

Original Research Article

Effect of Gamma Irradiation on Amino Acids profile, Minerals and Some Vitamins

Present in Pigeon Pea (*Cajanus Cajan*) Flour

Abstract

Effects of gamma irradiation on amino acids profile, minerals and some vitamins in Pigeon pea flour were investigated at gamma doses (0, 5, 10, 15 and 20kGy). The application of gamma irradiation did not have positive effect on the amino acids of the pigeon pea flour and showed a significant reduction at all levels of the delivered doses ($P < 0.05$). Although, the raw and gamma irradiated flour meet the Food and Agricultural Organization-World Health Organization recommended pattern of essential amino acids. The effect of irradiation process was not significant on the mineral content of the flour and has slight effect on some vitamins analysed in the work. In conclusion, gamma irradiation has little or no significant effects on the amino acids profile, minerals and vitamins tested in this work and it can still be recommended as a preservative method for pigeon pea flour.

Keywords Amino acids, pigeon pea, flour, Irradiation, vitamin, minerals

Introduction

Legumes are an economically valuable source of protein in the developing countries, wherein animal proteins are scarce. Being rich in proteins, carbohydrates, calorific value, fibre, minerals and vitamins, legumes constitute staple food in many countries (Deshpande, 1992). Exploitation of underexplored wild legumes is an important approach to combat the protein-energy malnutrition in developing countries. Pigeon pea (*Cajanus cajan*) is a locally available, affordable and under-utilized grain legume of the tropics and sub-tropics. Pigeon pea varieties have protein content in the range of 23 - 26% (Oshodi *et al.*, 1985). The protein content is comparable with ~~those in~~ other legumes like cowpea and groundnut which have been used in complementing **maize**. It is rich in mineral quality and fibre content. In agricultural

science and food technology, recent research has elucidated new potential applications for radiation. For example, high doses of ionising radiation have been shown to inhibit growth of microbial infestations in seeds. There are also many reports supporting the use of gamma irradiation as a fungicidal agent (Aziz *et al.*, 2007). However, the viability and sometimes the developmental process of the seedling or the plant have been seriously hampered by radiation (Casarett, 1996). There are insufficient reports about possible effects of gamma irradiation on nutritional value of the underutilised leguminous seeds made into flour associated with seed sterilisation. Seeds of different plants that are consumed as food has varying nutrient values which are dependent on the basic constituents of seed proteins. Macronutrients in irradiated foods undergo little change, and as a consequence, the normal nutritional values of proteins, lipids and carbohydrates of foods are maintained [WHO, 1999]. Vitamins have varying sensitivity to radiation treatments and are the only components of foods that need to be considered from a dietary impact standpoint. Like thermal treatments, radiation processing of foods can cause some loss of vitamins. Vitamins have been found to be affected by irradiation because vitamins presented different sensitivities regarding the treatment with ionizing energy (Ana Paula Dionise *et al.*, 2009).

The amino acids are very important to human health and of the twenty standard proteinogenic amino acids, eight are called essential amino acids because the human body cannot synthesise them from other compounds at the level needed for normal growth, so they must be obtained from food (Elshazali *et al.*, 2010). The amino group ($-NH_2$) is the most radiosensitive portion of the amino acids (Siddhuraju *et al.*, 2002). Extensive research showed that the macronutrients (carbohydrates, proteins and lipids) content are relatively stable against irradiation doses up to 10 kGy. However, Lee *et al.* (2005) reported that gamma irradiation affects proteins by causing conformational changes, oxidation of amino acids, rupturing of covalent bonds and formation of protein free radicals. Also, chemical changes in the proteins caused by gamma irradiation include fragmentation, cross-linking, aggregation and oxidation by oxygen radicals that are generated in the radiolysis of water. This effect could be related to the structure of each amino

acid, simple amino acids increased upon irradiation, such as glycine, which undergo reductive deamination and decarboxylation (Erkan and Ozden, 2007). In addition, aliphatic amino acids with increasing chain length provide additional C–H bonds for interaction with OH radicals which reduces the extent of oxidative deamination (Erkan and Ozden, 2007). Wang & vonSonntage (1991) reported that sulphur containing as well as aromatic amino acids are, in general, the most sensitive to irradiation, while simple amino acids could be formed by destruction of other amino acids. However, there is a scarcity of information relating to the effects of processing with ionising energy on most underutilized legumes. Therefore, the present work was undertaken to explore the effects of irradiation on amino acids, minerals and some vitamins contents of raw and processed pigeon pea flour.

Materials and Method

Pigeon pea was purchased at Odori market in Iwo, Osun State. The gamma irradiation process at Energy Centre in Abuja at different doses (5, 10, 15, and 20 kGy). The samples were exposed to gamma rays generated by a cobalt-60 source (Gammacell 220, MDS Nordion, Ottawa, Canada) following the procedures described by Helinski *et al.* (2008) with a dose rate of ca. 2 Gy/min at 25°C and normal relative humidity. Double side irradiation (exposure to both sides) was performed for uniform dose delivery. A dosimetry system was used to measure the dose received by the batch based on the Gafchromic HD-810 film (International Specialty Products, NJ, USA; FAO/IAEA/USDA, 2003). Three dosimeters were included with each batch of pigeon pea seeds and read after irradiation with a Radiachromics reader (Far West Technology Inc., CA, USA). All experiments were repeated 3 times and 3 replicates of each sample type were irradiated. The irradiated samples were milled with hammer mill and packaged inside low density polythene bags. All of the flour samples were stored in a deep freezer (-20° C) until further analysis.

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74 **Mineral analysis**

75 Minerals (sodium, potassium, calcium, magnesium, iron, copper, zinc, manganese and selenium) of the
76 seed flour were determined employing the AOAC (2000) method on digestion of flour with a mixture of
77 concentrated nitric acid, sulphuric acid and perchloric acid (10:0:5:2, v/v) using an atomic absorption
78 spectrophotometer (GBC 904AA; Germany). The total phosphorus was determined as orthophosphate by
79 the ascorbic acid method after acid digestion and neutralization using phenolphthalein indicator and
80 combined reagent (APHA 1995). The absorbance was read at 880 nm (Spectronic 21 D, Miltonroy, NY,
81 USA) and KH₂PO₄ (Merck, India Limited, Mumbai, India) served as the standard.

82 **Amino acid analysis**

83 Amino acids of the pigeon pea flour samples were assessed by the method outlined by Hofmann *et al.*
84 (1997, 2003). A known quantity of pigeon pea flour was hydrolysed with 6 N HCl (15 ml) for 4 h at 145°
85 C. After cooling, HCl was removed in a rotoevaporator combined with a diaphragm vacuum pump
86 (MC2C; Vacuubrand GmbH, Wertheim, Germany). Internal standard, trans -4-(aminomethyl) -
87 cyclohexanecarboxylic acid was added to each sample. The derivatisation step consisted of esterification
88 with trifluoroacetylation (Brand *et al.*, 1994). Samples of standard amino acids were weighed out in
89 reaction vials and dried using CH₂Cl₂ under a gentle stream of helium and slow heating in an oil bath
90 (40-60° C) to remove the traces of water. A 12 ml freshly prepared acidified isopropanol (acetyl chloride,
91 3 ml+2-propanol, 12 ml) was added and the mixture was heated at 100° C for 1 h. After cooling, the
92 reagent was eliminated with a gentle stream of helium at 60° C, followed by combined evaporation with
93 three successive aliquots of CH₂Cl₂ to remove propanol and water. The dry residue was
94 trifluoroacetylated with 200 ml trifluoroacetic anhydride overnight at room temperature. An aliquot of
95 this solution was used without further treatment for gas chromatography-combustion-isotope ratio mass

spectrometry. The GC-C-IRMS/MS measurements were carried out with a Hewlett-Packard 58590 II gas chromatograph, connected via a split with a combustion interface to the IRMS system (GC-C-II to MAT 252; Finnigan MAT, Bremen, Germany) for the isotopic determination of nitrogen and through a transfer line with a mass spectrometer (GCQ; Finnigan MAT) for qualitative analysis and quantification of the amino acids. The capillary column of GC was a 50 m x 0.32 mm i.d. x 0.5 µm BPX5 (SGE), operating with a carrier gas flow of 1.5 ml/min with the following temperatures and pressure: initial 50° C (1 min), increased to 100° C at 10° C/min (10 min), increased to 175° C at 3° C/min (10 min), increased to 250° C/min (10 min); head pressure, 13 psi (90 kPa). The essential amino acid (EAA) score was determined employing the formula:

$$\text{EAA score} = [\text{EAA (mg) in 100 mg test protein}] / [\text{EAA (mg) in 100 mg FAO/WHO (1991) reference pattern}] \times 100$$

Protein digestibility

The Akesson and Stahmann (1964) method was employed to determine the in vitro protein digestibility (IVPD) of pigeon pea flours (100 mg). The defatted flours were incubated (37C, 3 h) with pepsin (3,165 units/mg protein; Sigma-Aldrich, St. Louis, MO, USA) (1.5 mg/2.5 ml of 0.1N HCl) followed by inactivation (0.25 ml of 1 N NaOH). Incubation was continued (24 h, 37° C) with trypsin (16,100 units/mg protein; Sigma-Aldrich, St. Louis, MO, USA) and a-chymotrypsin (76 units/mg protein; Sigma-Aldrich, St. Louis, MO, USA) (2 mg each/2.5 ml potassium phosphate buffer, pH 8.0, 0.1 M) followed by inactivation (0.7 ml of 100% Trichloroacetic acid (TCA)). Zero-time control was maintained by inactivating the enzyme before addition of substrate. The inactivated reaction mixtures were centrifuged and the supernatant was collected. The residue was washed (2 ml, 10% TCA) and centrifuged. The combined supernatant was washed twice with 10 ml diethyl ether, and the ether layer was removed by aspiration. The aqueous layer was heated on a boiling water bath (15 min) to remove traces of ether and

the solution was made up to 25 ml with distilled water. The nitrogen content (in 5 ml aliquots) was determined to calculate protein in the digest:

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In vitro digestibility (%) = (protein in digest) / (protein in defatted flour) x 100

The protein digestibility corrected amino acid score (PDCAAS) of EAA was calculated based on EAA requirements for adults (FAO/WHO 1991)

PDCAAS (%) = [(amino acid content in food protein) x true digestibility] / (amino acid content of reference pattern)

Vitamins Analysis

Ascorbic acid was extracted and estimated according to the method described by Sadasivam and Manickam (1996). 3 g of each sample was mixed with 25 ml of 4% oxalic acid and filtered. Bromine water was added drop by drop to 10 ml of the filtrate until it turned orange-yellow to remove the enolic hydrogen atoms. The excess of bromine was expelled by blowing in air. This filtrate was made up to 25 ml with 4% oxalic acid and used for ascorbic acid estimation. Two ml of the extract was made up to 3 ml with distilled H₂O in a test tube. One ml of 2% 2, 4-dinitrophenyl hydrazine reagent and a few drops of thiourea were added. The contents of the test tube were mixed thoroughly. After 3 hours incubation at 37° C, 7 ml of 80% H₂SO₄ was added to dissolve the osazone crystals and the absorbance was measured at 540 nm against a reagent blank. The ascorbic acid content present in the sample was calculated by referring to a standard graph and expressed as milligrams per 100 grams of powdered samples.

For the extraction of thiamin, riboflavin and niacin, method of AOAC was followed (AOAC, 2000). Content present was calculated by comparison with standard curve developed using standards for vitamins, such as thiamine hydrochloride, riboflavin, and nicotinic acid, purchased from sigma St, Louis, USA.

Vitamin A in form of β -Carotene was determined using the method of AOAC (2000), which involved extraction of the samples with acetone and hexane. Concentrations were determined by comparison with a standard curve developed using β -Carotene from sigma St, Louis, USA.

Statistical analysis

Each determination was carried out on three separate samples, analysed in triplicate, and the mean taken. Data obtained from the mineral analysis, amino acids profiles and vitamins analysis were assessed using analysis of variance (ANOVA) and the means were separated by lowest standard deviation (LSD) test (SPSS version 16, 2008). Significant level was accepted at 5%.

Results and Discussion

Mineral Composition

The calcium content of un-irradiated pigeon pea flour was (119 mg/100 g), potassium (38.80 mg/100 g), Zinc (28.57mg/100 g) and manganese (14.33 mg/100 g) were the major minerals in the pigeon pea flour (Table 1). The study showed no decrease occurred in the mineral concentrations of pigeon pea flour upon irradiation ($P < 0.05$). However, calcium, potassium, zinc, and manganese did not show some slight increase ($P < 0.05$). Ca(120.90 to 122.13 mg/100g), K(40.13 to 40.15 mg/100g), Zn(27.70 to 33.30 mg/100g) and Mn(14.10 to 18.53 mg/100g). The moderate increase observed occurred from dose 5 kGy to that of 20 kGy except that of Zinc where the lowest value was recorded at dose 15 kGy. This result was directly opposite to the findings of Bhat *et al.* (2009). They recorded a decrease in mineral content of irradiated lotus seed flour at dose 0-30 kGy. Generally, minerals do not degrade on irradiation, but a change in their oxidation state might occur due to their varied extractability in a particular solvent (Bhat *et al.* 2009). Apart from this, pigeon pea seed being a biological sample, variations in mineral concentrations might naturally be present between each individual seed, which might give rise to varied results on irradiation. The mineral constituent of legumes is dependent on the soil edaphic features as well as the efficacy of mineral uptake (Vadivel and Janardhanan 2002). The mineral composition of

167  Pigeon pea can be considered to be present in adequate quantities when compared with the
168 Recommended Dietary Allowances, which infers that any diet containing two-thirds of the
169 Recommended Dietary Allowances is deemed as a potential source of minerals.

170 **Amino Acids**

171 The application of gamma irradiation did not have positive effect on the amino acids of the pigeon pea
172 flour, it showed a significant reduction at all of the delivered doses ($P < 0.05$). In Table 3-2, the amino acid
173 composition of the raw and gamma irradiated pigeon pea flour has been compared with the FAO-WHO
174 (1991) reference patterns and soybeans (Bau *et al.*, 1994). The acidic amino acids, glutamic acid and
175 aspartic acid, were the major amino acids present in the flour. The sulfur-amino acids cysteine and
176 methionine gave a value of 2.38% compared with the FAO/WHO pattern of 2.5% and tyrosine and
177 phenylalanine gave 8.78% against 6.3% of FAO/WHO. Except for tryptophan, the rest of the essential
178 amino acid in the raw and gamma irradiated samples are in line with the Food and Agricultural
179 Organization-World Health Organization-United Nations University (FAO-WHO-UNU) (1985) pattern.
180 The tyrosine in the raw flour was higher than soybeans (2.63 - 1.24 %), but lower in the irradiated sample
181 compared with soybeans (1.00, 1.15 and 1.24 ). The changes in the concentration of amino acids
182 induced by irradiation may probably be due to free radicals that might be formed in association with
183 splitting of the peptide bonds, deamination and decarboxylation reactions of amino acids followed by
184 chains of chemical reactions forming other new radicals (Elias and Cohen, 1977). This finding is not in line
185 with the report of Abdel-Ghaffar *et al.*, 2013, who reported an increase in some amino acid content of soy
186 flour. According to Baudoin and Maquet (1999), ecological conditions are known to markedly influence
187 the total nitrogen of seeds, which in turn affect the relative proportions of the essential amino acid. The
188 essential amino acid score and the PDCAAS were also significantly decreased with irradiation ($P < 0.05$),
189 indicating the limitation of available amino acids on digestion with enzymes. Even though gamma

irradiation decreased the essential amino acid of the pigeon pea flour, the irradiated seed flour still fulfilled the FAO-WHO (Brand *et al.*, 1994) essential amino acid requirement pattern.

In vitro protein digestibility

Table 3.3 presents the EAA, IVPD and PDCAAS of raw and irradiated pigeon pea flour. Cystine and methionine were the limiting amino acids in raw flour, which further decreased on irradiation ($P < 0.05$). The IVPD of raw flour (60.67%) was significantly affected by irradiation doses. There was a dose increase in IVPD through all the irradiated samples (64 to 75%). The increase can be attributed to the degradation of proteins into fractions susceptible to enzymes or partial destruction of trypsin inhibitors. Similarly, decrease in some of the major anti-nutrients (e.g. phenols, protease inhibitors, phytates, oxalates, lectins, goitrogens) might have contributed to increase IVPD. Abu-Tarboush (1998) correlated the elevated IVPD of soybean defatted flour with decreased anti-nutrients such as trypsin and chymotrypsin. The PDCAAS also revealed a significant dose-dependent decrease on irradiation ($P < 0.05$), indicating the limitation of available amino acids on digestion with enzymes.

Vitamins

Table 3.4 summarizes the vitamins (β -carotene, thiamin, riboflavin, niacin and ascorbic acid) content of both raw and irradiated pigeon pea flour (*Cajanus cajan*). A slight decrease in vitamin β -carotene occurred throughout the doses of irradiation and the decrease became significant in ascorbic acid at doses of 15 and 20 kGy. For un- irradiated sample of Vit A and the ascorbic acid, the values were 469.67 and 25.00mg/100g, respectively. The irradiated samples showed a dose dependent decrease, although the decrease was slight from 5 kGy to 20 kGy. Vit A (463.83-458.95mg/100g), Vit B1 (0.25-0.18mg/100g), Vit B2 (0.27-0.21mg/100g), Vit B3 (2.99-2.86mg/100g) and Vit C (24.00-18.69mg/100g). From the result, it showed that pigeon flour is richer in β -carotene and ascorbic acid. The remaining vitamins; thiamine, riboflavin and niacin followed the same trend as the first two vitamins. The result followed the report of Tresina *et al.* (2012) who reported a significant decrease in vitamins (niacin and ascorbic acid)

214 which occurred only at high doses of irradiation. The reduction in the vitamins may be due to the ionizing
215 radiation reacts with water in the food, causing the release of electrons and the formation of highly
216 reactive free radicals. The free radicals interact with vitamins in ways that can alter and degrade their
217 structure and/or activity (Murano, 1995).

218 **Conclusion**

219 The observations about amino acids profile, minerals and some vitamins present in pigeon pea (*Cajanus*
220 *cajan*) flour in the studied samples tend to suggest that radiation processing up to 20 kGy had little or no
221 effects (amino acids profile and vitamins) on their values and had no effect on the minerals of the flour.
222 Therefore, radiation can be applied to alleviate any problem relating to production of the flour. Moreover,
223 radiation process when compared to chemicals or heat treatment emerges as an attractive and healthy
224 alternative.

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307 Table 3.1, Effects of Gamma Irradiation on Mineral content of Pigeon Pea Flour (mg/100g)

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Dose kGy	Minerals						
	Na	K	Fe	Cu	Zn	Mn	Ca
0	6.99±0.03 ^c	38.80±0.27 ^a	8.30±0.10 ^b	4.57±0.06 ^b	28.57±0.20 ^b	14.33±0.20 ^a	119.67±0.12 ^a
5	5.95±0.05 ^a	40.13±0.06 ^b	7.07±0.06 ^a	5.17±0.15 ^{bc}	31.73±0.15 ^d	14.10±0.10 ^a	120.90±0.85 ^a
10	6.70±0.20 ^b	40.12±0.02 ^b	8.43±0.20 ^b	4.73±0.12 ^d	30.57±0.08 ^c	18.67±0.58 ^c	121.53±1.16 ^a
15	7.07±0.15 ^c	40.12±0.08 ^b	8.7±.60.58 ^{bc}	5.10±0.10 ^b	27.70±0.10 ^a	15.43±0.15 ^b	121.33±2.40 ^a
20	7.09±0.17 ^c	40.15±0.06 ^b	9.37±0.058 ^d	5.47±0.06 ^d	33.30±0.10 ^c	18.53±0.12 ^c	122.13±2.16 ^a

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310 Values are means and standard deviation of three determinations (n=3)

311 Means followed by the same letter within the same column are not significantly (p>0.05) different
312 according to LSD test

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314 Table 3.2 Amino acid profile of Pigeon pea flour treated with gamma irradiation (mg/100 mg crude
315 protein).

Amino Acids	Dose					FAO/WHO Pattern ^a	Soybean ^b
	Control	5 kGy	10 kGy	15 kGy	20 kGy		
Glutamic acid	9.23	5.06	4.97	4.88	4.82		16.9 ^c
Aspartic acid	11.56	6.59	6.44	6.37	6.34		11.3 ^d
Serine	3.59	2.49	2.47	2.36	2.32		5.67
Threonine	3.12	2.41	2.41	2.32	2.30	3.4	3.76
Proline	3.17	2.22	2.20	2.18	2.16		4.86
Alanine	3.79	2.86	2.81	2.75	2.65		4.23
Glycine	3.07	2.17	2.15	2.06	2.02		4.01
Valine	5.85	3.45	3.42	3.40	3.35	3.5	4.59
Cystine	1.19	0.12	0.10	0.08	0.06	2.5 ^e	1.70
Methionine	1.19	0.08	0.04	0.02	0.01		1.22
Isoleucine	3.47	2.47	2.40	2.35	2.30	2.8	4.62
Leucine	6.78	4.50	4.44	4.32	4.30	6.6	7.72
Tyrosine	2.63	1.15	1.05	1.03	1.00	6.3 ^f	1.24
Phenylalanine	6.15	4.01	3.98	3.88	3.78		4.84
Tryptophan	ND	ND	ND	ND	ND	1.1	3.39
Lysine	7.79	5.72	5.45	5.20	5.15	5.8	6.08
Histidine	3.66	2.12	2.09	2.09	2.07	1.9	2.50
Arginine	5.86	3.89	3.76	3.54	3.43		7.13
Total amino acid	82.10	51.31	50.18	48.83	48.06		
Total EAA	41.83	29.92	29.14	28.23	27.75		

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317 Notes: Data presented as the mean, n=3. ND, not determined. ^aFAO/WHO (1991). ^bBau et al. (1994).
318 ^cGlutamic acid_glutamine. ^dAspartic acid_asparagine. ^eMethionine_cystine. ^fPhenylalanine_tyrosine.
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320 Table 3.3 Essential amino acid score and protein digestibility corrected amino acid score ^a of gamma-
321 irradiated Pigeon pea flour.

EAA	Dose				
	Control	5 kGy	10 kGy	15 kGy	20 kGy
EAA Score					
Threonine	91.76	70.88	70.88	68.24	67.64
Valine	167.14	98.57	97.71	97.14	95.57
Cystine +	95.20	8.00	5.60	4.00	2.80
Methionine					
Isoleucine	123.93	88.21	85.71	83.93	82.14
Leucine	102.72	68.18	67.27	65.46	65.15
Tyrosine +	139.37	81.91	79.84	77.94	75.87
Phenylalanine					
Lysine	134.31	98.62	93.97	89.66	88.79
Histidine	192.63	111.58	110.00	110.00	108.95
IVPD (%)	60.67±1.60 ^a	64.00±1.73 ^b	69.00±1.00 ^c	72.33±1.53 ^d	75.00±2.00 ^e
PDCAAS (%)					
Threonine	45.61	35.23	35.23	33.91	33.62
Valine	83.07	48.99	48.56	48.28	47.57
Cystine +	47.31	39.80	27.80	19.90	15.90
Methionine					
Isoleucine	61.59	43.84	42.60	41.71	40.83
Leucine	51.06	33.89	33.44	32.53	32.38
Tyrosine +	69.26	40.71	39.68	38.73	37.71
Phenylalanine					
Lysine	66.75	49.02	46.70	44.56	44.13
Histidine	95.74	55.46	54.67	54.67	54.15

322 ^a Calculated according to FAO/WHO (1991).

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Table.3.4 Vitamins content of Pigeon Pea (*Cajanus cajan*) flour treated with gamma irradiation
(mg 100 g⁻¹)

Components	Dose				
	Control	5 kGy	10 kGy	15 kGy	20 kGy
Carotene (Vit A)	469.67± 2.08 ^b	463.83± 2.02 ^a	462.00± 2.00 ^a	460.21± 2.19 ^a	458.95± 1.69 ^a
Thiamin (Vit B1)	0.30± 0.20 ^b	0.25± 0.50 ^{ab}	0.19±0.03 ^a	0.19± 0.03 ^a	0.18±0.02 ^a
Riboflavin (Vit B2)	0.31± 0.04 ^b	0.27±0.03 ^{ab}	0.22±0.02 ^a	0.21±0.03 ^a	0.21±0.03 ^a
Niacin (Vit B3)	3.20± 0.20 ^b	2.99±0.10 ^{ab}	2.98±0.20 ^{ab}	2.90±0.04 ^a	2.86±0.09 ^a
Ascorbic acid (Vit C)	25.00± 1.00 ^b	24.00±2.00 ^b	22.00±2.00 ^{ab}	20.08±0.88 ^a	18.69±1.52 ^a

Means ± SE (N = 3) means in the column with unlike superscript differs significantly (p<0.05)