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Research Article

Comparative studies of antioxidants and amino-acids concentrations in the fruits of two cultivars of watermelon (*Citrullus lanatus* L.)

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Abstract

Importance of fruits and vegetables in diet and health cannot be overemphasized. Cultivation practices can however influence the nutritional compositions of fruits and vegetables. Hence, field and laboratory experiments were carried out to determine and compare the antioxidants and amino-acids concentrations of a hybrid and an indigenous cultivar of watermelon in Osun State University, Teaching and Research Farm, Nigeria. Influence of NPK 15:15:15 fertilizer at different rates (0, 100, 200 and 300 kg/ha) was evaluated on 'Kaolack' (hybrid) and 'Rothmas' (local) cultivars of watermelon. 200 kg/ha of the fertilizer was found to bring out the optimal yield of both cultivars. Hence, at 0 kg/ha and 200 kg/ha, mature fruits of the cultivars were analyzed for the antioxidants and amino-acids using standard analytical methods. Antioxidant properties of 'Rothmas' were found to be significantly (P≤0.05) higher than those of 'Kaolack' with the exception of the radical scavenging ability and lycopene content of 'Kaolack'. Fruits that received no fertilizer across the cultivars exhibited more antioxidant potency compared to fruits under 200 kg. Results of amino acids concentration showed that, threonine, serine, glutamic-acid, alanine, valine, phenylalanine and arginine in 'Rothmas' were significantly higher compared to the contents in 'Kaolack'. Except for tryptophan, alanine, lysine and phenylanine, fruits that received no fertilizer had higher contents of amino acids. Where fertilizer is needed to boost soil fertility, 100 kg/ha of NPK is recommended, this can be augmented with the organic nutrient or green manure if necessary, depending on the available nutrients in the soil before cultivation. This is very critical so as not to compromise the health-giving substances in the fruits. Local landraces of fruits and vegetables obviously have to be brought into limelight through research.

Keywords: cultivar; diet; health substances; protein; vegetable; watermelon

Introduction

Watermelon (*Citrullus lanatus*) belongs to the family Cucurbitaceae. It is native to Africa and was then introduced to Asia, Europe, and the Americas. Even though watermelon is commonly classified as a vegetable, it is botanically considered as a fruit and used primarily as a dessert (Chafik *et al.*, 2020). Watermelon contains carotenoid, a precursor of vitamin A which is very important to the body. β -carotene has a protective role

against cancer (Halter, 1989) and coronary heart disease (Guoyao *et al.*, 2009). Watermelon also contains antioxidants, flavonoids, lycopene, pytofluene, lutein, neurosporene, zeaxanthin, phytoene and cryptoxanthin (De Lannoy, 2001). Potassium is also available in watermelon, which helps in the control of blood pressure and to prevent stroke (IITA, 2013).

Agronomic practices such as fertilizer application (type and amount), cultivar selection, planting season/date, etc. however influence not only crop yield but also the health substances contained in fruits and vegetables (Oloyede *et al.*, 2012; Oloyede *et al.*, 2014; Oloyede *et al.*, 2015). Hence, this study determined the effect of NPK fertilizer rates and cultivar types on the antioxidants and amino acids of *Citrullus lanatus*.

Materials and Methods

Field study

Field experiment was carried out from September to December, 2016 at the Teaching and Research Farm of Osun State University, Nigeria. The experimental design used was a two factorial experiments, as a randomized complete block design (RCBD), in three replicates. At the experimental site, the soil had total N of 1.58%, K was 4.25 cmol/kg, while P value evaluated in ppm was 19. Influence of NPK 15:15:15 fertilizer at different rates (0, 100, 200 and 300kg/ha) was evaluated on 'Kaolack' (hybrid) and 'Rothmas' (local) cultivars of watermelon. 200 kg/ha of the fertilizer was found to bring out the optimal yield of both cultivars. Hence, at 0 kg/ha and 200 kg/ha, mature fruits of the cultivars were analyzed for the antioxidants and essential aminoacids using standard analytical methods.

Determination of selected antioxidant properties

The radical scavenging ability of the fruit extract was determined using the stable radical DPPH (2, 2-diphenyl-1-picrylhydrazyl hydrate) as described by (Brand-Williams $\it et al.$, 1995). The reaction of DPPH with an antioxidant compound which can donate hydrogen, leads to its reduction (Blois, 1958). The change in color from deep violet to light yellow was measured spectrophotometrically at 517nm. The proanthocyanidin content of the extract was determined spectrophotometrically. Extracts were diluted to provide a spectrophotometric reading between 0.1 and 0.8 absorbance units. Carotenoid was measured using the method of Godwin $\it et al.$ (2015). 500 $\it pl$ of testing sample is taken in a test tube and added with 5 ml of the stock solution. BHT (90 $\it pg/ml$) is employed as a positive control agent. The absorbance of the mixture is noted at 470 nm. The reaction mixture is then incubated for 2 h at 50 °C. After incubation the absorbance was measured again at 470 nm (t = 120 min). Total lycopene was measured according to the method of Fish $\it et al.$ (2002). The method of determining the total phenolic content was described by Singleton and Rossi (1965) using the Folin-Ciocalteu's phenol reagent which is an oxidizing reagent.

Amino acids determination

Amino acid content was determined based on the method described by Moore *et al.* (1958) using Technicon Sequential Multi-sample (TSM) Amino Acid Analyzer, Model GFL 1083 (Technicon Instruments Corporation), Germany and with nor leucine as an internal standard. The hydrolysate was vacuum dried to remove buffer solution before loading into the TSM. Compressed nitrogen was passed into the TSM to serve as a segmented stream flow of the amino acid which helped the analyzer to detect any amino acid found without mixing up the amino acids.

Results and Discussion

Regarding the antioxidant properties of the two cultivars of watermelon fruits, the radical scavenging ability of 'Kaolack' was 92.3% while that of 'Rothmas' was 78.9% (Table 1). Lycopene content was higher in 'Kaolack' compared to 'Rothmas'. However, the phenol and proanthocyanidin were higher in 'Rothmas' compared to 'Kaolack'. Both cultivars had same carotenoid content (0.07 g/100 g). Moreover, both cultivars of watermelon evaluated have abundant antioxidant properties. Oxidation, a chemical reaction that transfers electrons from a substance to an oxidizing agent, produces free radicals (Muller *et al.*, 2007) and initiates chain reactions that may damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, inhibit other oxidation reactions, or enhance the endogenous antioxidant defenses of the organism (Lü *et al.*, 2010). Consumption of fruits and vegetables which contain these compounds have been scientifically validated to help slowdown the aging process and reduce the risk factors of many diseases including cancer, heart disease, stroke, high blood pressure, cataracts, osteoporosis, diabetes and urinary tract infections (Liu *et al.*, 2004; Barjesteh *et al.*, 2007).

Table 1. Cultivar effect on the antioxidant concentration of watermelon on wet weight basis

Cultivars	DPPH (%)	Proantocyanidin (Mg/ml)	Carotenoid (%)	Lycopene (Mg/kg)	Phenol (Mg GAE/g)
'Rothmas'	78.79 ^b	9.53ª	0.07^{a}	3.08 ^b	2.86ª
'Kaolack'	92.31ª	6.92 ^b	0.07^{a}	4.16 ^a	1.60 ^b
LSD (0.05)	8.29	0.88	NS	0.26	0.06

Table 2 showed the effect of NPK fertilizer on the antioxidant properties of watermelon fruits. The radical scavenging abilities of the fruits that received 0 and 200 kg/ha were 88% and 83% respectively, though not statistically significantly different (P≤0.05). Proanthocyanidin and carotenoid contents did not differ statistically as well. Phenol and lycopene contents of the fruits that received no fertilizer were higher than those that received 200 kg/ha. Similar trends were observed in the antioxidant activities of pumpkin fruits. Application of 0 to 100 kg/ha of NPK 15:15:15 were recommended as opposed to higher rates (150-250 kg/ha) which were found to compromise the phenol, flavonoid, anthocyanin and proanthocyanidin concentrations of pumpkin fruits (Oloyede *et al.*, 2012; Oloyede *et al.*, 2014).

Table 2. NPK effect on the antioxidant concentration of watermelon on wet weight basis

NPK	DPPH	Proantocyanidin	Carotenoid	Lycopene	Phenol
	(%)	Mg/ml	%	Mg/kg	Mg GAE/g
0	83.04ª	8.62ª	0.09^{a}	3.89ª	2.69ª
200	88.05ª	7.82^{a}	0.05ª	$3.34^{\rm b}$	1.77 ^b
LSD (0.05)	NS	0.88	NS	0.26	0.06

Table 3 revealed the effects of cultivars on the essential amino-acids concentrations (mg/100 g) in watermelon. Respective values of 2.07 and 2.19 of tryptophan in 'Rothmas' and 'Kaolak' cultivars were observed. 'Kaolack' cultivar had statistically higher concentrations of methionine (6.00), isoleucine (21.04), leucine (24.91) and lysine (58.54). Threonine and phenylanine contents did not differ statistically in the two cultivars. Table 4 showed the effects of cultivars on the non-essential amino-acids concentrations (mg/100 g) in watermelon. 'Rothmas' had statistically higher glutamic acid content (75.08) while 'Kaolack' had statistically higher proline (29.03), tyrosine (2.48) and aspartic acid (49.22). Serine, glycine, alanine, cysteine, and arginine contents did not differ statistically in the two cultivars. Similar report was given on cultivar effects on snake tomato (*Trichosanthes cucumerina*) chemical compositions (Oloyede and Adebooye 2005).

Table 3. Cultivar effect on the essential amino acid concentration of watermelon on wet weight basis

Cultivar	Tryptophan	Threonine	Valine	Methionine mg/100g	Isoleucine	Leucine	Phenylanine	Lysine
'Rothmas'	2.07^{b}	34.64ª	21.14 ^a	5.54 ^b	19.60 ^b	23.36 ^b	2.17 ^a	57.41 ^b
'Kaolack'	2.19 ^a	34.02 ^a	20.61 ^b	6.00 ^a	21.04^{a}	24.91ª	2.16 ^a	58.54ª
LSD (0.05)	0.01	2.06	0.21	0.14	0.66	0.99	0.02	0.93

Table 4. Cultivar effect on the non-essential amino acid concentration of watermelon on wet weight basis

Cultivars	Serine	Glutamic acid	Proline	Glycine	Alanine mg/100g	Cysteine	Tyrosine	Arginine	Aspartic acid
'Rothmas'	20.97ª	75.08ª	27.91 ^b	13.07 ^a	22.38ª	2.22ª	2.36 ^b	62.99ª	46.99 ^b
'Kaolack'	20.50 ^a	73.52 ^b	29.03 ^a	13.67 ^a	22.10 ^a	2.22ª	2.48a	63.12ª	49.22a
LSD (0.05)	0.64	1.24	0.93	0.97	0.59	0.04	0.02	0.93	0.7

Table 5 revealed the effects of NPK 15:15:15 fertilizer on the essential amino-acids concentrations (mg/100 g) in watermelon. Respective values of 55.64 and 60.31 of lysine in fruits under 0 and 200 kg/ha of NPK were observed. Fruits under 0 kg/ha of fertilizer had statistically higher concentrations of methionine (6.10), isoleucine (20.77), leucine (24.89) and valine (21.14), while tryptophan, phenylanine and lysine with respective values of 2.15, 2.21 and 60.31 were statistically higher in fruits under 200 kg/ha. Threonine content was indifferent to fertilizer application.

Table 5. NPK effect on the essential amino acid concentration of watermelon on wet weight basis

NPK	Tryptophan	Threonine	Valine	Methionine mg/100g	Isoleucine	Leucine	Phenylanine	Lysine
0	2.11 ^b	33.77ª	21.14 ^a	6.10 ^a	20.77^{a}	24.89a	2.11 ^b	55.64 ^b
200	2.15ª	34.89 ^a	20.61 ^b	5.44 ^b	19.88 ^b	23.34 ^b	2.21 ^a	60.31 ^a
LSD (0.05)	0.01	2.06	0.21	0.14	0.66	0.99	0.02	0.93

Table 6 showed the effects NPK 15:15:15 fertilizer on the non-essential amino-acids concentrations (mg/100 g) in watermelon. Fruits under 0 kg/ha of fertilizer had statistically higher concentrations of serine (21.44), glutamic acid (75.37) proline (29.62), cysteine (2.28) and tyrosine (2.51). Alanine (23.08) and aspartic acid (48.30) were however statistically higher in fruits under 200 kg/ha. Arginine and glycine contents were not influenced statistically fertilizer application. Amino acids are organic compounds composed of nitrogen, carbon, hydrogen and oxygen, along with a variable side chain group. The body needs different amino acids to grow and function properly.

Table 6. NPK effect on the non-essential amino acid concentration of watermelon on wet weight basis

NPK	Serine	Glutamic acid	Proline	Glycine mg/100g	Alanine	Cysteine	Tyrosine	Arginine	Aspartic acid
0	21.44 ^a	75.37 ^a	29.62ª	13.38ª	21.40 ^b	2.28ª	2.51a	64.75ª	47.91 ^b
200	20.02 ^b	73.23 ^b	27.32 ^b	13.36ª	23.08ª	2.16 ^b	2.33 ^b	61.36ª	48.30 ^a
LSD (0.05)	0.64	1.24	0.93	0.97	0.59	0.04	0.02	0.93	0.7

Note: Means on the same column with different letters are significantly different (P<0.05).

NS: Not significantly different at (P<0.05).

Unlike nonessential amino acids, essential amino acids can't be made by the body and must be obtained through diets. When protein is eaten, it is broken down into amino acids, which are then used to help the body with various processes such as building muscle and regulating immune function (Health line, 2021). According to Oloyede *et al.* (2015) agronomic input especially fertilizer types and rates influence the health substances in fruits and vegetables and there is a need to apply moderate dose to boost the yield while the health substances in the plants remain uncompromised.

Conclusions

Both 'Rothmas' and 'Kaolack' cultivars of watermelon evaluated have abundant antioxidant properties. There is a need to bring the local landraces of fruits and vegetables obviously into limelight through research. 200 kg/ha of NPK 15:15:15 limited some of the concentrations of antioxidants and amino-acids parameters evaluated at the location where this study was carried out. Where fertilizer is needed to boost soil fertility, 100 kg/ha of NPK is recommended, this can be augmented with the organic nutrient or green manure if necessary, depending on the available nutrients in the soil before cultivation. This is very critical so as not to compromise any of the health-giving substances in the fruits.

Authors' Contributions

Conceptualization: F.M.; Data curation; Formal analysis; Investigation; Methodology: F.M and A.B.; Project administration: F.M.; Laboratory analyses: G.G.; Resources: The 3 authors; Supervision F.M.; Writing: F.M. and A.B.; Writing review and editing: The 3 authors. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Ak T, Gulcin L (2008). Antioxidant and radical scavenging properties of *curcumin*. Chemico-Biological Interactions 174(1):27-37. https://doi.org/10.1016/j.cbi.2008.05.003

- Artés-Hernández F, Robles PA, Gómez PA, Tomás Callejas A, Artés F (2010). Low UV-C illumination for keeping overall quality of fresh-cut watermelon. Postharvest Biology and Technology 55(2):114-120. https://doi.org/10.1016/j.postharvbio.2009.09.002
- van Waalwijk van Doorn-Khosrovani SB, Janssen J, Maas LM, Godschalk RW, Nijhuis JG, van Schooten FJ (2007). Dietary flavonoids induce MLL translocations in primary human CD34+ cells. Carcinogenesis 28(8):1703-1709. https://doi.org/10.1093/carcin/bgm102
- Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidants activity. LMT Food Science and Technology 28:25-30. https://doi.org/10.1016/S0023-6438(95)80008-5
- Chafik H, Imen T, Riadh I (2020). Watermelon. In: Jaiswal AK (Ed). Nutritional composition and antioxidant properties of fruits and vegetables. Academic Press, pp 515-531. https://doi.org/10.1016/B978-0-12-812780-3.00032-5
- Charoensiri R, Kongkachuichai R, Suknicom S, Sungpuag P (2009). Beta-carotene, lycopene, and alpha-tocopherol contents of selected Thai fruits. Food Chemistry 113(1):202-207. https://doi.org/10.1016/j.foodchem.2008.07.074
- De Lannoy (2001). Crop Production in Tropical Africa. Romain HR (Ed). Published by Directorate General for International Cooperation (DGIC), Brussels, Belgium, pp 236-238.
- Edwards AJ, Vinyard BT, Wiley ER, Brown ED, Collins JK, Perkins-Veazie P, ... Clevidence BA (2003). Consumption of watermelon juice increases plasma concentrations of lycopene and β-carotene in humans. The Journal of Nutrition 133(4):1043-1050. https://doi.org/10.1093/jn/133.4.1043
- Grotewold E (2006). The genetics and biochemistry of floral pigments. Annual Review in Plant Biology 57:761-780. https://doi.org/10.1146/annurev.arplant.57.032905.105248
- Fish WW, Davis AR (2003). The effects of frozen storage conditions on lycopene stability in watermelon tissue. Journal of Agricultural and Food Chemistry 51(12):3582-3585. https://doi.org/10.1021/jf030022f
- Wu G, Bazer FW, Davis TA, Kim SW, Li P, Marc Rhoads J, ... Yin Y (2009). Arginine metabolism and nutrition in growth, health and disease. Amino Acids 37(1):153-168. https://doi.org/10.1007/s00726-008-0210-y
- Floriano-Sánchez E, Floriano-Sánchez E, Villanueva C, Noel Medina-Campos O, ... Pedraza-Chaverrí J (2006). Nordihydroguaiaretic acid is a potent in vitro scavenger of peroxynitrite, singlet oxygen, hydroxyl radical, superoxide anion and hypochlorous acid and prevents in vivo ozone-induced tyrosine nitration in lungs. Free Radical Research 40(5):523-533. https://doi.org/10.1080/10715760500419365
- Haces ML, Hernández-Fonseca K, Medina-Campos ON, Montiel T, Pedraza-Chaverri J, Massieu L (2008). Antioxidant capacity contributes to protection of ketone bodies against oxidative damage induced during hypoglycemic conditions. Experimental Neurology 211(1):85-96. https://doi.org/10.1016/j.expneurol.2007.12.029
- Halliwell B, Gutteridge JM, Aruoma OI (1987). The deoxyribose method: a simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. Analytical Biochemistry 165(1):215-219. https://doi.org/10.1016/0003-2697(87)90222-3
- Halter SA (1989). Vitamin A: its role in the chemoprevention and chemotherapy of cancer. Human Pathology 20(3):205-209. https://doi.org/10.1016/0046-8177(89)90124-X
- Health Line (2021). Retrieved 2021 December 1st from: https://www.healthline.com/nutrition/essential-amino-acids#roles-in-your-body
- Hossain MA, Asada K (1985). Monodehydroascorbate reductase from cucumber is a flavin adenine dinucleotide enzyme. Journal of Biological Chemistry 260(24):12920-12926. https://doi.org/10.1016/S0021-9258(17)38813-0
- Huh YC, Choi HS, Solmaz I, Sari N, Kim S (2014). Morphological characterization of Korean and Turkish watermelon germplasm. Korean Journal of Agricultural Science 41(4):309-314. https://doi.org/10.7744/cnujas.2014.41.4.309
- IITA (2013). Growing watermelon commercially in Nigeria- an illustrated guide. International Institute of Tropical Agriculture (IITA), pp 1-16.
- Ilic D, Misso M. (2012). Lycopene for the prevention and treatment of benign prostatic hyperplasia and prostate cancer: A systematic review. Maturitas 72:269-276. https://doi.org/10.1016/j.maturitas.2012.04.014
- Kang HS, Kim KR, Jun EM, Park SH, Lee TS, Suh JW, Kim JP (2008). Cyathuscavins A, B, and C, new free radical scavengers with DNA protection activity from the Basidiomycete *Cyathus stercoreus*. Bioorganic & Medicinal Chemistry Letters 18(14):4047-4050. https://doi.org/10.1016/j.bmcl.2008.05.110
- Liu RH (2004). Potential synergy of phytochemicals in cancer prevention: mechanism of action. The Journal of Nutrition 134(12):3479S-3485S. https://doi.org/10.1093/jn/134.12.3479S

- Lü JM, Lin PH, Yao Q, Chen C (2010). Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. Journal of Cellular and Molecular Medicine 14(4):840-860. https://doi.org/10.1111/j.1582-4934.2009.00897.x
- Muller FL, Lustgarten MS, Jang Y, Richardson A, Van Remmen H (2007). Trends in oxidative aging theories. Free Radical Biology and Medicine 43(4):477-503. https://doi.org/10.1016/j.freeradbiomed.2007.03.034
- Obadoni BO, Ochuko PO (2002). Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. Global Journal of Pure and Applied Sciences 8(2):203-208. https://doi.org/10.4314/gjpas.v8i2.16033
- Okwu DE, Okwu ME (2004). Chemical composition of *Spondias mombin* Linn plant parts. Journal of Sustainable Agriculture and Environment 6(2):140-147.
- Oloyede FM, Adebooye OC (2005). Effect of season on growth, fruit yield and nutrient profile of two landraces of *Trichosanthes cucumerina* L. African Journal of Biotechnology 4(6):1040-1044.
- Oloyede FM, Agbaje GO, Obuotor EM, Obisesan IO (2012). Nutritional and antioxidant profiles of pumpkin (*Cucurbita pepo* Linn.) immature and mature fruits as influenced by NPK fertilizer. Food Chemistry 135(2):460-463. https://doi.org/10.1016/j.foodchem.2012.04.124
- Oloyede FM, Adebooye OC, Obuotor EM (2014). Planting date and fertilizer affect antioxidants in pumpkin fruit. Scientia Horticulturae 168:46-50. https://doi.org/10.1016/j.scienta.2014.01.012
- Oloyede FM, Adebooye OC, Agbaje GO (2015). Fertilizer, crop yield and bioactive compounds. In: Shishir S, Pant KK, Bajpai S (Eds). Fertilizer Technology. Vol. 1. Studium Press LLC, pp 42-58.
- Rhodes B, Zhang X (1995). Gene list for watermelon. Cucurbit Genet Coop.
- Thulaja NR (2005). Watermelon, National library board, Singapore North Carolina University. Watermelon, a paper presented at Charpel Hill.
- USDA (2002). Food and Nutritonl information centre. National Agricultural library, United states Department of Agriculture. http://fnic.nal.usda.gov/6
- Van den Berg H, Faulks R, Granado HF, Hirschberg J, Olmedilla B, Sandmann G, ... Stahl W (2000). The potential for the improvement of carotenoid levels in foods and the likely systemic effects. Journal of the Science of Food and Agriculture 80(7):880-912. <a href="https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<880::AID-JSFA646>3.0.CO;2-1">https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<880::AID-JSFA646>3.0.CO;2-1
- Zhou K, Raffoul JJ (2012). Potential anticancer properties of grape antioxidants. Journal of Oncology 803294. https://doi.org/10.1155/2012/803294





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