Sugar Content and Physical Characterization of Four Selected African Nightshade (Solanum nigrum) Edible Berries

Elijah H. Kamau, Julius M. Mathara, and Glaston M. Kenji

***Abstract*—Fruits constitute a major part of the diet in many parts of the world, highly recommended for the nutritional value derived from them. Fruit maturity is an important determinant of the quality as it affects the appearance, an aspect of quality considered by most consumers. Sugar content, colour, size and firmness are some of the quality indicators associated with maturity. While the ripening and maturity indicators are well documented for climacteric fruits, non-climacteric fruits such as berries lag behind. African nightshade (Solanum nigrum L.) edible berries are among them. This study evaluated the quality parameters of the edible berries of four varieties of African nightshade and found out that they accumulate glucose and fructose as they ripen with glucose being the most abundant sugar. Sucrose is only present during the senescence stage. Size remained relatively constant within each variety while firmness decreased progressively after veraison. Black NS differed with the others in colour besides fructose and sucrose content at 29.35 and 388.40 mg/100g, respectively. Giant NS recorded the highest glucose content at 172.44 mg/100g when ripe. Conclusively, the African nightshade berries are characteristically similar to other non-climacteric fruits adopted as part of the normal diet and should be considered as a valuable addition to the diet.**

***Index Terms*—Berries, African nightshade, Ripening, Firmness, Colour.**

I. Introduction

Fleshy fruits are classified into climacteric and non-climacteric types and they are important worldwide crops accounting for a major fraction of the world's agricultural output as well as a major component of the diet [1]. Quality of fresh fruits and their products is a function of a combination of various attributes which give value in terms of human food [2]. The importance of each of the quality attributes is determined by the commodity and its intended use and this often varies amongst producers, handlers and consumers. These qualities include but are not limited to appearance, firmness and shelf-life [2-3]. Final fruit quality and storage life are largely affected by the fruit maturity at harvest [2].

Maturation is the developmental stage leading to attainment of physiological maturity [4-5]. Immature fruits are of an inferior quality since they are more prone to mechanical damage and shriveling besides having an inferior flavor [3]. Overripe fruits, on the other hand, are likely to be soft and mealy with an insipid flavor. Proper timing during fruit picking is important as early or late picking can lead to physiological disorders during storage [3].

For most fruits, advances in maturity are accompanied by several coordinated physiological, biochemical and structural processes [1,4,6]. These processes lead to changes in colour, size and chemical composition of the fruits. Various studies have addressed the metabolism of sugars and changes in biochemical and physiological properties in apples [7], sea buckthorn fruits [8-9], strawberries [1], pomegranate [4,10-11] and grapevines [12-14] among others. These studies documented the increase in size, change in skin colour, softening and changes in sugar fractions of the respective fruits.

Despite these advances in understanding fruit ripening, Jia et al. [1] observed that the regulation of ripening in the fleshy fruits is not well understood while [13] opined that control of berry ripening is still not well documented especially for the non-climacteric fruits. This is also true for the edible berries of African nightshade (*Solanum nigrum L.*) which, for long, have not been explored as a food item [15]. The documented information has described the crop as a leafy vegetable and therefore the fruits have not been given much attention. Few studies, however, have analyzed specific parts of the African nightshade berries such as the seeds and skin [16] and proven that they have a nutritional value that is comparable to other fruits that are regularly consumed in the diet. However, these studies were not controlled, the berries were mainly harvested in the wild and they did not address the changes during berry development and ripening. This study addresses this knowledge gap by using controlled trials to document the changes in the African nightshade berries during the ripening process, keen to evaluate the possibility of incorporating them in the household diet.

II. MATERIALS AND METHODS

A. Sample Preparation

Four varieties of the African nightshade (Solanum nigrum L.) were planted in the experimental field at the University of Eldoret. The Completely Randomized Design (CRD) was employed during planting on site as described by Bvenura and Afolayan [17]. There were four (4) blocks each subdivided into three (3) subplots to allow for three replications. Within the main blocks, there were subplots

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about 4 m by 2 m in size. These were separated by margins of about 1m within the replicate blocks. The distance between blocks was about 1.5 m. Four selected varieties of African nightshade were planted in the plots. These comprised of Giant Nightshade (Simlaw seeds), Black Nightshade – local variety (Simlaw seeds), Improved variety (JKUAT), Agriculture variety (KARLO-Kakamega). These were planted with a spacing of about 60 cm by 45cm. Drip irrigation was installed within the plots and appropriate crop husbandry was employed till the berries ripened at 12 weeks. Berries for the analyses were picked at four ripening stages; green, colour break, ripe and at senescence.

B. Sugars

Sugars were analyzed as described by Ribera-Fonseca, Noferrini, Jorquera-Fontena and Rombola [18] using the high performance liquid chromatography (HPLC). The samples were prepared by grinding about 10 g of the berries and placing this in a 100 ml conical flask. This was followed by addition of 50 ml of 96% ethyl alcohol. After refluxing this mixture for 1 hour at 1000C, the slurry was filtered and the conical flask rinsed with 5 ml of 80% ethyl alcohol. This filtrate was then evaporated at 600C to attain dryness before being dissolved in 10 ml of distilled water. From this solution, 2 ml was taken and combined with 2 ml acetonitrile. This was then filtered through a 0.45 micro-filter and placed in vials, ready for injection into the HPLC system.

C. Firmness

Berry firmness was determined as described by Lee and Lee [19] using the Rheometer (Compac-100, Sun Scientific Co., Japan). The mode was set at 20 with maximum load of 10 kg and R/H hold of 2.0 mm and the P/T press was set at 300 mm/min. For each ripening stage of each variety of African nightshade, the measurements were taken in 5 replicates.

D. Size

Berries' seizes were determined using the method described by Li et al. [20] using Vernier calipers. Fruits were chosen at random from each variety at each stage of ripening with the fruits in the mid-upper parts of the shoot being the most preferred. This measurements were taken 5 times for each variety at each of the four ripening stages and the mean values obtained.

E. Colour

Berry colour was determined using the method by Leite et al. [21]. This was done using a colour spectrophotometer (Model CR – 200 – Minolta, Japan). The parameters L* for lightness, a* for redness and b* for yellowness were recorded with a white tile used as a reference. All the values were taken in triplicate.

F. Statistical Analyses

Data was analyzed using the one way Analyses of Variance (ANOVA) using Genstat Version 14. The means were separated using Tukey's significance difference test using a probability value of $P \leq 0.05$.

III. RESULTS AND DISCUSSIONS

A. Sugars

Berry sugars observed a rapid accumulation in the last stages of ripening which could be associated with up-regulation of sugar-related genes such as cell wall invertases, hexose transporters and vacuolar invertases [6, 22]. The content of fructose in most of the berries increased from the green to ripe stage and then observed a decline. KARLO Agric. showed the sharpest decline at this stage. Black NS was exceptional from this trend as the content peaked at colour break, decreased at the ripe stage and then rose again at the ripe stage (Table 1). Nevertheless, the content at the senescence was statistically similar to the content at colour break, which was the highest recorded of the varieties. This is an indication that Black NS was clearly superior in terms of fructose content compared to the other varieties. There was a continuous, steady increase in glucose content for all the varieties from the ripe stage through to senescence. Only JKUAT variety showed a decrease in the glucose content at senescence, though this was not statistically significant from the ripe stage which recorded the highest content for this particular variety. Glucose content was very low at the green and colour break stages for all the varieties. However, on hitting the ripe stage, the content observed a major spike. Compared to the other varieties, KARLO Agric was significantly inferior in glucose content, scoring the highest value of 22.20 mg/100g at senescence compared to Giant NS' 172.44 mg/100g at the same stage.

TABLE 1: DEVELOPMENTALLY-RELATED CHANGES IN SUGARS (MG/100G) IN FOUR SELECTED VARIETIES OF AFRICAN NIGHTSHADE *(SOLANUM NIGRUM L.)* EDIBLE BERRIES

Variety	**Fructose**	**Glucose**	**Sucrose**
Green			
KALRO Agic.	17.00±0.62^{de}	0.25±0.00^{a}	ND
Black N.S.	24.21±0.18^{hi}	3.28±0.02^{a}	ND
Giant N.S.	14.99±0.46^{c}	2.78±0.05^{a}	ND
JKUAT Impr.	16.29±0.70^{cd}	2.38±0.03^{a}	ND
Colour Break			
KALRO Agric.	18.74±0.53^{ef}	11.74±0.07^{a}	0.60±0.01^{a}
Black N.S.	30.65±0.20^{k}	8.95±0.14^{a}	2.00±0.06^{a}
Giant N.S.	21.23±0.94^{g}	7.76±0.28^{a}	ND
JKUAT Impr.	16.76±0.07^{d}	2.38±0.07^{a}	0.70±0.01^{a}
Ripe			
KALRO Agric.	27.03±1.13^{j}	20.17±0.70^{a}	252.3±8.50^{d}
Black N.S.	25.79±0.45^{ij}	156.29±62.72^{bc}	283.8±6.42^{f}
Giant N.S.	29.36±0.66^{k}	165.13±5.13^{c}	267.8±0.32^{e}
JKUAT Impr.	20.07±0.61^{fg}	125.07±3.85^{bc}	261.0±11.06^{de}
Senescence			
KALRO Agric.	13.15±0.66^{b}	22.20±0.37^{a}	66.00±2.58^{b}
Black N.S.	29.35±0.42^{k}	161.45±7.00^{c}	388.40±7.40^{g}
Giant N.S.	23.94±0.22^{h}	172.44±3.79^{c}	211.30±3.90^{c}
JKUAT Impr.	9.43±0.29^{a}	111.38±3.42^{b}	206.20±3.17^{c}

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
ND = Not detected

The trends in reducing sugars in this study agree with previous works. A study on seabuckthorn berries indicated that glucose and fructose constituted nearly the whole of sugar fraction in the berries [23]. However, glucose was the major sugar. Cultivars showed different trends in sugars with ripening though at the end, all of them recorded decreasing sugar levels. The variability in sugar accumulation among different genotypes was also reported by [8].

In another study, juice made from the pomegranate fruits contained 12-16% sugar mainly comprising of fructose and glucose with the latter being more predominant [10,11]. A similar observation was made by Fawole and Opara [24] where fructose and glucose increased during maturation but with ratios of glucose to fructose (G/F) ranging from 0.67-0.85 to 0.72-0.86 in Bhangwa and Ruby fruits grown in South Africa, respectively. Al-maiman and Ahmad [10] further agreed by reporting that in 'Taifi' cultivar of pomegranate, glucose levels were higher than fructose at the unripe, half-ripe and full ripe stages of development. Davies and Robinson [25] indicated that during the ripening of grape berries, sucrose that is transported from the leaves accumulates in the vacuoles of the berries (sink cells) as glucose and fructose and this could explain the influx of the two sugars during fruit development.

No sucrose was detected at the green stage for all the varieties but traces were recorded at colour break for all the varieties except Giant NS. This was followed by a major spike at the ripe stage with the varieties recording values in the range of 252 – 283mg/100g. At senescence, there was a significant decrease in sucrose content for three of the varieties with the exception of Black NS which continued to record a significant rise in the content, peaking at 388 mg/100g at senescence, the highest value recorded for the sugars. Sucrose accumulation in the current study showed a trend where there was little or no traces in the first stages of development but observed a major hike during the senescence stage (overripe).

This increase in sucrose is a deviation from previous studies on berries which did not report on the increase in sucrose. Besides the fact that sucrose is a major sugar in the fruit development [7,14], this phenomenon could also be explained by the fact that sucrose is an important signal in the regulation of berry ripening as demonstrated by Jia et al. [1]. This study revealed that apart from the traditional understanding of the role of sugars in plants where they are metabolic resources for carbon skeleton construction and energy sources, sugar signaling could also be involved in other functions such as seed germination, embryogenesis, vegetative and reproductive growth as well as senescence [1,26].

In the sink cells, the sucrose cycle is, also known as the sucrose-sucrose cycle or the futile sucrose cycle, is an important pathway [7]. The cycle involves the action of sucrose synthase and invertase for the liberation of hexoses (glucose and fructose). These hexoses are then phosphorylated and there is interconversion between hexose phosphates and UDP-glucose. Finally, there is the re-synthesis of sucrose through the enzymes sucrose-6-phosphate synthase (SPS) and sucrose-6-phosphate phosphatase [7,14,26]. This cycle allows for connection of sugar with other metabolic pathways such as the glycolysis, TCA cycle, starch and cellulose synthesis [7]. Since the biochemical regulation of the sucrose-sucrose cycle and the associated transport system is not fully understood [7], it can be hypothesized that the accumulation of sucrose in the African nightshade berries at senescence is as a result of under-regulation of sugar related enzymes and genes during deterioration at senescence. Consequently, the sucrose from the leaves is not converted to glucose and fructose and the sucrose-sucrose cycle stops. As such, sucrose accumulates in the sink cells in its initial form. Given that sugars in fruits are not only essential for fruit growth and development but also for the overall quality of the fruit [7], more studies could be carried out to investigate the accumulation of sucrose during senescence in Solanum nigrum edible berries.

B. Colour

Fruit colour is one of the appearance aspects that consumers use to evaluate for quality [2]. The colour characteristics of the berries were as indicated in Table 2. KARLO Agric., Giant NS and JKUAT varieties had similar colour characteristics in nearly all the stages of development.

High L* values were recorded at the green stage but decreased as they ripened. Black NS, however, showed a different trend where the L* value was small at the green stage but increased continuously to senescence. The a* value increased for all the varieties although Black NS still recorded higher values compared to the rest. KARLO Agric., Giant NS and JKUAT varieties had the highest a* values at colour break and then the value decreased to 1.57 to 1.77 while the value for Black NS increased to the last stage, reaching 46.23. These changes in colour are due to accumulation of anthocyanins in the berries [27] while the differences in colour parameters could be attributed to the fact that while all the varieties were green while unripe, Black NS acquired a bright orange colour upon ripening while all the other varieties acquired a purplish-black colour. This could have an impact on the acceptability of the berries by consumers [2, 28].

TABLE 2: COLOUR CHANGES IN 4 VARIETIES OF AFRICAN NIGHTSHADE EDIBLE BERRIES WITH RIPENING

Variety	L*	a*	b*
Green			
KALRO Agric.	66.47±19.19^{d}	-12.53±0.93^{ab}	1.55±1.36^{a}
Black N.S.	32.23±6.41^{ab}	-10.9±0.56^{b}	15.7±2.26^{b}
Giant N.S.	53.77±4.04^{cd}	-17.57±0.38^{a}	27.13±0.72^{cd}
JKUAT Impr.	44.53±1.66^{abc}	-14.77±2.60^{ab}	21.13±2.80^{bc}
Colour Break			
KALRO Agric.	38.8±0.75^{abc}	-1.27±0.31^{c}	4.57±0.95^{a}
Black N.S.	41.63±0.83^{abc}	4.47±1.16^{d}	33.97±1.04^{d}
Giant N.S.	35.7±1.15^{abc}	2.60±1.40^{cd}	3.90±3.30^{a}
JKUAT Impr.	65.63±13.82^{d}	-12.33±5.33^{b}	21.77±8.78^{bc}
Ripe			
KALRO Agric.	28.20±2.89^{d}	3.83±0.49^{d}	2.07±0.72^{a}
Black N.S.	49.40±0.40^{bcd}	15.60±0.50^{e}	43.27±1.14^{e}
Giant N.S.	33.20±0.70^{ab}	5.20±0.53^{d}	4.53±3.61^{a}
JKUAT Impr.	29.03±1.81^{a}	3.63±1.15^{cd}	2.07±0.21^{a}
Senescence			
KALRO Agric.	28.23±0.37^{a}	1.87±1.26^{cd}	1.77±0.55^{a}
Black N.S.	52.80±0.44^{cd}	21.60±0.20^{f}	46.23±0.32^{e}
Giant N.S.	28.80±1.35^{a}	2.57±0.72^{cd}	1.67±0.49^{a}
JKUAT Impr.	27.33±0.40^{a}	0.87±0.15^{cd}	1.57±0.45^{a}

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

C. Firmness

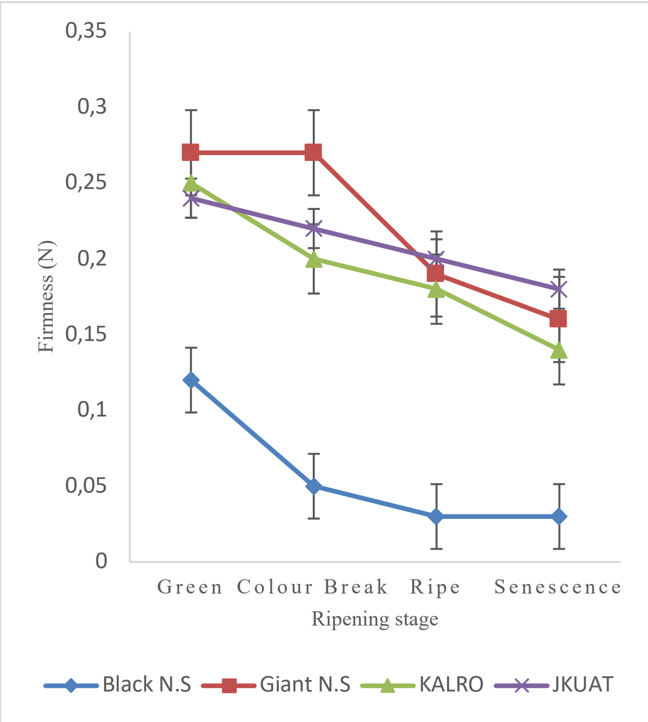

Fig. 1: Changes in berry firmness of four varieties of *Solanum nigrum* against the ripening stages

Fruit ripening is achieved through chlorophyll degradation and cell wall lysis [29]. During ripening, fruit softening is a function of coordinated processes of modification of the polysaccharides making up the cell wall and the middle lamella, leading to the weakening of the structure [30] as well as an increase in ethylene production [31]. In this study, all the four varieties recorded a decrease in firmness from the green stage to senescence. Giant NS had the most firm fruits at colour break. However, it was observed, as shown in Figure 1, that Giant NS, KALRO Agric and JKUAT varieties were not significantly different in firmness of their fruits at the green, ripe and senescence stages. Black NS was significantly different from the other varieties at all stages, recording the lowest firmness values of all the varieties at all stages. This is consistent with the conclusion by Brummell et al. [32] that softening and textural changes that occur during ripening are characteristic of particular species. For all the varieties, fruit softening was initiated at veraison and remained on a downward trend to senescence, a trend that was also reported [32] while studying the cell wall changes in ripening of peach fruits.

Trends observed in this study correspond with previous related studies. Castelllarin and others [12] studied the changes in grapes and concluded that berry firmness reached peak at full grown but unripe stage and then decreased and remained on a stable decline upto the end of the study. This study showed that majority of the softening in grapes seemed to take place just prior to the change of colour. The possible reason for the softening could be cell wall modification [13]. While analyzing the changes in firmness of grape berries during ripening, Nunan and colleagues [33] observed that at veraison, there was a rise in the protein content and an approximate 3-fold increase in the hydroxyproline content.

Of the pectic polysaccharides, type 1 arabinogalactan decreased by about 80% from 20 mol% to 4 mol% of total cell wall. Galacturonan increased by almost 2-fold and became more soluble [33]. This is further corroborated by other workers [32] who concluded that softening of mature fruits prior to ripening was associated with depolymerization of matric glycans that are both loosely and tightly attached to cellulose as well as loss of Gal from cell wall fractions. This continued during ripening and was coupled by major losses of Ara from the loosely bound matric glycan fraction. At senescence, the excessive softening of peach fruits was associated with dramatic depolymerization of chelator-soluble polyuronides. The depolymerization was facilitated by the increase in solubilization of the chelator-soluble polyuronides [32]. Given the consistency in the trends in berry ripening in this study and previous studies, it can be concluded that the changes can be attributed to changes in composition of cell wall polysaccharides as well as significant modification in specific polysaccharides. More studies can be carried out on the African nightshade berries to ascertain the specific changes that take place as they ripen.

D. Size

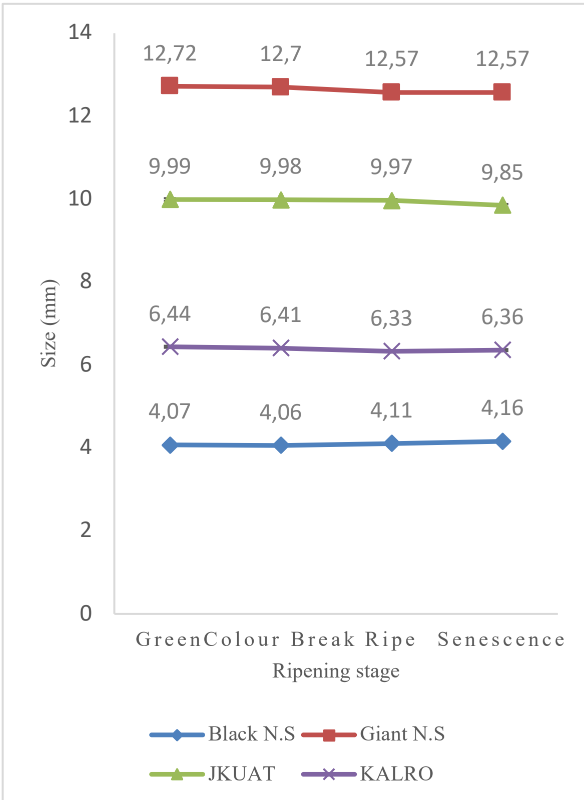

Fig. 2: Changes in berry size (mm) of four varieties of *Solanum nigrum* against the ripening stages

Berry sizes were as indicated in figure 2. The berry sizes were significantly different amongst the varieties. There were very gradual decreases in berry size for the Giant NS, KALRO and JKUAT varieties. However, Black NS showed a different trend where the berry sizes seemed to increase slightly with each ripening stage. While the berry sizes were significantly different amongst the varieties at each stage, the size within each variety did not show any significant change in size with ripening. Previously, grape berries were recorded to increase in diameter and weight in the early development stages [12] but not in the latter stages of development. Fruit parts such as weight and volume are known to increase with development [24], which is the observation made in this study.

IV. CONCLUSION

Findings from this study indicate that African nightshade edible berries have comparable characteristics with other fleshy, non-climacteric fruits that are commonly consumed in the household diet. They accumulate reducing sugars as they develop but have higher glucose than fructose fraction. Sucrose is present in high amounts at senescence. These characteristics could contribute to organoleptic quality and appeal to consumers. Black NS was considerably different from the other varieties, having a different colour, smaller size, higher sugar content and less firmness compared to the other varieties. This could imply that its application in the diet or other commercial purposes could be different from the others. Though not commonly consumed, the berries could be a valuable addition to the household diet through dietary diversification and for commercial exploitation through value addition and processing. However, research should be carried out to determine the exact pathways responsible for the ripening and accumulation of sugars in the berries.

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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