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Characterization of the Chemical and Phytochemical Profiles during Fruit Development and Ripening in Selected Cultivars of African Nightshade (*Solanum Nigrum Complex*) Edible Berries

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Abstract

Solanum nigrum complex is a green, indigenous leafy vegetable that grows in many parts of the world and its utilization can deliver more nutrients and phytochemicals into the diet. Even though it can help in alleviating the burden of hidden hunger, only the leafy part is utilized whereas the plant has edible berries. This study sought to address the problem of underutilization by looking at the benefits that can be derived from the berries. Four varieties of the plant were harvested through four stages; green, colour break, ripe and at senescence. Chemical analyses of the berries were done to determine the content and changes in macro and micro-nutrients and the phytochemical content of the berries as they ripened. Analysis of Variance (ANOVA) was used to determine the significant difference between nutrient and phytochemical composition of the different *S. nigrum* varieties at different ripening stages. The results show that fibre, ash and protein increased at senescence while carbohydrates decreased. Magnesium was the most abundant mineral. As berries ripened, oxalates, total phenols, flavonoids and phytates decreased while Vitamin C, tannins and total carotenoids increased. Conclusively, the berries in this study have comparable nutritional value with other commonly consumed fruits and could, therefore, be incorporated into the family pot.

Keywords: African nightshade, Berries, Ripening, Indigenous vegetables, Phytochemicals

1. Introduction

African nightshade (*Solanum nigrum L.* complex) is arguably one of the largest and most variant groups within the genus *Solanum* (Poczai et al., 2010). Originating from the New World tropics, particularly in South America, most of the variants are considered as invasive weeds especially in the Americas and Europe (Defelice, 2003; Poczai et al., 2010; Sarma & Sarma, 2011). In many African and Asian countries, African nightshade is a nutrient powerhouse and an important source of income for many small scale farmers (Ojiewo et al., 2013). It is popularly classified as one of the African Indigenous Vegetables (AIVs) which are reported to be key sources of micronutrients and phytochemicals (Mavengahama et al., 2013; Nyadanu & Lowor, 2015; Ojiewo et al., 2015). Besides strongly featuring as one of the food items that can help to meet the macro and micro-nutrient needs of the masses (Grubben et al., 2014; Ruel-Bergeron et al., 2015), its antimicrobial qualities have led to its application in managing respiratory tract infections and tonsillitis where its antibacterial activity has been proven with isolated bacteria (Matasyoh et al., 2014; Modilal et al., 2015).

Despite its documented benefits, only its leaves are used as vegetables (Mwai et al., 2007; Ojiewo et al., 2013). Its berries are not considered as a potential source of the micronutrients. Consequently, little or nothing has been studied on the nutrient composition and possible dietary applications of the African nightshade berries. This study sought to bridge this gap by characterizing the nutrient composition and phytochemical content of the berries. It considers how these are altered through the fruit development and ripening with a view of how these berries can be incorporated into the daily household diets so as to harness the optimum nutritional benefits of this crop.

2. Material and Methods

2.1 Sample Preparation

Field trials were conducted between February – May 2017 in the experimental field at the University of Eldoret. The Completely Randomized Design (CRD) was employed during planting on site (Bvenura & Afolayan, 2016). Four selected varieties of African nightshade were planted in the plots; Giant Nightshade (Simlaw seeds), Black Nightshade – local variety (Simlaw seeds), Improved variety (JKUAT), Agriculture variety (KARLO-Kakamega). Upon maturity at 12weeks, the berries in the four ripening stages were harvested on weekly basis, dried and then ground into fine powder. These were then stored in air tight containers awaiting laboratory tests.

2.2 Chemical Analyses

All the chemical analyses carried out in triplicates using standard Association of Official Analytical Chemists (AOAC) methods.

2.2.1 Determination of Proximate Composition

Protein analysis was carried out using the Kjeldahl method as outlined by AOAC Method

984.13. (Zhang et al., 2015). Crude fat content was determined using the Soxhlet extraction, AOAC Method 920.29. Crude fibre was analyzed using the AOAC Method 978.10 while ash determination was done using AOAC Method 923.03 (Codex Alimentarius Commission, 1999). Carbohydrate content was analyzed by the difference method (FAO/WHO, 1998).

2.2.2 Minerals

The concentration of Zinc (Zn), Calcium (Ca), Magnesium (Mg), Phosphorus (P), Iron (Fe), Sodium (Na) and potassium (K) was determined using the Atomic Absorption Spectrophotometry (UV-1800, Shimadzu Co-operation, Kyoto, Japan), AOAC Method 985.35 (Codex Alimentarius Commission, 1999).

2.2.3 Vitamin C

Vitamin C was determined using the HPLC (20A Series, Shimadzu Co-operation, Kyoto, Japan) AOAC Method 967.22 as described by (Koyuncu & Dİlmaçünal, 2010). The mobile phase was water adjusted to pH 3 with phosphoric acid and the separation carried out at flow rate of 0.4 ml/min and the UV detector set at 254 nm.

2.2.4 Phytochemicals

The phytochemicals that were analyzed included flavonoids, Total phenols, oxalates, Total carotenoids, phytates and tannins. These were analyzed as described by (Chandra et al., 2014; Jeyasree et al., 2014; Zhang et al., 2015). The HPLC system connected to a conductivity detector (elution rate 1.0 ml/min, mobile phase: A – deionized water, B – 200 mmol/l NaOH; gradient program 0 – 3 minutes: 87% A, 3 – 11 minutes: 50% A, 11.1 – 15 minutes: 87% A). Phytate standards Inositol (Sigma-P5681 MSDS) was used and to obtain the phytate value by matching 25 µl of the standard with retention time of peaks of the berry extracts based on calibrations curves of aqueous standards which contained 0.125% sulphuric acid.

Total phenols were determined using the formula by Zhang et al. (2015) where total phenolic concentration was then determined by measuring the absorbance at 765 nm using UV-Visible spectrophotometer and the values expressed as Gallic acid equivalents (GAE). Flavonoids were determined using the method by (Chandra et al., 2014). The absorbance of the reaction mixture was measured at 420 nm using UV-Vis spectrophotometer and the concentration expressed as mg quercetin equivalents (QE)/g. Quantification of condensed tannins was then done using spectrophotometry with (+)-catechin (upto 0.2 mg.ml) used as a reference standard and expressed as mg/g of (+)-catechin equivalents (CE). Total oxalates were determined in triplicate using the method described by Vũ et al. (2013)while carotenoid content was determined using the method described by Leong and Oey (2012) using UV-VIS at 450 nm.

2.2.5 Titrable Acidity (TTA)

TTA was determined using the method described by Sadler and Murphy (2010). The berries were crushed using mortar and pestle to obtain a slurry. About 2.5 g of the slurry was weighed out into a 100 ml beaker and 50 ml of distilled water added. The sample was then titrated with 0.1 N NaOH in the presence of phenolphthalein indicator until the end-point was

reached, signified by a change of indicator colour to pink. The volume (ml) of NaOH used was recorded and used in determining TTA using the formula:

% acid = (mls NaOH used) x (0.1 N NaOH) x (milliequivalent factor) x (100)/grams of sample

3. Results and Discussion

3.1 Proximate Composition

Table 1 shows the proximate composition of the berries as they ripened. The ash content did not differ significantly in all varieties at all stages. However, it was observed that within each variety, there was a slight decrease in the ash content from the green stage through to the ripe stage preceding a significant rise in the ash content from ripe to senescence stages. This trend in ash reduction with ripening was also observed in prickly pears (Barbera et al., 1992), though the values recorded were slightly lower than those recorded in this study. Fat content decreased through the ripening stages of the berries, a phenomenon also reported by (Barbera et al., 1992). This decrease could be explained by the effect of ripening on lipid metabolism (Balic et al., 2018) where the lipid membranes, specifically thylakoid membranes, are degraded to provide energy during the process of senescence. Variety differences could account for these disparities (Skrovankova et al., 2015).

With reference to crude fibre, three of the varieties (Giant nightshade, JKUAT variety and KARLO Agriculture) were within the same range. However, Black NS had significantly lower content, almost half of the content in the other varieties which could be attributed to the varietal differences (Skrovankova et al., 2015). It was observed that this trend continued through all the ripening stages albeit the fact that ripening did not seem to have a significant effect on the fibre. Carbohydrates were the most abundant of the berry components. Black NS emerged superior in carbohydrate content, significantly overtaking the other varieties at all stages of berry ripening. It was also observed that with the exception of Black NS, the other varieties showed a steady decrease in the carbohydrate content through the ripening stages. Black NS, however, showed a different trend where an increase was noted at the ripe stage. The trend in the protein content showed a steady increase through the four ripening stages for Giant NS and KARLO Agric varieties. Black NS and JKUAT varieties recorded an insignificant decrease from the green to colour break stage followed by a consistent increase in protein content to senescence. For all varieties, a major spike was observed between the ripe and senescence stages. Black NS had lower protein content compared to the other varieties at all stages but the difference was significant at ripe and senescence stages. The trend in protein content for the berries differed with that of Barbera et al. (1992) who noted a decrease in protein content of prickly pears, implying that berries had a different trend to pears during maturation.

Table 1. Proximate Composition (%) of S. nigrum berries at different ripening stages

Variety	Proximate				
	Ash	Crude Fibre	Oil	Carbohydrates	Protein
Green					
Black N.S.	6.41±0.26^{bcd}	10.06±0.56^{a}	3.39±0.12^{abcde}	72.02±1.56^{fg}	9.16±0.31^{abc}
Giant N.S.	7.64±0.16^{defg}	20.81±0.60^{d}	3.75±1.37^{cdef}	54.59±2.65^{abcd}	13.21±1.89^{cd}
JKUAT	8.57±0.45^{efgh}	21.43±1.82^{d}	3.24±0.14^{abcde}	55.94±1.86^{abcde}	11.81±1.39^{bcd}
KARLO Agric.	6.90±0.48^{bcde}	17.25±0.74^{cd}	3.64±0.83^{bcdef}	60.23±1.18^{cdef}	11.98±1.02^{bcd}
Colour Break					
Black N.S.	5.86±0.37^{abc}	12.16±1.18^{ab}	2.44±0.10^{abcde}	71.42±2.41^{efg}	9.01±1.15^{ab}
Giant N.S.	7.53±0.60^{cdefg}	17.37±2.22^{cd}	5.48±0.18^{fgh}	61.87±6.98^{cdefg}	14.82±2.83^{d}
JKUAT	7.71±0.85^{defg}	18.56±1.07^{cd}	4.18±0.02^{efg}	61.54±10.69^{cdef}	10.44±0.97^{abc}
KARLO Agric.	7.01±0.13^{bcdef}	16.89±1.04^{cd}	1.90±0.13^{abc}	60.23±2.58^{cdef}	12.67±1.17^{bcd}
Ripe					
Black N.S.	4.60±0.87^{a}	9.84±0.63^{a}	1.68±0.11^{a}	77.25±1.81^{g}	7.10±0.54^{a}
Giant N.S.	5.45±0.90^{ab}	16.21±3.41^{bc}	5.78±1.29^{gh}	57.54±6.09^{abcdef}	15.01±0.60^{d}
JKUAT	6.56±0.23^{bcd}	17.17±1.02^{cd}	2.05±0.01^{abcd}	59.10±3.86^{bcdef}	15.71±1.46^{d}
KARLO Agric.	5.77±0.38^{ab}	18.03±1.35^{cd}	1.74±0.04^{ab}	59.87±3.77^{cdef}	15.26±2.06^{d}
Senescence					
Black N.S.	5.84±0.58^{ab}	11.92±0.83^{ab}	2.37±0.50^{abcde}	64.70±1.91^{defg}	15.17±0.10^{d}
Giant N.S.	10.05±0.70^{h}	17.49±2.82^{cd}	3.99±0.63^{defg}	48.72±10.89^{abc}	25.75±1.98^{e}
JKUAT	8.61±0.15^{fgh}	19.10±0.93^{cd}	6.46±0.28^{h}	43.01±1.28^{a}	22.83±1.33^{e}
KARLO Agric.	9.03±0.68^{gh}	19.30±1.73^{cd}	5.52±0.44^{fgh}	44.16±5.21^{ab}	21.99±2.68^{e}

Values are Means ± Standard Deviation. Values with different superscript letters along the same column are significantly difference at p=0.05 as assessed by Tukey's significant difference

Table 2. Mineral Content (Mg/100g) of S. nigrum berries at different ripening stages

Variety	Minerals						
	Ca	Fe	K	Mg	Na	Zn	P
Green							
KALRO Agric.	42.51±3.89^{bcd}	1.02±0.08^{bc}	13.69±0.82^{a}	91.30±4.51^{abcd}	2.54±0.18^{b}	0.88±0.02^{def}	33.03±2.31^{bc}
Black N.S.	77.66±2.93^{g}	1.36±0.03^{def}	25.18±2.44^{bc}	177.50±10.26^{g}	2.20±0.19^{b}	1.10±0.08^{ghi}	40.73±1.14^{c}
Giant N.S.	46.86±2.30^{d}	0.77±0.03^{ab}	12.09±0.43^{a}	84.70±4.81^{abc}	1.32±0.04^{a}	0.40±0.07^{b}	22.36±1.32^{a}
JKUAT Impr.	42.59±0.58^{bcd}	0.69±0.04^{a}	8.21±0.18^{a}	73.30±5.30^{a}	2.78±0.28^{b}	0.76±0.06^{cde}	17.41±1.67^{a}
Colour Break							
KALRO Agric.	47.71±0.38^{de}	1.14±0.09^{cd}	22.48±0.66^{b}	111.00±9.02^{ef}	3.79±0.12^{c}	0.90±0.08^{efg}	60.01±2.94^{def}
Black N.S.	55.07±2.07^{ef}	1.40±0.03^{def}	30.16±0.60^{cde}	224.30±8.96^{h}	5.25±0.22^{e}	1.15±0.14^{hi}	60.99±1.81^{ef}
Giant N.S.	45.63±0.96^{cd}	1.32±0.05^{de}	26.69±0.21^{bcd}	90.80±3.62^{abcd}	2.23±0.26^{b}	0.62±0.05^{c}	91.46±4.68^{j}
JKUAT Impr.	57.93±3.31^{f}	0.78±0.04^{ab}	24.08±0.60^{b}	96.70±4.02^{bcde}	3.47±0.50^{c}	0.67±0.04^{cd}	31.84±2.06^{b}
Ripe							
KALRO Agric.	48.56±2.37^{de}	1.41±0.07^{def}	32.54±2.58^{e}	126.90±5.05^{f}	4.42±0.14^{d}	1.00±0.04^{fgh}	78.00±2.65^{hi}
Black N.S.	56.87±3.02^{f}	1.64±0.14^{f}	44.46±1.08^{f}	167.70±4.52^{g}	6.71±0.89^{f}	1.15±0.08^{hi}	82.80±0.44^{i}
Giant N.S.	37.84±0.59^{bc}	1.94±0.07^{g}	34.74±1.55^{e}	101.90±7.61^{cde}	3.43±0.26^{c}	0.76±0.03^{cde}	53.68±3.17^{de}
JKUAT Impr.	42.48±2.72^{bcd}	0.96±0.04^{abc}	35.22±1.58^{e}	108.50±8.93^{def}	4.84±0.14^{de}	0.73±0.03^{cde}	75.95±4.23^{hi}
Senescence							
KALRO Agric.	35.44±0.79^{ab}	1.55±0.02^{ef}	43.99±3.33^{f}	108.00±6.42^{def}	6.36±0.34^{f}	1.47±0.07^{j}	52.00±3.01^{d}
Black N.S.	62.00±4.21^{f}	2.49±0.20^{h}	63.21±3.13^{h}	259.90±11.03^{i}	6.45±0.30^{f}	0.83±0.09^{cdef}	65.16±1.24^{fg}
Giant N.S.	34.94±2.27^{ab}	1.64±0.12^{f}	31.21±1.05^{de}	86.40±2.96^{abc}	6.45±0.10^{f}	1.30±0.05^{ij}	57.26±3.30^{def}
JKUAT Impr.	29.69±2.03^{a}	1.01±0.11^{bc}	53.93±3.21^{g}	77.40±4.60^{ab}	4.40±0.08^{d}	0.06±0.08^{a}	73.23±4.44^{gh}

Values are Means±Standard Deviation. Values with different superscript letters along the same column are significantly different at p=0.05 as assessed by Tukey's significant difference

3.2 Minerals

The different varieties showed different trends with reference to calcium content. Nevertheless, the values recorded (Table 2) were comparable to those obtained in other works (Sarma & Sarma, 2011). Black nightshade, which had the highest content at the green stage, recorded a sharp decrease at colour break which then remained steady at ripe stage. This was also the only variety to record an increase in calcium content from the ripe stage to senescence, though this was still lower than the calcium content at the green stage of this veriety. Black nightshade also reigned superior over the other varieties in iron content having significantly higher values at all stages, with the highest value of 2.49 Mg/100g at senescence and a low of 1.36 Mg/100g while green.

These values were slightly lower than the average value of 3.8 Mg/100g reported by other worker (Sarma & Sarma, 2011). Remarkably, nearly all varieties showed a similar trend in the iron content through the four stages of ripening, recording iron content at senescence which was significantly higher than the content at the green and colour break stages. A similar trend was again observed in the potassium content. Black nightshade was superior to the rest, recording a range of 25.18 – 63.21 Mg/100 g while JKUAT variety had the least values while green at 8.21 Mg/100g. These values are in the range reported by (Sarma & Sarma, 2011). Magnesium was by far the most abundant mineral in the berries across all varieties with black nightshade emerging the most loaded with magnesium at all the stages of ripening. All the varieties showed a dip in the content to nearly match the values recorded at

the green stage. The high content of magnesium in the berries is of nutritional importance because of the dietary minerals, magnesium is the fourth most abundant and the second most abundant intracellular cation (Volpe, 2013). It is an important co-factor in over 300 metabolic reactions (Emila & Swaminathan, 2013; Laires et al., 2004; Long & Romani, 2015). In terms of distribution, 50% of magnesium is in the bones while about 49% is in the tissue and organs (Baaij et al., 2015).

Changes in sodium content in the ripening berries showed a very close similarity in all the varieties. From the green to the ripe stage, all the varieties showed a steady increase in sodium content to record the highest concentration at senescence. Zinc values were way lower than those reported by Sarma and Sarma (2011) and this could be attributed by the genotypic differences between the varieties and other growth parameters (Valadon et al., 1975). Phosphorus was present in substantial quantities in all of the varieties and did not vary much with ripening.

The trends observed in this study are consistent with findings from previous studies on berry ripening. Tosun et al. (2008) concluded that potassium, calcium and zinc changed moderately during ripening but magnesium, copper and iron presented significant changes throughout the ripening. The most significant change was observed in magnesium because it is a central atom of the chlorophyll molecule (Marschner, 1995) and its insertion into the porphyrin structure is the first step in chlorophyll biosynthesis. As the fruits develop, calcium content of the cell wall increases to the fully grown but immature stage then this drops as the binding form of calcium in the tissue changes just before ripening (Tosun et al., 2008). A similar trend is observed in magnesium. However, after ripening, the two minerals again accumulate in the cell wall and this could explain the fluctuations that were observed in the content of the two minerals in the berries. Conclusively, the mineral content of the berries is comparable to other berries and fruits (Bvenura & Afolayan, 2016; Rop et al., 2011) and this could imply that the nightshade berries, if incorporated into the diet, can help to meet the nutritional needs of the consumers using a diversified diet.

3.3 Phytochemicals

Table 3. Phytochemical Content (Mg/g) of S. nigrum berries at different ripening stages

Variety	Total Phenols	Tannins (Mg/100g)	Flavonoids	Oxalates	Phytates
Green					
KALRO Agric.	0.95±0.01^{i}	8.18±0.47^{ef}	2.47±0.03^{de}	9.18±0.34^{fg}	5.53±0.40^{g}
Black N.S.	0.77±0.03^{fg}	4.43±0.31^{abc}	7.20±0.27^{i}	9.84±0.87^{fg}	3.91±0.08^{f}
Giant N.S.	0.84±0.04^{gh}	4.50±0.18^{abc}	2.51±0.11^{e}	11.87±0.14^{h}	5.35±0.46^{g}
JKUAT Impr.	0.42±0.01^{ab}	7.16±0.34^{cdef}	1.26±0.13^{bc}	12.16±0.32^{h}	4.26±0.21^{f}
Colour Break					
KALRO Agric.	0.46±0.02^{bc}	5.80±1.22^{bcde}	3.19±0.09^{f}	8.60±0.52^{def}	3.16±0.06^{e}
Black N.S.	1.20±0.01^{j}	16.76±0.70^{h}	1.31±0.05^{bc}	9.48±0.52^{fg}	1.10±0.05^{cd}
Giant N.S.	0.39±0.00^{a}	8.11±1.58^{ef}	2.17±0.03^{d}	10.16±0.10^{g}	3.12±0.13^{e}
JKUAT Impr.	0.49±0.06^{cd}	11.50±0.51^{g}	2.18±0.09^{d}	8.92±0.49^{efg}	2.88±0.05^{e}
Ripe					
KALRO Agric.	0.53±0.02^{de}	20.97±0.48^{i}	1.47±0.04^{c}	7.39±0.35^{cd}	0.88±0.03^{bc}
Black N.S.	1.30±0.01^{k}	2.41±0.60^{a}	0.76±0.02^{a}	6.13±0.13^{bc}	0.85±0.40^{abc}
Giant N.S.	0.75±0.01^{f}	5.84±1.79^{bcde}	4.55±0.15^{g}	5.84±0.43^{ab}	1.43±0.07^{cd}
JKUAT Impr.	0.82±0.01^{gh}	22.64±1.64^{i}	5.02±0.13^{h}	7.83±0.76^{de}	0.86±0.02^{abc}
Senescence					
KALRO Agric.	0.70±0.01^{f}	8.84±1.47^{fg}	1.12±0.05^{b}	5.60±0.06^{ab}	0.39±0.05^{ab}
Black N.S.	0.87±0.01^{h}	5.13±0.31^{abcd}	1.05±0.01^{ab}	5.63±0.20^{ab}	0.26±0.01^{a}
Giant N.S.	0.58±0.01^{e}	7.62±0.38^{def}	1.52±0.01^{c}	5.23±0.16^{ab}	1.55±0.07^{d}
JKUAT Impr.	0.73±0.01^{f}	3.80±0.12^{ab}	1.33±0.01^{bc}	4.64±0.52^{a}	0.39±0.03^{ab}

Values are Means ± Standard Deviation. Values with different superscript letters along the same column are significantly difference at p=0.05 as assessed by Tukey's significant difference

The oxalate values reported in this study (Table 3) were similar to those reported for kiwi fruits (Vũ et al., 2013). The concentration of oxalates was higher in Giant nightshade and JKUAT varieties at the green stage. As the fruits started ripening, the concentration in all the varieties started decreasing and maintained this trend to senescence, a trend also reported by (Vũ et al., 2013) who observed that green kiwi fruits had higher content than golden kiwi fruits. The low levels implies that intake of African nightshade berries in the diet would not increase the risk of formation of kidney stones (Massey, 2007).

Phytate content in the berries was low, ranging from 0.26 to 5.53 Mg/g with the highest values recorded at the green stage followed by a downward trend in concentration as the berries ripened. This concentration levels are within the range of the contents recorded in Brazilian fruits (Marin et al., 2009). This low levels make the berries a good addition to the diet as they ensure there is greater availability of minerals such as calcium, iron and zinc which would otherwise be bound within the phytic complexes, making them non-bioavailable (Ellis et al., 1987; Saha et al., 1994). Tannin values were quite low compared to those reported in 18 Brazilian fruits (15.7 mg/100g in Cagaita to 472.2 mg/100g in Baru nut) (Marin et al., 2009). Since the tannin content is affected by soil composition and other

environmental factors (Davidsson, 2003), these could have led to the disparities observed in this study.

The levels of total phenols were quite varied amongst the varieties. At the green stage, black nightshade did not differ much from the other varieties but the continuous rise in phenolic levels saw it emerge superior at the other ripening stages. The decrease recorded as the fruits could be due to increase in polyphenol oxidase activity (Rop et al., 2011). A similar trend in different strawberry varieties was observed by other workers (Aaby et al., 2012) where phenolic content varied amongst cultivars but with minor changes in berries of the same cultivar as they ripened. The total phenolic content varied from 0.39 (Giant nightshade at colour break) to 1.30 mg/g (ripe Black nightshade), a range that corresponded to that reported in strawberry at 0.57 to 1.33 mg/g (Aaby et al., 2012). Other reports indicate that the phenolic content in berries increased in berries until the fruits set after which the content decreased gradually (Cheng & Breen, 1991). However, they noted that even though there was a continuous decrease in the concentration of total soluble phenols, the amount steadily increased and this could explain why the berries in this study had a high amount of phenols at senescence.

Flavonoids and phenols are directly related to the antioxidant activity of berries (Wang et al., 2009) and as such, their concentration in these berries is of interest to the consumers. Flavonoid concentration had a similar trend in KARLO agriculture and JKUAT varieties. The content increased as the berries started breaking their colours and then decreased through the last stages of development. Amongst the varieties, black nightshade posted the highest value while green (7.20 Mg/g) but also posted the least value (0.76 Mg/g) when ripe. Given this trend, it can be concluded that the best antioxidant capacity of the berries can be attained at colour break, which is in agreement to the conclusion by Jain et al. (2003).

3.4 Vitamin C, TTA and Total Carotenoids

Results for Vitamin C, TTA and total carotenoids are as indicated on Table 4. Vitamin C concentration was comparable amongst all varieties at the green, ripe and colour break stages. Though the values in this study were lower than those recorded in medlar fruits (Rop et al., 2011), they fall within the range of 5 to 50 mg/100 g vitamin C recorded in strawberries and raspberries (Skrovankova et al., 2015), which are termed as the richest sources of vitamin C amongst berries. The trend in concentration at all stages was also similar with all varieties manifesting an increase in concentration through the ripening stages, which affirms the position by Wang et al. (2009) that immature berries contain lower levels of acids than ripe berries.

An inverse trend was observed in the percentage of titrable acidity. This reduced significantly in all the varieties as they ripened. Levels in this study are comparable to those reported by (Tosun et al., 2008) who recorded TTA of blackberries in the range of 5.78% (ripe) to 14.80% (red). The decrease in acidity as the berries ripen is consistent with the findings by Balic et al. (2018) and Barbera et al. (1992) who recorded a decrease in acidity of grape berries and prickly pears, respectively, as they ripened. This decrease as berries ripen could be due to the effects of respiration or conversion of organic acids into sugars (Tosun et al., 2008).

Table 4. Vitamin C (Mg/100g), TTA (%) and Total Carotenoids (Mg/100g) contents of S. nigrum berries at different ripening stages

Variety	Total Carotenoids	TTA	Vit. C
Green			
KALRO Agric.	0.20±0.10^{c}	15.92±0.49^{g}	1.55±0.03^{a}
Black N.S.	0.23±0.01^{c}	3.76±0.15^{b}	3.91±0.04^{ab}
Giant N.S.	0.16±0.00^{b}	5.30±0.45^{c}	3.57±0.15^{ab}
JKUAT Impr.	0.09±0.01^{a}	9.76±0.74^{e}	3.25±0.05^{ab}
Colour Break			
KALRO Agric.	0.28±0.01^{d}	11.74±0.75^{f}	2.13±0.05^{a}
Black N.S.	0.21±0.01^{c}	3.52±0.26^{b}	7.04±0.15^{abc}
Giant N.S.	0.49±0.01^{g}	3.89±0.05^{b}	5.34±0.07^{ab}
JKUAT Impr.	0.37±0.01^{e}	9.25±0.26^{e}	4.39±0.11^{ab}
Ripe			
KALRO Agric.	0.28±0.00^{d}	9.69±0.12^{e}	4.05±0.03^{ab}
Black N.S.	2.29±0.01^{i}	3.07±0.06^{ab}	11.63±0.23^{bcd}
Giant N.S.	0.38±0.01^{e}	3.55±0.17^{b}	4.51±0.01^{ab}
JKUAT Impr.	0.34±0.01^{e}	6.63±0.40^{d}	5.12±0.03^{ab}
Senescence			
KALRO Agric.	0.42±0.01^{f}	7.58±0.24^{d}	8.12±0.16^{abc}
Black N.S.	1.10±0.03^{h}	2.16±0.01^{a}	16.22±0.32^{cd}
Giant N.S.	0.23±0.01^{c}	3.14±0.05^{ab}	18.63±0.47^{d}
JKUAT Impr.	0.27±0.02^{d}	5.29±0.41^{c}	9.45±0.01^{abcd}

Values are Means±Standard Deviation. Values with different superscript letters along the same column are significantly difference at p=0.05 as assessed by Tukey's significant difference

The content in carotenoids increased from the green to colour break stages, with the exception of black nightshade which recorded a decrease at this stage. The concentration then increased to ripe stage and then started reducing as the berries reached senescence. This observation is consistent with an earlier study done by Valadon et al. (1975) who observed that carotenoids of berries increase on ripening as does the content in guavas (Jain et al., 2003). This is attributable to the fact that as fruits ripen, chlorophyll disappears and chloroplasts degenerate to chromoplasts. Control of carotenoid synthesis is removed and oxidative processes take place leading to appearance of different carotenoids (Valadon et al., 1975).

4. Conclusion

The content of oxalates, total phenols, flavonoids and phytates decreased as the fruits ripened while the content of Vitamin C, tannins and total carotenoids increased with ripening. Since the berries are consumed ripe, it is evident that ripening reduces the anti-nutritional factors while enhancing the much needed vitamin C. Black nightshade had significantly higher carotenoid content compared to the other varieties. This higher content gave the berries an orange-yellow color while all other varieties were purple-black. It also had lower percentage of titrable acidity. Though different amongst different varieties, berries of the African Nightshade have macro and micro-nutrient content that is comparable to other berries and fruits. Given their nutritional value which is largely consistent with other commonly consumed berries, the African nightshade berries should be incorporated in the diet through dietary diversification to enhance optimal utilization of this popular indigenous plant.

Ethical Statements

Declaration of Conflicting Interests: The Authors declare that there is no conflict of interest

Ethical Review: The study does not involve any human or animal testing.

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