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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Effect of Thermal Processing on Antinutrients in Common Edible Green Leafy Vegetables Grown in Ikot Abasi, Nigeria

Imaobong Inyang Udousoro, Roland U. Ekop and Efiok Johnson Udo
Department of Chemistry, University of Uyo, Akwa, Ibom State, Nigeria

Abstract: The effects of time and temperature variation on the levels of anti-nutrients in five popularly consumed green leafy vegetables in Akwa Ibom State, Nigeria, were investigated. The vegetables were obtained from Ikot Abasi, Akwa Ibom State in Nigeria, and they included Utazi leaf (*Gongronema latifolium*), Bitter leaf (*Vernonia amygdalina*), Curry leaf (*Ocimum canum sims*) Bush apple leaf (*Heinsia crinata*) and Waterleaf (*Talinum triangulare*). The vegetables were subjected to heating conditions of 50°C and 90°C for 5 minutes and 15 minutes, and the levels of selected anti-nutrients (phytate, cyanides, tannins, oxalates) assessed. Heating conditions reduced the levels of all the investigated anti-nutrients in vegetables in the studies; the greatest reduction in level was observed in oxalates. In the vegetables, *Talinum triangulare* recorded the greatest reduction in anti-nutrients levels, tannins (84.4%), phytates (84.1%), cyanides (35.8%), total oxalate (57.1%) and soluble oxalate (75%). Heating at 90°C for 15 minutes was the most impacting for all the vegetables. The reduction of tannins ranged from 25.4 to 84.4%, phytates from 43.7 to 84.1%, cyanides from 22.1 to 40.7%, total oxalates from 57.1 to 75.0%, soluble oxalates from 66.7 to 78.3%. This study reveals that the most effective thermal processing temperature and time for anti-nutrient reduction in the selected vegetables was 90°C for 15 minutes. The data generated will help dieticians and other health workers in the formulation of different diets to meet the varied needs of patients in clinics and health establishments.

Key words: Green leafy vegetables,Nigerian diet, anti-nutrients, thermal processing

INTRODUCTION

Green leafy vegetables are an essential part of the Nigerian diet and about 60 different species of green leafy vegetables are consumed in Nigeria (Kubmarawa *et al*., 2009). These vegetables generally have high nutritional contents, and they are consumed for health and nutritional benefits (Otitoju *et al*., 2012). However, vegetables contain anti-nutritional factors that can affect the availability of nutrients to the human body. These anti-nutritional factors interfere with metabolic processes and reduce the bioavailability of nutrients from plants or plant products used as human foods (Abara, 2003; Agbaire and Emoyan, 2012). Plants generally contain chemical compounds (such as saponins, tannins, oxalates, phytates, trypsin inhibitors and cyanogenic glycosides) which are known as secondary metabolites and which are biologically active (Soetan and Oyewole, 2009). However, a substance cannot be classified as an anti-nutritional factor based on the intrinsic characteristic of the compound; an anti-nutrient exerts its adverse effects generally at the level of the digestive process of humans (Kumar, 1992).

Tannins are water-soluble phenolic compounds (Akande *et al*., 2010) that chelate Fe and Zn and limit the absorption of these nutrients. Tannins may precipitate proteins from aqueous solution by inhibiting digestive enzymes (Soetan and Oyewole, 2009) and have been found to interfere with digestion by displaying anti-trypsin and anti-amylase activity. Phytate decreases the bioavailability of proteins and essential elements such as Ca, Mg, Zn, Fe, and P by forming insoluble complexes, which are not readily absorbed by the gastrointestinal tract (Akande *et al*., 2010; Agbaire and Oyewole, 2012). Oxalates interfere with magnesium metabolism and react with proteins to form complexes, which have an inhibitory effect in peptic digestion (Akande *et al*., 2010). Oxalate binds to calcium to form insoluble calcium oxalate crystals; these prevent the absorption and utilization of calcium by the body thereby causing diseases such as rickets and osteomalacia (Ladeji *et al*., 2004; Agbaire, 2012). Cyanogens glycosides on hydrolysis yields toxic hydrogen cyanide (HCN). The cyanide ions inhibit several enzyme systems and depress growth through interference with certain essential amino acids and utilization of associated nutrients (Soetan and Oyewole, 2009). A high level of hydrogen cyanide has been implicated in cerebral damage and lethargy in man (Ekop, 2007; Agbaire and Emoyan, 2012).

Processing techniques can increase the nutritive quality of plant foods by reducing specific anti-nutrients. Cooking is the major form of processing vegetables in

Corresponding Author: Imaobong Inyang Udousoro, Department of Chemistry, University of Uyo, Akwa Ibom State, Nigeria

the South-South region of Nigeria. The direct effects of temperature and duration of processing on the reduction of anti-nutrients in vegetables therefore need to be investigated, since improper processing of plant food may expose humans to high levels of anti-nutrients. Thermal processing is one of the domestic techniques, which may reduce anti-nutrients in green leafy vegetables. However, the effect it has on degrading phytates depends on the plant species, temperature, and pH (Hotz and Gibson, 2007). Heat processing, specifically moist heat (cooking, extrusion, autoclaving and microwave) decreased to a substantial extent, the levels of hydrogen cyanide, tannins and phytate (Habiba, 2002; Embaby, 2010, 2011). In addition, the levels of phytate and tannins decreased significantly after roasting (Fagbemi *et al.*, 2005; Embaby, 2011). According to Yadav and Sehgal (2002), blanching of green leafy vegetables induces moderate losses of 5-15% of phytate in vegetables.

Furthermore, it is desirable to establish optimum time and temperature to process food in order to derive maximum health benefits for the country which, currently, is scanty. Such findings may help nutritionists and dieticians to formulate different diets to meet the varied needs of patients in clinics and health establishments. The effects of heat processing conditions at varied temperatures and times on anti-nutrients in five common edible leafy vegetables grown in Ikot Abasi, Nigeria are investigated in this study.

MATERIALS AND METHODS

Sample collection and identification: Five green leafy vegetables were harvested from farms in Ikot Abasi, in Akwa Ibom State of Nigeria, and were identified and classified in the Department of Botany and Ecological Studies in the University of Uyo (Table 1).

Sample preparation and treatment: The leaves of the vegetables were destalked, washed with tap water, then rinsed with distilled-deionized water, sliced and divided into five parts of 500 g each. Four parts of the sliced leaves (one part of sliced vegetable to four parts of distilled-deionized water) were heated at 50°C for 5, 50°C for 15 minutes, 90°C for 5 and 90°C for 15 minutes. The leaves were rinsed with cold distilled-deionized water to stop further heating, and then sun-dried. The remaining one part was sun-dried with no treatment. The dried vegetable leaves were ground into powder, passed through 0.5 mm sieve and then stored in airtight containers for analysis.

Determination of Anti-nutrients: Tannin was determined using the Folin-Dennis Spectrophotometric method described by Pearson (1976). The method described by Oberlease (1973) was used for phytate content determination. Hydrogen cyanide was estimated using alkaline extraction method described by AOAC (1990). Total and soluble oxalates were estimated using potassium permanganate titration method after extraction with HCl for total oxalate and water for soluble oxalate followed by precipitation as described by Baker (1952).

RESULTS AND DISCUSSION

Anti-nutrient levels in the untreated green leafy vegetables are summarized in Table 2. The results obtained were converted to percentage for easier comparison (Fig. 1-5).

Phytate molecule is negatively charged and is reported to bind essential, nutritionally important cations such as Fe, Zn, Mg and Ca (Embaby, 2011). This forms insoluble complexes, thereby making minerals unavailable for absorption (Frontela *et al.*, 2008). In the investigated green leafy vegetables, phytates levels are the lowest in the untreated samples, as presented in Table 2. The results obtained for untreated samples by Agbaire (2012) in the South-South part of Nigeria for *Vernonia amygdalina* and *Talinum triangulare* are notably lower than the values obtained in the present study (Table 3). Results reported by Adeboye and Babajide (2007) from the South Western part of Nigeria for *Vernonia amygdalina* and *Gongronema latifolium* are, however, considerably higher than those obtained in this study (Table 3). In all the samples, heating caused the phytate levels to decrease; some studies conducted revealed that phytate levels remain unchanged or increased after heat treatments (Yagoub and Abdalla, 2007; Martin-Cabrejas *et al.*, 2009; Embaby, 2010). The wide disparity in the anti-nutrient levels stems from the fact that the formation of anti-nutrients is genetically controlled (Franceschi and Nakata, 2005) and depends on factors such as the soil type, climate and fertilizer used.

Tannins, which range from 162.96 mg/100g to 427.16 mg/100g, are generally the most abundant anti-nutrients found in the vegetables (Table 2). Similar levels to those in the present study were reported by Onyeka and Nwambekwe (2007) for *Vernonia amygdalina* and *Gongronema latifolium* (Table 3).

From our investigations on the five leaf samples, it is observed that oxalates, especially soluble oxalates, have the highest reduction among the studied anti-nutrients.

Table 1: Investigated vegetables with herbarium identification number

Scientific name	English name (Local name)	Family	Herbarium No.
Gongronema latifolium	Not available (Utazi)	Asclepiadaceae	Udo, UUH 1573
Vernonia amygdalina	Bitter leaf (Etidot)	Asteraceae	Udo, UUH 1569
Ocimum canum sims	Curry leaf (Iko)	Labiatae	Udo, UUH 1561
Heinsia crinata	Bush apple leaf (Atama)	Rubiaceae	Udo, UUH 1572
Talinum triangulare	Waterleaf (Mmong-mmong ikong)	Portulaceae	Udo, UUH 1570

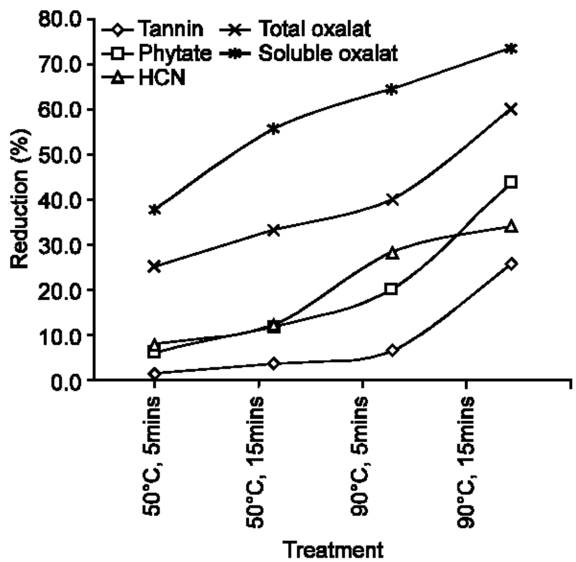

Fig. 1: Percentage reduction of anti-nutrients in *Gongronema latifolium*

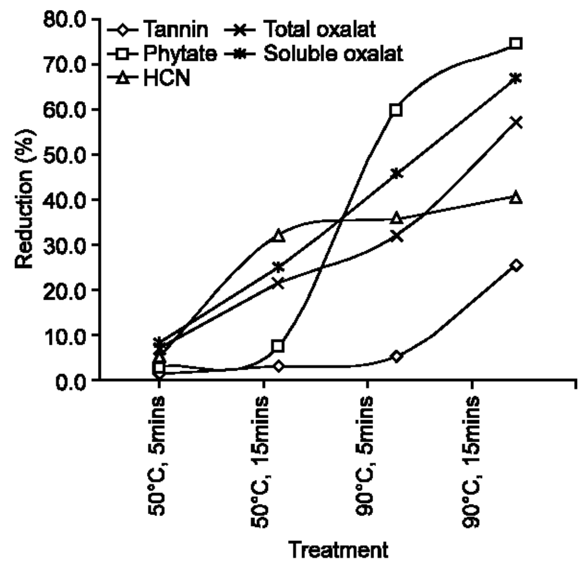

Fig. 3: Percentage reduction of anti-nutrients in *Ocimum canum sims*

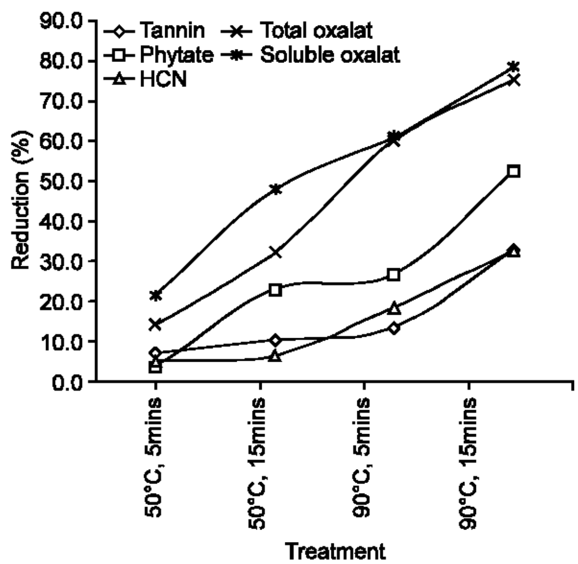

Fig. 2: Percentage reduction of anti-nutrients in *Vernonia amygdalina*

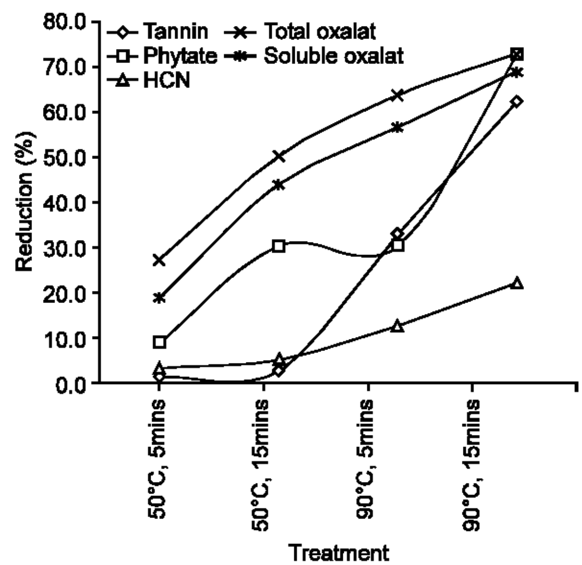

Fig. 4: Percentage reduction of anti-nutrients in *Heinsia crinata*

This could be attributable to the fact that soluble oxalates in the vegetable are removed when the vegetables are washed. However, Lisiewska *et al.* (2011) suggested that the decrease in the level of soluble oxalates by washing is nullified if the water containing the displaced oxalates is still used in cooking.

Gongronema latifolium: Samples of *Gongronema latifolium* (*utazi*) were found to have the lowest levels of anti-nutrients among the investigated green leafy vegetables. After heating for five minutes at 50°C, there is no significant reduction in the levels of the anti-nutrients except oxalates (Fig. 1); while the percentage reduction of tannin, phytates and HCN are less than 10%, oxalates reduced by greater than 25%. Generally, significant reduction of anti-nutrients occurs after heating the vegetable leaves for 15 minutes at 90°C, the reduction in tannin rises from 6.8 to 25.8%. Furthermore, phytates and oxalates are observed to reduce significantly after heating at 90° for 15 minutes. The optimum condition is deduced to be heating at 90°C for 15 minutes.

Vernonia amygdalina: There is a minimal decrease in the levels of the studied anti-nutrients in *Vernonia amygdalina* (*bitter leaf*) at 50°C. Tannin content in *Vernonia amygdalina* recorded mere 10.4% level of reduction on heating at 50° (Fig. 2). After heating for 15 minutes at 90°C, tannin is observed to reduce to 32.8%. The levels of cyanide are reduced when the sample was heated at 90°C. Thus, the optimum temperature for effectively reducing the levels of anti-nutrients is 90°C.

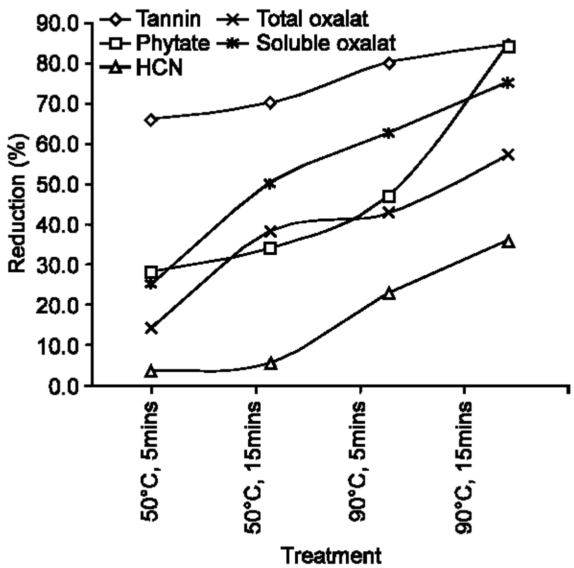

Fig. 5: Percentage reduction of anti-nutrients in Talinum triangulare

Ocimum canum sims: Among the green leafy vegetables investigated, samples of *Ocimum canum sims* (*curry leaf*) are observed to have the highest levels of anti-nutrients prior to heating (Table 2). A remarkable and significant decrease in levels of the phytate is seen when the samples were heated at 90°C (Fig.3). At 50°C, the levels of phytates reduce to 7.7% and 74.6% at 90°C. Cyanide levels reduce significantly after heating the samples for 15 minutes at 50°C. Heating the samples further did not cause much reduction in the cyanide levels. The effective condition for reduction of these anti-nutrients would be heating at a temperature of 90°C for 15 minutes.

Heinsia crinata: For the level of phytates in *Heinsia crinata* (*bush apple leaf*), it is observed that there was no remarkable reduction heating at 50°C for 5 minutes (30.3% reduction) and 90°C for 15 minutes (30.4% reduction).Cyanide and oxalates have steady reduction in levels as the samples were heated at higher

Table 2: Levels of anti-nutrients in untreated and treated samples at various process temperatures and time

Gongronema latifolium	Tannin (mg/100g)	Phytate (mg/100g)	HCN (mg/100g)	Total oxalate (mg/100g)	Soluble oxalate (mg/100g)
Untreated	162.96	40.01	129.87	132.00	98.80
50°C, 5 minutes	160.49	37.46	119.04	98.80	61.60
50°C, 15 minutes	156.79	35.24	113.64	88.00	44.00
90°C, 5 minutes	151.85	29.53	93.07	29.20	35.20
90°C, 15 minutes	120.98	22.54	85.49	52.80	26.40
Vernonia amygdalina					
Untreated	308.64	33.34	162.34	246.40	202.40
50°C, 5 minutes	286.42	32.06	153.67	211.20	158.40
50°C, 15 minutes	276.04	25.72	151.51	107.20	105.60
90°C, 5 minutes	266.66	24.45	132.03	98.80	79.20
90°C, 15 minutes	207.41	15.87	109.31	61.60	44.00
Ocimum canum sims					
Untreated	427.16	41.27	231.60	246.40	211.20
50°C, 5 minutes	420.98	40.01	218.61	228.80	193.60
50°C, 15 minutes	413.58	38.10	150.93	193.60	158.40
90°C, 5 minutes	403.70	16.51	148.26	167.70	114.40
90°C, 15 minutes	318.52	10.47	137.44	105.60	70.40
Heinsia crinata					
Untreated	264.19	41.91	186.15	193.60	140.80
50°C, 5 minutes	260.49	38.10	179.65	140.80	114.40
50°C, 15 minutes	256.79	29.21	176.41	96.80	79.20
90°C, 5 minutes	124.69	25.39	162.34	70.40	61.60
90°C, 15 minutes	100.00	9.52	145.02	52.80	44.00
Talinum triangulare					
Untreated	333.33	43.81	175.32	184.80	140.80
50°C, 5 minutes	113.58	31.40	168.83	158.40	105.60
50°C, 15 minutes	100.00	28.87	165.50	114.40	70.40
90°C, 5 minutes	65.43	23.15	135.28	105.60	52.80
90°C, 15 minutes	51.85	6.97	112.55	79.20	35.20

Table 3: Comparison of anti-nutrients levels in untreated samples from different locations

Botanical name	Common name	Tannin	Phytate	HCN	Total oxalates	Soluble oxalates	Reference
Gongronema latifolium	Utazi	162.96	40.01	129.87	132	98.8	Ikot Abasi (Present study)
		1967	5320		9536		Abeokuta (Adeboye and Babajide, 2007)
		190					Umuahia, (Onyeka and Nwambekwe, 2007)
Vernonia amygdalina	Bitter leaf	308.64	33.34	162.34	246.4	202.4	Ikot Abasi (Present study)
		0.004	0.859	0.022	0.23		Delta (Agbaire, 2012)
		2733	5524		7836		Abeokuta (Adeboye and Babajide, 2007)
					220		Umuahia (Onyeka and Nwambekwe, 2007)
Ocimum canum sims	Curry leaf	427.16	41.27	231.6	246.4	211.2	Ikot Abasi (Present study)
Heinsia crinata	Bush apple leaf	264.19	41.91	186.15	193.6	140.8	Ikot Abasi (Present study)
Talinum triangulare	Waterleaf	333.33	43.81	175.32	184.8	140.8	Ikot Abasi (Present study)
		0.018	0.649	0.013	0.26		Delta (Agbaire, 2012)

temperatures for longer periods (Fig. 4). However, tannins levels significantly decrease when the sample is heated at 90°C. Thus, the optimum condition for the reduction of anti-nutrients in *Heinsia crinata* is determined to be 90°C for 15 minutes.

***Talinum triangulare*:** A higher reduction in the levels of all investigated anti-nutrients is observed in this leafy vegetable. The level of cyanide after heating for 15 minutes at 50°C is 5.6%, at 90°C, the level of cyanide reduces to 22.8% (Fig. 5). Tannin and phytate each recorded 84% reduction after heating for 15 minutes at 90°C. *Talinum triangulare* (*waterleaf*) has large cell saps in the leaves due to the very high moisture content of the vegetable (Saidu and Jideobi, 2009). It may be inferred that anti-nutrients present in the large cell saps are removed by draining of the water used in heating and cooling the vegetable.

Heating at high temperatures for longer periods resulted in lower levels of all investigated anti-nutrients. Heating the vegetables at higher temperatures and for longer periods (beyond 90°C for 15 minutes) may lead to further reduction in anti-nutrient levels; but there is risk the beneficial nutrients in the vegetables might be denatured at such temperatures. Consequently, the highest temperature (90°C) below the boiling point of water was chosen for the study.

Conclusion: Anti-nutrients present in the investigated vegetables could affect the bioavailability of essential nutrients if these vegetables are not properly processed. In this study, heating conditions resulted in a reduction in all studied anti-nutrients. Washing is found to reduce significantly, the levels of oxalates in the studied vegetables. As a result, it is suggested that the water used in washing the vegetables should not be used in cooking as earlier suggested by Lisiewska *et al.* (2011). Heating at 90°C for 15 minutes was most effective in reducing the levels of all studied anti-nutrients. Heating for at least 15 minutes at 90°C is highly recommended before the consumption of these selected leafy vegetables. Cyanide levels were the least reduced anti-nutrients. Increase in heating time than the one used in this study may be necessary to reduce cyanide significantly, but this could call for further research on the effect of heating on the overall nutrients and mineral compositions of the selected leafy vegetables. The investigation should provide more insight on the holistic improvement in the nutritional quality of vegetables with respect to the duration and temperature of heat processing.

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