Nutritional and anti-nutritional evaluation of ten genotypes of pepper (*Capsicum annuum* L.) grown in a derived savanna ecology of Nigeria

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ABSTRACT

The selection of pepper genotypes for high yield could be exploited together with high nutritional content and considerable secondary metabolite level. The present study was aimed at evaluating the nutrient and anti-nutrient compositions of ten genotypes of pepper through laboratory assessment. According to the nutrients analyzed, total carotenoid content ranged from 6 - 34 mg / 100 g, β -carotene from 4 - 7 mg / 100 g and ascorbic acid from 83 - 100 mg/ 100g. Most parameters evaluated varied significantly. Moisture content ranged from 84.2% to 77.4%, ash content from 1.5% to 0.8%, protein from 7.5% to 4.6%, fat from 2.7% to 1.8% and fiber from 11.7% to 6.2%. Results also showed that mineral content had the following range: Na with 0.31 – 0.82 mg/ 100 g, K with 24.50 – 40.70 mg/ 100 g, Zn with 21.11 – 26.31 mg/ 100 g, Fe with 0.41 – 0.65 mg/ 100 g and Ca with 5.96 – 121.98 mg/ 100 g. The antinutrients analyzed were also found to be present in varied concentrations across the pepper genotypes. All the genotypes evaluated had values to supply sufficient vitamin A and β – carotene for daily recommendations.

Keywords: Antinutrients, *Capsicum annuum*, Food analysis, Food composition, Genotypes, Nutrients, Recommended Daily Allowance.

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INTRODUCTION

Aboriginal vegetable cultivation is an essential part of the subsistence farming system generally practiced in Nigeria and West Africa at large (Maga *et al.*, 2012; Abu and Odo, 2017). In Nigeria, pepper is placed third

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among the cultivated vegetable crops after onions and tomatoes (Abu *et al.*, 2013). It plays an essential function in the nutritional stability of rural and urban dwellers by supplying vitamins and minerals in their diets (Uguru, 1999). Pepper is used at length in food flavouring in the daily diet of over 120 million Nigerian irrespective of their socio-economic status (Benson *et al.*, 2014). In Nigeria, pepper fruits are consumed in two forms (fresh or dry). Before use, the fresh fruits can be sliced or ground while the dry fruits are ground. It is used in cooking of soup and stew, which are among the most important compliments of major cereals and root crops (Asawalam, 2007; Benson *et al.*, 2014).

Pepper is extremely a rich source of ascorbic acid (Vitamin C) and carotenoids (Abu *et al.*, 2013). The ascorbic acid content in pepper has been reported to range from 46 - 395 mg 100 g⁻¹ (Howard *et al.*, 2000; Uguru, 2000). The differences in ascorbic acid among pepper cultivars is attributed to variations in moisture content of the fruits as ascorbic acid is a water-soluble compound that could decline as the fruits dehydrate (Bosland and Votava, 2012). Few decades in the past, the recommended daily allowance (RDA) of vitamin C was 60 mg/day (NRC, 1989), but currently, RDA of vitamin C is now 90 mgday⁻¹ for men and 75 mgday⁻¹ for women (HMD-NASEM, 2018).Capsicum peppers considered as one of the richest sources of Vitamin C in vegetables are, however, also sources of Vitamins E₁, provitamin A₁, Thiamin, Riboflavin and Niacin.

The diverse and brilliant colours of pepper fruits originate from the carotenoid pigments present in the thylakoid membranes of the chromoplasts of photosynthetic tissues and the chromoplasts of flowers, fruits and roots. Carotenoids are lipid-soluble compounds in peppers, 95% of the total provitamin A in green pods and 93% in mature red pods are β -carotene (Howard *et al.*, 2000). The β -carotene content of some pepper cultivars varies from 42.4 to 144.7 mg 100 g⁻¹ (Uguru, 2000). Wall *et al.* (2001) working with 57 pepper cultivars reported a range of 0 to 166 μ gg⁻¹ fresh weight for β -carotene of ripe fruit and a value of 4 to 1173 μ gg⁻¹ fresh weight for total carotenoids.

In selecting pepper genotypes for consumption, high yield could be exploited together with high carotenoid and vitamin contents. The World Health Organisation had earlier reported that after total energy deficiency, vitamin A and protein deficiency are estimated to be the common dietary problems in the world. The World Bank had also reported that vitamin A deficiency is the most common form of malnutrition after protein

deficiency. Peppers are important sources of both provitamin A and vitamin C. The yellow, orange and red colours of *Capsicum* fruit originate from carotenoid pigments produced during ripening. Red pepper carotenoids are used in food industries as natural red colouring, but also have immense nutritional value as provitamin A and antioxidants. Since the improvement of peppers for nutrient content has been the goal of several breeding programmes (Wall *et al.*, 2001), the general objective of this work was to evaluate ten pepper genotypes in the laboratory with the specific aim of studying their nutrient and anti-nutrient compositions.

MATERIALS AND METHODS

Study area

The field characterisation of the genotypes was conducted at the research farm of the Department of Crop Science, Faculty of Agriculture, University of Nigeria, Nsukka. This is located in a plateau at an average elevation of approximately 440 meters above sea level, a geographical location of 6°51′56″N Latitude and 7°24′22″E Longitude. The average temperature in Nsukka is 24.9 °C, average annual rainfall is 1579 mm (Climate data, 2019) with an annual average solar daily radiation of 4.95 kWh/m²/d and an annual average wind of 2.1 m/s (NASA, 2013). Nsukka is in the derived savanna vegetation of Nigeria, an area with ustic soil moisture regime and the soil around is characterized as well drained with very low total exchangeable base, cation exchange capacity (CEC) and base saturation. The soil is deep, coarse textured and low in organic matter with perennial leaching problem. The soils mostly belong to the order of Ultisol and Vertisol (Edeh *et al.*, 2013).

Sample collection

The experimental materials consisted of ten aromatic pepper genotypes of which five were obtained from the pepper germplasm of the Department of Crop Science, University of Nigeria Nsukka while the other five genotypes were bought from the local market. The ten accessions passed through three cycles of selection in the Botanical garden, Department of Botany, University of Nigeria, Nsukka before the commencement of the work. The seedlings for the field work were raised in a nursery inside the Botanical garden. The nursery medium was mixed in the proportion of 3: 2: 1 of top soil, poultry manure and river sand, respectively (Ojua *et al.*, 2019; Abu *et*

al., 2019). The field characterization of the genotypes was conducted at the research farm of the Department of Crop Science, Faculty of Agriculture, University of Nigeria, Nsukka. The genotypes were assessed for three consecutive years under rain fed conditions in a randomized complete block design (RCBD) with three replications. The seedlings of the different genotypes were transplanted to the field after four weeks (Abu et al. 2006; Abu et al. 2011). Ripe fresh fruits were then harvested at the end of the third year and used for the nutrient analysis at the Department of Crop Science Teaching and Research Laboratory, of the University of Nigeria, Nsukka.

Carotenoids and Vitamins

The analysis for total carotenoids, β - carotene and ascorbic acid were carried out using standard methods as outlined by the Association of Official Analytical Chemists (2005) and UV – VIS spectrophotometer (Model; 752P, Made: Techmel and Tachmel, USA.).

Proximate analysis

Proximate analysis (proteins, fat, fibre, moisture and ash) was done for each of the pepper genotypes. Protein was determined by the Kieldahl method as outlined by Pearson (1976). This method involved digestion of the sample in Tetraoxosulphate (VI) acid, which was neutralized with Sodium hydroxide. This frees the ammonia, which was removed from the mixture by steam distillation. The distillate from the Kjeldahl mixture was received in boric acid solution and titrated with standard HCL. For each ammonia molecule found in the distillate, one nitrogen atom was present in the sample. The crude protein in the sample was calculated by multiplying the total nitrogen by an empirical factor (N × 6.25). The percentage fat in the sample was determined as outlined in Pearson (1976). This was by extraction from dried, ground sample using light petroleum in Soxhlet extraction apparatus under controlled conditions. The Weende method for the determination of crude fibre is outlined in Pearson (1976). The crude fibre of a sample is the residue remaining after protein, starch, fat and digestible carbohydrates have been hydrolyzed out of the sample. The ash of a sample is the inorganic residue remaining after the organic matter has been burnt away. The percent ash of each sample was determined as in Pearson (1976), while moisture was determined by indirect distillation method by measuring weight loss due to evaporation of water (AOAC, 2005).

Minerals and anti-nutrient analysis

The minerals which include Na, K, Zn, Fe and Ca, and some antinutritional factors (tannin, phenol, phytate, saponin, oxalate) were determined using the methods as outlined in Pearson (1976) and AOAC (2005).

Statistical Analysis

Difference in the levels of carotenoids, β - carotene, ascorbic acid, proteins, fat, fibre, moisture, ash, Na, K, Zn, Fe, Ca, tannin, phenol, phytate, saponin, oxalate among genotypes were subjected to a one-way Analyses of Variance (ANOVA) using IBM SPSS Statistics 20 software and means were separated using Least Significant Different Test (LSD).

RESULTS AND DISCUSSION

Carotenoids and Vitamins

The values obtained for total carotenoid, $\beta\text{-}carotene$ and vitamin C are presented in Table 1. The total carotenoid varied from 6.00 to 34.00 mg/100 g; genotypes UNS3 and Nsky-re had the highest carotenoid content. The highest β - carotene content was recorded in genotype Tatase (17.30 \pm 0.35 mg/100 g) and the lowest in Shombo (4.07 \pm 0.09 mg/100 g). The ascorbic acid content of the fresh fruits ranged from 83.33 \pm 2.03 mg/1 00 g in Tarugu to 100.00 \pm 0.00 mg/ 100 g in Shombo which was significantly (P < 0.05) the higher (Table 1).

The presence of nutritional or anti-nutritional factors has been reported by numerous researchers (Uguru, 2000; Bosland and Votava, 2000; Kumar et al., 2003; Finger et al., 2010; Ogunlade et al., 2012; Wahua et al., 2013 and 2014). Mozarfor (1994) who attributed the wide range in the levels of the nutritional composition being reported by different researchers to differences in cultivars, maturity, growing practices, climate, post-harvest handling and analytical methods. This could explain the disparity between the levels of the nutritional qualities obtained as a result of capsicum genotype differences in this study and previous similar reports (Uguru, 2000).

Table 1. Total carotene, β – carotene and Vitamin C (ascorbic acid) content of	•
pepper fruits grown in the derived savanna ecology of Nigeria.	

Pepper Genotypes	Total carotenoid (mg/100g)	β –carotene (mg/100g)	Vitamin C (ascorbic acid) (mg/100g)
UNS2	$21.27 \pm 1.25e$	$11.23 \pm 0.34d$	$90.67 \pm 0.33b$
UNS3	$34.30\pm0.25a$	$12.30\pm0.12c$	$84.33 \pm 0.88 de$
Nsky-1p	$22.03 \pm 1.11e$	$7.30 \pm 0.40 h$	$87.67 \pm 0.88c$
Shombo	$6.20\pm0.15h$	4.07 ± 0.09 j	$100.00 \pm 0.00a$
Nsky-se	$27.17 \pm 0.15 d$	$9.97 \pm 0.03e$	$87.67 \pm 0.33c$
Tarugu	$18.33 \pm 0.86 f$	$9.27 \pm 0.50g$	$83.33 \pm 2.03e$
Tatase	$31.13 \pm 0.07c$	$17.30\pm0.35a$	$91.67 \pm 0.88b$
Dangarawa	$32.90 \pm 1.30b$	$16.30 \pm 0.10b$	$84.00\pm0.00\text{de}$
Oshosho	$12.23 \pm 0.35g$	$5.10 \pm 0.12i$	$85.33 \pm 0.88d$
Nsky-rw	$33.90 \pm 0.10ab$	$12.00\pm0.15c$	86.00 ± 1.73 cd
LSD	1.26	0.46	1.74

^{*}significant means were separated with different alphabets on each vertical array using Least Significant Difference Test (LSD). NS = not significant.

Total carotenoids and β -carotene which are good precursors of vitamin A are reported to be available in pepper (Uguru, 2000). According to Howard et al. (2000), the level of β -carotene is cultivar specific whereby some cultivars of hot pepper have as much as 12 mg/kg total carotenoids, while others are below the detectable level. National Research Council (1989) reported that between 4.8 and 6.0 mg of β -carotene would supply 100 %of RDA for vitamin A in adult females and males respectively. This implies that all the genotypes excepting Shombo contained greater amounts of β carotene than the RDA for vitamin A for the average adult assuming that β -carotene was fully absorbed and converted to vitamin A in the body. Howard et al. (2000) also reported that the provitamin A value of ripe pepper fruit can be 3 to 15 times as high as that of green fruits depending on the fruit type, cultivar and growing condition. The total carotenoid levels of fruits are relevant for breeding high pigment pepper genotypes for the food industry. Carotenoids are added to a range of food products, cosmetics, and pharmaceutical as natural colorants. Pepper fruits have been known to contain higher ascorbic acids compared to other vegetables (Bosland and Votava, 2000; Finger et al., 2010). The result of this present study reveals variation in ascorbic acid, ranging from 83 mg/100 g to 100 mg/100 g among the selected pepper genotypes evaluated. This range falls in-between the ranges reported before such as some Indian pepper lines (Kumar et al., 2003; Topuz and Ozdemir, 2007), and in some Turkish pepper types (Balkaya and Karaagac, 2009). This is an indication that these pepper fruits contain enough ascorbic acid to meet or exceed the adult Recommended Daily Allowance (RDA) of 90 mg and 75 mg per day for man and women, respectively. Therefore, consumption/intake of these peppers in diets per 100g would be enough to meet the vitamin C requirements of an individual per day. The values of vitamin C in these pepper genotypes confirm the report that peppers are among the richest known plant sources for vitamin C.

Proximate composition

The fruits of the ten genotypes widely varied in percentage protein, fat, fibre, moisture and ash (Table 2). The protein ranged from 7.47 - 4.82%. Tatase genotype contained 7.47 \pm 0.74% and was not significantly different from other genotypes evaluated except for Nsky-1p whose percentage content was 4.82 \pm 0.76%. The percentage fat ranged from 2.65 \pm 1.01% in Nsky-se to 1.80 \pm 0.92% in Nsky-1p while the highest and lowest values obtained for fibre were 11.65 \pm 3.72% in UNS3 and 6.15 \pm 1.36% in Nsky-re. Fat and fibre contents were not significantly different (P > 0.05) across all genotypes.

Moisture content significantly varied (Table 2), where Tatase had the highest content of $87.30 \pm 2.77\%$ and the least moisture content was observed in Oshosho genotype (77.40 \pm 0.46%). Tatase genotype contained the highest ash content of $1.50 \pm 0.06\%$ and was significantly different (P < 0.05) from the values obtained in Nsky-se. Comparing the results obtained in this study with previous reports on the proximate composition of *C. annuum*, current results also corroborate Ogunlade *et al.* (2012) for parameters such as moisture, but ash content was lower in the current study, while protein, fat and fibre were higher. Pepper is not a perfect source of protein because the protein content across the pepper varieties was much lesser when compared to some commonly consumed protein crops in Nigeria (Aremu *et al.*, 2011). However, pepper needs to be combined with other foods of high protein value in order to meet the protein requirements of individuals (Emmanuel-Ikpeme *et al.*, 2014).

The higher fiber content suggests that these genotypes may be useful in the regulation of intestinal transports and increase dietary bulk due to their capacity to absorb water. Fibre is also known to help in fighting cancer and reducing serum cholesterol (Emmanuel-Ikpeme *et al.*, 2014). Ash content was relatively low indicating that these genotypes may not be considered

as good sources of minerals when compared to values (2 - 10 %) obtained for cereals and tubers (Oulai *et al.*, 2014).

Mineral composition

Table 3 shows the compositional levels of the following mineral elements – Na, K, Zn, Fe, Ca. The highest Na content was 0.82 mg/100 g (UNS2), K – 41.20and 40.70 mg/100 g (Tatase and Tarugu), Zn – 26.41 mg/100 g (Dangarawa), Fe - 0.65 mg/100 g (Nsky-1p, and Tarugu) and Ca – 12.98 mg/100 g (Nsky-1p).

Following the report of Rubio *et al.* (2002), who gave a summary of the data published about pepper mineral content of macroelements, the selected genotypes all had mineral content within the range although K content was relatively lower in the present study. According to Geissler and Powers (2005), iron plays many biochemical roles in the body, ranging from oxygen binding in hemoglobin and acting as an important catalytic center in many enzymes as the cytochrome oxidase. The iron contents of the studied genotypes were lower than the recommended dietary allowance for males (1.37 mg/day) and females (2.94 mg/day) (FAO/WHO, 1988). To meet the recommended dietary needs for iron, it could suffice to say that these pepper genotypes need to be complemented with other vegetables or consumed more frequently and in larger quantity especially the non-pungent types.

Anti-nutrient composition

Results in Table 4 show the antinutrients factors, tannin which ranged from 0.04 to 0.31 mg/g, phenol (1.21-1.89 mg/g), phytate (0.01-0.07 mg/g), saponin (0.21-0.35 mg/g) and oxalate (23.90-39.70 mg/g) with various concentrations among the genotypes.

The tannin content of *C. annuum* (of the current study) was much lower than the tannin content of legumes reported before (Oboh, 2006). Phenolic content, on the other hand, corroborates previous reports (Adedayo *et al.*, 2010). The phytate content was also within range comparing to the content in some vegetables reported before (Oulai *et al.*, 2014).

Plants are generally known to contain antinutrients acquired from fertilizer and pesticides and several naturally-occurring chemicals (Soetan and Oyewole, 2009). Some of these chemicals called secondary metabolites

have been shown to be highly biologically active. Tannin cause a decline in feed consumption by animals, bind dietary protein and digestive enzymes to form complexes that are not readily digestible. They also cause decreased palatability and reduced growth rate (Soetan and Oyewole, 2009). Phytic acid however considered an anti-nutritional factor, is a common storage form of phosphorus in seeds and in a few tubers and fruits (Adedayo *et al.*, 2010).

CONCLUSION

In conclusion, this study was geared toward laboratory assessment of nutrient and anti-nutrient compositions of ten genotypes of C. annuum; it was observed that all the genotypes evaluated contained both nutrients and anti-nutrients at varied proportions which agreed with the reports of other researchers. From the observations, Tatase was rich in β -carotene, protein, ash, K as well as tannin and phenol; UNS3 was rich in total carotenoid; Shombo in vitamin C. Consumption of all the pepper genotypes evaluated in this study could supply the needed nutritional needs of consumers.

Table 2. Proximate analysis (%) of pepper fruits grown in the derived savanna ecology of Nigeria.

Pepper Genotypes	Protein	Fat	Fibre	Moisture	Ash
UNS2	6.53 ± 0.74 ab	2.45 ± 0.78	11.55 ± 4.24	78.90 ± 0.17 bc	$0.75 \pm 0.14ab$
UNS3	$5.91 \pm 0.38ab$	2.30 ± 1.15	11.65 ± 3.72	78.00 ± 2.66 bc	$0.80 \pm 0.40 ab$
Nsky-1p	$4.82 \pm 0.76b$	1.80 ± 0.92	9.60 ± 3.06	79.55 ± 1.47 bc	$1.30 \pm 0.29 ab$
Shombo	$7.44 \pm 0.76a$	2.65 ± 1.01	10.00 ± 3.00	80.75 ± 0.84 bc	$0.95 \pm 0.32ab$
Nsky-se	$5.47 \pm 0.64ab$	2.55 ± 1.18	7.80 ± 2.66	$84.20 \pm 0.81ab$	$0.60\pm0.23b$
Targuru	$6.96 \pm 0.48ab$	1.85 ± 0.95	8.95 ± 3.32	79.55 ± 1.99 bc	$1.35 \pm 0.20ab$
Tatase	$7.47 \pm 0.74a$	2.05 ± 0.72	8.80 ± 3.75	$87.30 \pm 2.77a$	$1.50 \pm 0.06a$
Dangaraw	6.53 ± 0.74 ab	2.45 ± 1.24	7.60 ± 3.06	81.15 ± 3.95 abc	$0.80 \pm 0.00 ab$
Oshosho	$5.73 \pm 0.23ab$	1.90 ± 0.98	8.70 ± 2.54	$77.40 \pm 0.46c$	$1.10 \pm 0.40 ab$
Nsky-rw	$7.00 \pm 1.01ab$	2.20 ± 0.69	6.15 ± 1.36	82.35 ± 0.49 abc	1.10 ± 0.06 ab
LSD	2.01	NS	NS	5.79	0.74

^{*}significant means were separated with different alphabets on each vertical array using Least Significant Difference Test (LSD). NS = not significant.

Table 3. Mineral contents (mg / 100 g) of pepper fruits grown in the derived savanna ecology of Nigeria.

Pepper Genotypes	Na	K	Zn	Fe	Ca
UNS2	0.82 ± 0.08	26.90 ± 0.40 de	21.00 ± 0.58	0.54 ± 0.03 abc	$8.21 \pm 0.12d$
UNS3	0.42 ± 0.06	$37.10 \pm 0.52b$	21.13 ± 1.27	$0.41 \pm 0.02d$	11.00 ± 0.65 b
Nsky-1p	0.40 ± 0.16	25.90 ± 0.40 ef	25.32 ± 1.14	$0.65 \pm 0.02a$	$11.98 \pm 0.01a$
Shombo	0.44 ± 0.09	$28.30 \pm 0.69d$	21.19 ± 0.83	0.49 ± 0.08 bcd	$9.31 \pm 0.18c$
Nsky-se	0.49 ± 0.08	27.60 ± 0.87 de	23.10 ± 0.98	$0.62 \pm 0.01a$	$5.96 \pm 0.02e$
Tarugu	0.33 ± 0.09	$41.20 \pm 0.52a$	26.31 ± 2.74	$0.65 \pm 0.04a$	$8.41 \pm 0.24d$
Tatase	0.37 ± 0.20	$40.70 \pm 0.52a$	24.29 ± 1.81	$0.59 \pm 0.07ab$	$11.00 \pm 0.06b$
Dangarawa	0.59 ± 0.04	$32.40 \pm 0.58c$	26.41 ± 2.18	0.42 ± 0.03 cd	$8.11 \pm 0.06d$
Oshosho	0.31 ± 0.10	$24.50 \pm 0.06 f$	21.11 ± 0.16	0.45 ± 0.02 cd	$7.99 \pm 0.01d$
Nsky-rw	0.64 ± 0.11	$28.30 \pm 0.81d$	22.00 ± 1.29	$0.41 \pm 0.02d$	$9.01 \pm 0.01c$
LSD	NS	1.71	NS	0.12	0.68

^{*}significant means were separated with different alphabets on each vertical array using Least Significant Difference Test (LSD). NS = not significant.

Table 4. Anti-nutrient compositions of ripe pepper fruits (mg/g).

Pepper Genotypes	Tannin	Phenol	Phytate	Saponin	Oxalate
UNS2	$0.18 \pm 0.07a$	1.36 ± 0.13 bc	0.07 ± 0.006 a	$0.25 \pm 0.012b$	33.40 ± 1.16
UNS3	$0.04 \pm 0.02c$	$1.29 \pm 0.08c$	$0.03 \pm 0.012 bcd$	$0.21 \pm 0.006b$	30.10 ± 0.94
Nsky-1p	$0.31 \pm 0.12a$	$1.76\pm0.03ab$	$0.06\pm0.006ab$	$0.35\pm0.058a$	23.90 ± 1.25
Shombo	$0.27 \pm 0.09 ab$	$1.46 \pm 0.01 bc$	$0.05 \pm 0.023 abc$	$0.23\pm0.006b$	34.60 ± 3.31
Nsky-se	$0.05 \pm 0.02 bc$	$1.21\pm0.08c$	$0.00\pm0.017d$	$0.24 \pm 0.012b$	39.70 ± 2.36
Tarugu	$0.04 \pm 0.00c$	$1.88 \pm 0.15a$	$0.02 \pm 0.000 cd$	$0.27 \pm 0.006b$	26.70 ± 1.20
Tatase	$0.31 \pm 0.12a$	$1.87 \pm 0.33a$	$0.02 \pm 0.000 cd$	$0.26\pm0.017b$	34.80 ± 0.78
Dangarawa	$0.31 \pm 0.11a$	$1.89 \pm 0.08a$	$0.01 \pm 0.017d$	$0.24 \pm 0.006b$	39.70 ± 0.73
Oshosho	$0.22 \pm 0.06 abc$	$1.31 \pm 0.10c$	$0.03 \pm 0.017 bcd$	$0.22\pm0.006b$	31.70 ± 1.40
Nsky-rw	$0.29 \pm 0.02a$	$1.72\pm0.03a$	$0.02 \pm 0.000 cd$	$0.26\pm0.017b$	37.60 ± 0.99
LSD	0.22	0.40	0.03	0.06	NS

^{*}significant means were separated with different alphabets on each vertical array using Least Significant Difference Test (LSD). NS = not significant.

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