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Nutrients composition of calyces and seeds of three Roselle (*Hibiscus sabdariffa* L.) ecotypes from Niger

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The chemical composition of calyces and seeds of three ecotypes of Roselle from Niger was compared. The results indicate that calcium (Ca), potassium (K), sodium (Na), magnesium (Mg) and protein contents in calyces are significantly different ($P<0.005$) among ecotypes. The highest concentrations of K, Na, Mg and protein in calyces were recorded for ecotype E7 (35.66, 3.40, 6.01 and 101 mg/g d.w., respectively). Ecotype E9 had the highest Ca content in calyces (34.41 mg/g d.w.); while E3 and E7 had similar and lower contents. The protein content in calyces for E9 (52 mg/g d.w.) was approximately halved compared to those of E3 and E7. For all ecotypes, the concentrations of Ca, K, Mn, Na and Fe in the calyces were higher compared to those in the seeds. In contrast, P content was higher in seeds. The highest K, Na, Mg and P concentrations in seeds were registered for E7 and the lowest ones for E9. Ecotypes E3 and E9 recorded higher and similar Cu, Fe and Mn contents in calyces and in seeds compared to E7. The highest Zn concentrations in seeds were obtained for E3 and E7.

Key words: Niger, Roselle, seeds, calyces, protein, composition, micronutrients, macronutrients.

INTRODUCTION

Roselle or sorrel (*Hibiscus sabdariffa*), belonging to the family Malvacea is one of the most common flower plants grown worldwide. Probably native from Asia (India to Malaysia) (Mahadevan et al., 2009) or tropical Africa (Tounkara et al., 2011), Roselle is now cultivated in many tropical and subtropical regions of the world (Akanbi et al., 2009). It is an economically important plant, particularly in the Sahel zone of West Africa. The leaves, seeds and calyces are valued for its nutritional and medicinal uses (D'Heureux and Badrie, 2004). The most exploited part of Roselle plant is its calyces which may be green, red or dark red (Schippers, 2000). The green calyces are used for making vegetable stew (Babalola, 2000); while red and dark red ones are utilized in producing drinks, jellies, sauces, chutneys, wines, preserves and tea (Delgado-Vargas and Parcedes-Lopez, 2003). The calyces drink, which is receiving industrial attention internationally (Egharevba and Law-Ogbomo, 2007), is a readily available and inexpensive source of vitamin C (Babajide et al., 2004). Therefore, calyces of Roselle contain nine times more vitamin C than citrus (*Citrus sinensis*) (Amin et al., 2008). The seed of Roselle is a valuable food resource on account of its protein, calorie, fat and also substantial amount of fiber

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and valuable micro-nutrients (Akanbi et al., 2009). The seeds are subjected to a solid-state fermentation process to produce a meat substitute condiment called “Furudu” in Sudan (Yaquob et al., 2004), “mungza ntusa” in Nigeria (Omobuwajo et al., 2000), “bi-kalga” in Central Burkina and datou in Mali (Bengaly et al., 2006). In Niger, this condiment is called “dawadawa-botso” or “mari mi”, respectively in Hausa and Djerma language.

Roselle is one of the most important and popular medicinal plant which has several properties. The leaves are emollient and are much used in Guinea as a diuretic, refrigerant and sedative (Anhwange et al., 2006). The calyces, boiled in water are used as a drink in bilious attacks (Perry, 1980). They are also used to reduce hypertension (Onyenekwe et al., 1999). The seeds of Roselle are used as diuretic, laxative, tonic (Duke, 1985) and to treat debility (Perry, 1980). Despite the beneficial uses of this crop and its cosmopolitan nature, there is dearth of information about the crop. The objective of this study was to investigate the protein, micro and macro elements contents in calyces and seeds of three Roselle ecotypes from Niger.

MATERIALS AND METHODS

Plant material

Three Roselle ecotypes (E3, E7 and E9) collected in the south Sahelian zone of Niger were studied in field trial. Ecotypes A3 and A9 were collected in the area around the town of Dosso, and A7 in the area around the town of Maradi. Ecotypes E3 and E7 belong to the botanical type called “Waré” in Hausa dialect which is cultivated mainly for its developed calyces. Ecotype E9 belongs to the botanical type “Yakua”, which has medium sized calyces and cultivated mainly for its leaves and seeds. The colour of the calyces was black for E7, dark red for E3 and E7.

Growing conditions

The experiment was carried out under natural rainfall conditions during 2006 (from July to September) at the experimental station of the Agrhymet Regional Centre in Niamey, Niger (latitude 13° 29’ N, longitude 2° 10’ E, altitude 222 m). During the experiment, the daily temperature varied from 20.3 to 27.4°C for the minimum and 28.8 to 37.6°C for the maximum and the air humidity, from 29.4 to 69.5% and 74.7 to 97% respectively for minimum and maximum. Total rainfall during the crop cycle was 395 mm. The top 30 cm of the soil site had a pH in H_2O of 7.4. The soil was sandy with low moisture storage capacity and contained 0.20% of C, 0.162% of total N and 0.0479% of P (Ndiaye, 2002). In order to improve the water storage capacity of the soil moisture, 10 tons/ha of organic matter was applied. In addition, 100 kg N ha^{-1} of composite fertilizer NPK (15-15-15) was incorporated into the soil using a plow.

The experiment was laid out in a randomized complete block design with four replicates. The distance between two consecutive blocks was 2 m. Plot size was 10 × 6 m with 1 m margin round each plot. All plots consisted of 6 rows of plants with 1 m apart between rows and 1 m between plants within rows. Ten (10) seeds per hole were hand sown on July 14, 2006. After 22 days, the crop was tinned to two plants. The experimental plots were weeded with the aid of a hoe, five times to assure good conditions of plant growth

Plant sampling and analysis

Sample analysis

At harvest (115 days after sowing), five plants were randomly selected within the four central rows of each plot. The plants were then separated into different organs (leaves, stems, calyces and seeds) which were sun-dried. Thereafter, the calyces and seeds of three replicates were analysed for total N, Ca, Cu, Fe, K, Na, Mg, Mn, P and Zn at the Analytical Services Laboratory of ICRISAT Niamey (Sadoré, Niger) according to procedures of analysis (Houba et al., 1995). Samples were oven-dried (65°C overnight) and finely milled using the multi-beads shocker (Yasui Kikai, model MB500E). The quantitative determination of Cu, Ca, Fe, K, Na, Mg, Mn and Zn in the extract was done by atomic absorption spectrometry (Perkin-Elmer AAnalyst 400, Shelton, USA). The samples were nebulized into an air-acetylene flame where they were vaporized and the compounds were atomized.

Thereafter, the formed atoms were measured by atomic absorption at a wavelength of 324.7 nm for Cu, 422.7 nm for Ca, 248.3 nm for Fe, 766.49 nm for K, 589.0 nm for Na, 285.2 nm for Mg, 285.2 nm for Mn and 213.9 nm for Zn. Phosphorus was determined by the molybdate-blue method using ascorbic acid as the reductant (Houba et al., 1995). Color development was measured at 882 nm on a visible spectrometer (Turner model SP-850, Dublin, Ireland). The total N content was measured by colorimetric analysis by the salicylate/nitroprusside method (Houba et al., 1995). By this method, the ammonium reacts with salicylate in the presence of hypochlorite (oxidant) and nitroprusside (catalyst) to form an emerald green color, measured calorimetrically on an auto-analyzer Technicon AAII (Californie, USA) at 660 nm. The reaction took place in a buffered alkaline medium at pH: 12.8 to 13.0.

The total N content in calyces and seeds was converted to protein content as follows:

Protein content = total N content * 6.25.

Data analysis

Statistical analysis was performed using the GenStat software version 7.0. The analysis of variance (ANOVA) was carried out to determine the differences among the three ecotypes for nutrients and protein contents either for calyces or for seeds. Significantly-different means values were compared using the Student Newman Keuls test at 0.05 probability level.

RESULTS

The concentrations of Ca, K, Mg, Na, P and protein in calyces of the three ecotypes of Roselle are presented in Table 1. The results indicate that in calyces, the concentration of all theses components except P differed significantly ($P< 0.05$) among ecotypes. The highest Ca content in calyces, (34.41 mg/g dry weight, d.w.), was recorded for ecotype E9. Ecotypes E3 and E7 had similar Ca content but which was halved compared to those of E9. E7 had the highest K content in calyces (35.66 mg/g d.w.) and E9 the lowest one (28.75 mg/g d.w.). In addition, E9 had also the lowest Na and protein concentrations in calyces (respectively, 1.60 and 52 mg/g d.w.) which were approximately halved compared to those of

Table 1. Composition (mg/g dry weight) of Ca, K, Na, Mg, P and protein contents in calyces of three ecotypes (E3, E7 and E9) of Roselle from Niger.

Element	Ecotype		
	E3	E7	E9
Ca	17.45±3.93[b]	18.31±3.30[b]	34.41±3.40[a]
K	31.98±1.31[b]	35.66±1.88[a]	28.75±1.54[c]
Na	3.20±0.70[a]	3.40±0.35[a]	1.60±0.36[b]
Mg	4.53±0.66[b]	6.01±0.44[a]	5.01±0.57[b]
P	2.95±0.51	3.68±0.67	3.29±0.27
Protein	91±3.18[a]	101±4.19[a]	52±1.67[b]

Values in the same line followed by the same letter (s) are not significantly different (P< 0.05).

Table 2. Composition (mg/g dry weight) in Ca, K, Na, Mg, P and protein contents in seeds of three ecotypes (E3, E7 and E9) of Roselle from Niger.

Element	Ecotype		
	E3	E7	E9
Ca	4.70±0.10	5.10±0.53	Traces
K	19.52±0.77[b]	20.71±0.61[a]	16.86±0.77[c]
Na	2.90±0.29a	2.60±0.23a	0.90±0.26c
Mg	5.76±0.78	6.86±0.77	5.59±0.46
P	10.18±0.43[b]	11.99±0.48[a]	8.66±0.17[c]
Protein	250±3.33	252±9.26	271±8.62

Values in the same line followed by the same letter (s) are not significantly different (P< 0.05).

Table 3. Composition (µg/g dry weight) in Cu, Fe, Mn and Zn contents in calyces of three ecotypes (E3, E7 and E9) of Roselle from Niger.

Element	Ecotype		
	E3	E7	E9
Cu	73±7.25[a]	49±1.06[b]	67±3.71[a]
Fe	302±36.59[a]	111±54.25[b]	197±24.36[ab]
Mn	924±81.53[a]	580±50.40[b]	602±58.94[b]
Zn	37±4.24	42±7.65	43±2.29

Values in the same line followed by the same letter (s) are not significantly different (P< 0.05).

E3 and E7. The highest Mg concentration in calyces (6.01 mg/g d.w.) was recorded for E7 whereas E3 and E9 had similar and lower contents. The P content in calyces of the three ecotypes was similar. For all ecotypes, the concentrations of nutrients such as Ca, K and Na in the calyces were higher compared to those in the seeds. In contrast, the concentration of P was higher in seeds. The Mg content was similar in calyces and in seeds for the three ecotypes. The concentration of Ca in seeds was similar for E3 and E7 (Table 2). However, for E9 only traces of Ca was detected in seeds. Seeds Mg and protein contents did not show any significant differences among ecotypes. But ecotypes differed significantly ($P < 0.05$) for K, Na and P contents in seeds (Table 2).

E7 had the highest K and P contents, respectively; 20.71 and 11.99 mg/g d.w. Ecotype E9 recorded the lowest one (respectively 16.86 and 8.66 mg/g d.w.). The Na concentration in seeds of E9 was exceptionally low (0.90 mg/g d.w.), minus three times compared to those of E3 and E7 which was similar. Table 3 presents the composition of micro elements (Cu, Fe, Mn and Zn) in calyces of the three ecotypes. The Cu, Fe and Mn contents in calyces were significantly ($P < 0.05$) different among ecotypes. E3 and E9 had similar and higher Cu contents in calyces (around 70 µg/g d.w); whereas, E7 had lower one (49 µg/g d.w.). The variation of Fe and Mn contents in calyces among ecotypes was higher compared to those of Cu (Table 3). The highest Fe content content in calyces (302 µg/g d.w.) was recorded for E3. Ecotype E7 had minus three time concentration of Fe

Table 4. Composition (µg/g dry weight) in Cu, Fe, Mn and Zn contents in seeds of three ecotypes (E3, E7 and E9) of Roselle from Niger.

Element	Ecotype		
	E3	E7	E9
Cu	72±3.75^{a}	46±4.77^{b}	83±4.23^{a}
Fe	199±16.91^{a}	32±3.27^{b}	175±18.95^{a}
Mn	87±4.00^{a}	57±6.87^{b}	72±7.75^{ab}
Zn	88±3.88^{ab}	97±4.61^{a}	75±2.65^{b}

Values in the same line followed by the same letter (s) are not significantly different (P< 0.05).

content in calyces compared to those of E3. E9 had intermediate concentration of Fe in calyces (197 µg/g d.w.). The results indicated also similar and lower concentration of Mn in calyces for E7 and E9, 580 and 602 µg/g d.w., respectively compared to those of E3 (904 µg/g d.w.). The Zn content in calyces was not significantly different (40 µg/g d.w) among ecotypes. For the three ecotypes, the concentrations of Fe and Mn in the calyces were higher than those in the seeds. But the concentration of Zn in calyces was halved compared to seeds. The Cu content was similar.

The concentration of Cu in seeds was significantly ($P < 0.05$) different among ecotypes (Table 4). E3 and E9 recorded similar and higher concentrations of Cu content in seeds, 72 and 83 µg/g d.w., respectively. E7 had the lowest one (46 µg/g d.w.). The Fe content in seeds differed also significantly among ecotypes (Table 4). Moreover, the magnitude of variation in Fe content in seeds among ecotypes was important. Therefore, the Fe content in seeds varied from 32 µg/g d.w. (E7) to 199 µg/g d.w. (E3). The concentration of Fe in seeds for E9 was similar to E3. The data indicated significant differences among ecotypes in Mn contents in seeds. E3 and E9 had similar and higher Mn contents in the seeds. The highest Zn content in seeds was recorded for E7 (97 µg/g d.w.) and the lowest for E9 (75 µg/g d.w.).

DISCUSSION

The results indicate that all the analyzed nutrients were found in both calyces and seeds. However, the nutrients concentration in these plant organs varied among ecotypes. Ecotype E7 had the highest concentration of K, Na, Mg and P in the calyces and in the seeds while E9 had the lowest ones. In contrast, the highest Cu, Fe and Mn contents in the calyces and in the seeds were obtained for E3 and the lowest ones were recorded for E7. These results confirm variations in nutrient contents in calyces and seeds among Roselle varieties as previously reported by other colleagues (Cissé et al., 2009; Amin et al., 2008). For all ecotypes, the concentrations of nutrients such as Ca, K, Mn, Na, and Fe in the calyces were higher compared to those in the seeds. This result confirms those obtained by Haider et al. (2004). Although, for the three ecotypes, the protein and Zn contents in seeds were significantly higher compared to these in calyces, as previously recorded by Cissé et al. (2009). The Mg and Cu contents in calyces and seeds were similar. Moreover, the concentration of these nutrients is also similar to those of young leaves for the same cultivars (Atta et al., 2010a, 2010b).

The mineral content of *H. sabdariffa* indicates that the potassium was the most abundant inorganic cation in the calyces and in the seeds of Roselle, followed by Ca in the calyces and P in the seeds. Anhwange et al. (2006) found also that Roselle seeds are good source of P, Ca and Mg. K plays a critical role in the transmission of nerve impulses, muscle contraction and maintenance of normal blood pressure. A lack of K can result in liver ailments, pimpling of the skin, slow healing of sores and muscle weakness.

Calcium is major mineral constituent of the skeleton. The lack of the Ca in the body results in the breakdown of bones. Phosphorus is necessary for the innumerable biochemical reactions in which P serve as substrates or products such as ATP and as an integral part of phospholipids, phosphoproteins and phosphosugars (Taiz and Zeiger, 1998). The value recorded in this study for Ca, K, Fe, Mg and Mn contents in seeds was in good agreement with those reported by El Adawy and Khalil (1994). In Mali, Tounkara et al. (2011) reported also similar K, Fe, Mg, and Cu contents but higher Zn and Na contents in seeds. Nzikou et al. (2011) obtained higher concentrations of Ca, Na, P and lower ones for Mg and K in seeds of Roselle from Congo-Brazzaville. In India, Rao (1996) reported lower values for all measured nutrients in seeds except for Mn and Cu.

The protein content in seeds (25.2 and 27.2%) recorded in this study agrees with those reported by Nzikou et al. (2011); but was lower to those reported by Shaheen et al. (2012). Rao (1996) recorded higher protein contents in seeds of Roselle.

The range of protein content in seeds recorded in this study was higher to values obtained in conventional legume protein seed source such as cowpea (21 to 23%) (Henshaw and Sobowale, 1996) and bambara groundnut (23.4%) (Akpata and Ologhobo, 1994). This indicates that Roselle seeds are good source of protein for human nutrition. With the increasing world population in Sub Saharan Africa and the associated problems of nutrition and health, traditional vegetables such as Roselle may serve as sources of nutrition mainly for cheap protein. Since some cereals flours commonly consumed in the Sahel are deficient in calcium, for example, pearl millet (46 mg/100 g) and sorghum (15 mg/100 g) (FAO, 1995), the fortification of flours with Roselle seed flour might improve their dietary properties as suggested by El Adawy and Khalil (1994). In many countries, the seeds of Roselle are subjected to a solid-state fermentation process to produce a meat substitute condiment called dawadawa-botso in Niger. It is traditionally prepared by long cooking (up to 10 h) in alkaline water, followed by a 3 days solid-state fermentation (Bengaly et al., 2006). Dawadawa-botso is mostly used as meat-substitute in sauces accompanying cereals pastas in Niger. Therefore, this condiment should contribute to minerals intake of rural populations with low meat (and per se low hem iron) intakes. Abu-Tarboush et al. (1997) concluded that protein and lipid content is enough to be considered Roselle seeds as food grain.

Conclusion

Roselle calyces and seeds particularly for ecotype E7 is a rich source of protein and macronutrients such as K, Na and Mg. Ecotypes E3 and E9 are better source of micronutrients contents (Cu, Fe and Mn) compared to E7. Accordingly, Roselle could be used as supplement food or diet enrichment especially in the low protein diets. Since some cereals flours commonly consumed in the Sahel are deficient in some mineral elements such as calcium, the fortification of flours with Roselle seed flour might improve their dietary properties.

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