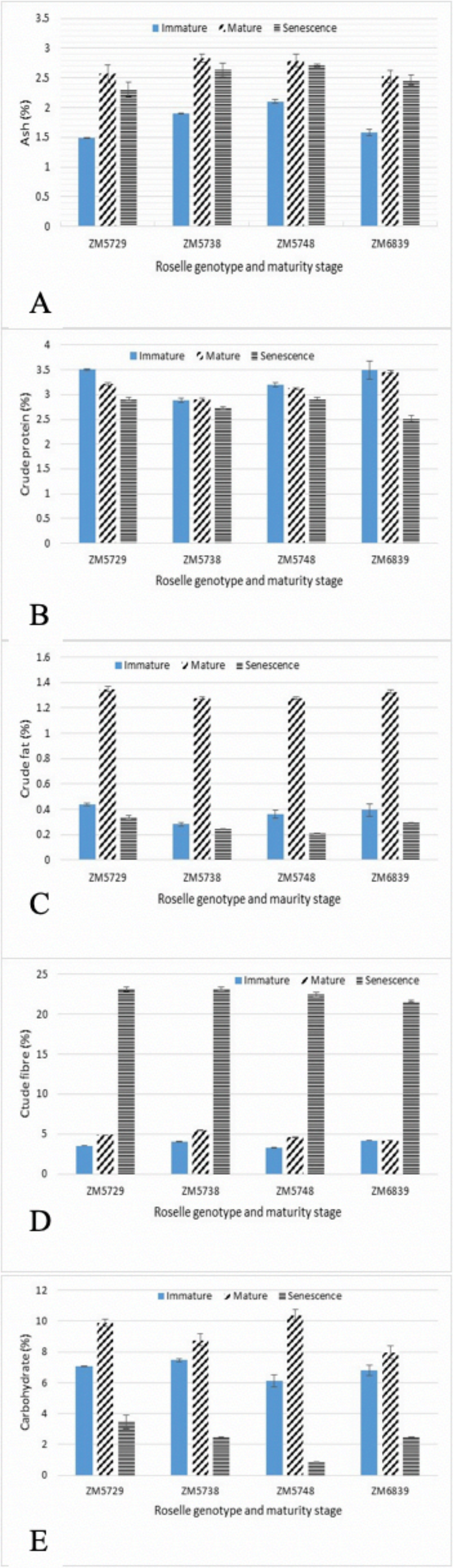


Figure 1. Interactive effects of maturity and genotypes on ash (A), crude protein (B), crude fat (C), crude fibre (D) and total carbohydrates (E) among the roselle *(Hibiscus sabdariffa)* genotypes.

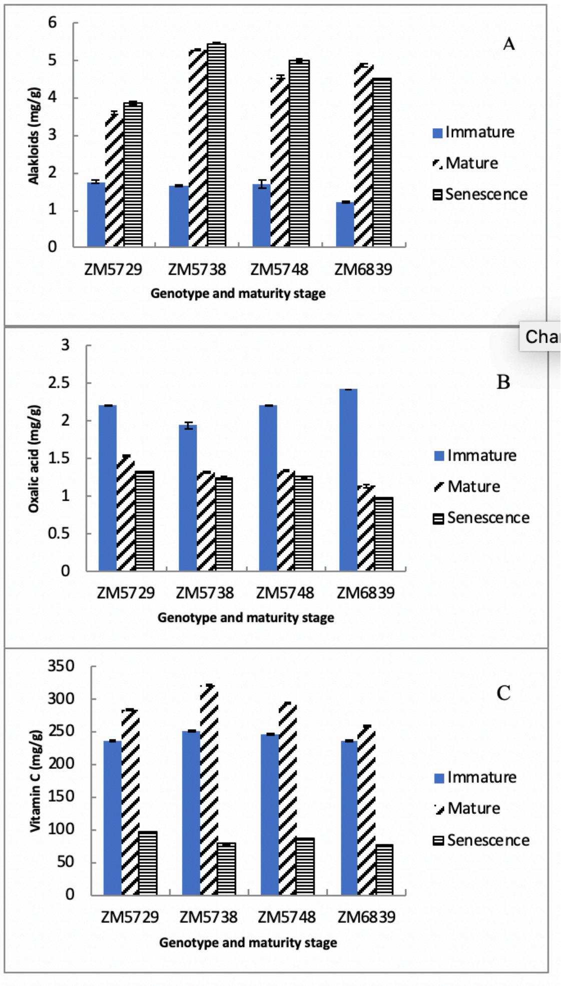


Figure 2. Changes in total alkaloids (A), oxalic acid (B), and vitamin C (C) due to maturity among different roselle *(Hibiscus sabdariffa)* genotypes

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