

Research Article

Physicochemical Characterization and Enhancement of the Antioxidant Potential of *Ocimum gratissimum* Enriched Pepper Soup Mix

¹Gibson Lucky Arueya, ¹Olayemi Irewolede and ²Oluwatosin Adaramoye

¹Department of Food Technology,

²Department of Biochemistry, University of Ibadan, Ibadan, Nigeria

Abstract: Scent leaf (*Ocimum gratissimum*) enriched pepper-soup mix continues to remain an indigenous soup delicacy consumed for its nutritional and health benefits. There is however paucity of scientific data underlying its significance which could be further explored to advantage. In this study, dry soup mix was prepared from scent leaf (15%), ginger (30%), black pepper (25%), African nutmeg (17.5%) and scent pepper (12.5%) and evaluated for proximate, anti-oxidative and sensory properties using standard methods. The mix was reformulated to include 10% modified cocoyam (*Colocasia esculenta*) flour (thickener), 5% fresh and dried tea (*Camellia sinensis*) leaf following which it was re-characterized. Concentration-dependent scavenging activity was observed for the entire samples with nitric oxide radical scavenging activity of scent leaf enriched soup (750 µg/mL) exhibiting the highest value 77% compared to 57.7% for soup containing cocoyam. Total phenol, flavonoid contents and hydrogen peroxide radical scavenging activity differed in all the reformulated samples ($p < 0.05$). A significant increase ($\geq 50\%$) in DPPH radical scavenging activity was observed as a result of tea leaf addition. The moisture, fat, crude fiber, ash, protein of the enhanced scent leaf enriched pepper-soup mix was 7.55, 28.4, 8.0, 6.2% and 11.2%, respectively. There was a significant difference ($p < 0.05$) in the sensory qualities (color, aroma, taste, consistency and overall acceptability) between scent leaf and enhanced tea leaf pepper soup mix. The antioxidant potential, nutrient content and sensory attributes of this enhanced pepper soup mix holds great promise in further combating oxidative stress as in some disease conditions and rapid ageing.

Keywords: Antioxidant, Nitric Oxide, *O. gratissimum*, radical scavenging activity, soup-mix

INTRODUCTION

Scent leaf enriched pepper soup is widely consumed in the tropics as appetizer, stimulant and refreshment or as comfort food in homes, restaurants, hotels and hospitals. It is especially eaten in periods of recuperation from illnesses. The use of plant materials as spices and condiments for medicinal purposes is as old as human society (Nweze *et al.*, 2004). The medicinal values of these plants lie in their component phytochemicals which produce definite physiological actions on the human body. Alkaloids, flavonoids, tannins and phenolic compounds are quite outstanding in this respect. Phenolic compounds are indeed well known as radical scavengers, metal chelators, reducing agents, hydrogen donors and singlet oxygen quenchers (Proestos *et al.*, 2006). It is a known fact that phenolic compounds in plants possess strong antioxidant activity and may help to protect cells against the oxidative damage initiated by free-radicals (Kahkonen *et al.*, 1999).

Ocimum gratissimum linn locally referred to as 'scent leaf' is a vegetable commonly consumed as a flavouring agent in soups. Traditionally, different kinds of anti-oxidative soups are prepared from various indigenous functional ingredients and vegetables for medicinal purposes prescribed for invalids as stimulant/appetizer or as a comfort food (Solomon and Williams, 2003). In recent years there is an increasing interest in exploring the anti-oxidative potentials of some of these soups that likely underscore their popular and wide traditional use. Rekha *et al.* (2010) observed that a soup mix formulation containing dill leaf associated with high phenolic content had its reducing power potential increased. In quantitative terms this was approximately two and a half fold rise in aqueous extract, but seven fold increases in methanolic extract. Cao *et al.* (2011) found that 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) scavenging activity of mung bean soup was about 145% higher than that of tea soup and 195% that of vitamin-C solution.

While the anti-oxidative properties of scent leaf are well established (Afolabi *et al.*, 2007; Aprioku and

Corresponding Author: Gibson Lucky Arueya, Department of Food Technology, University of Ibadan, Ibadan, Nigeria, Tel.: 234-805-9546-297; Fax: 234(02)8103043

This work is licensed under a Creative Commons Attribution 4.0 International License (URL: <http://creativecommons.org/licenses/by/4.0/>).

Obianime, 2008), the anti-oxidative potentials of soups enriched with it (such as the pepper soup mix) as widely consumed locally is unknown. There is therefore the need to characterize the pepper soup mix especially in view of the synergistic effect of antioxidants. This will provide base line data for further work such as in areas of product quality enhancement and shelf stability.

MATERIALS AND METHODS

The Materials used were *Ocimum gratissimum* (Scent leaf), *Camellia sinensis* (Tea leaf), *Zingiber officinale* (Ginger), *Capsicum annum L.* (Scent pepper), *Piper guinensis* (Black pepper), *Monodora myristica* (African nutmeg) and *Colocasia esculenta* (Cocoyam). All were purchased from the local market and identified by a Botanist in the herbarium, Botany Department, University of Ibadan. Tea leaf (clone 143) was obtained from Cocoa Research Institute of Nigeria (CRIN), Ibadan.

Sample preparation (dry soup mix): This was carried out as described by Afolabi *et al.* (2007). The leaves of scent leaf and tea leaf were gently washed in cold water and oven dried at 60°C for 11 ½ hours and 12 hours respectively.

The cocoyam was washed, peeled and cubed. The cubes were cooked using an autoclave at 121°C for 5 min. The cooked cocoyam was dried in a hot air drier at 60°C for 36 h.

Ginger, scent pepper and African nutmeg were sorted to remove extraneous materials and oven dried at 40°C for 6 h.

The dried samples were milled in a hammer mill (Apex Construction) and kitchen blender (15,000 rpm for 2 min). Each sample was sieved using a 180 mm mesh, packaged in an airtight container and stored under refrigeration before formulation and analysis.

Product formulation: The soup mix formulation was made by mixing different ingredients: scent leaf (15%), ginger (30%), black pepper (25%), African nutmeg (17.5%) and scent pepper (12.5%). The variant of this sample were enhanced with (10%) modified cocoyam flour (thickener), (5%) fresh and dried tea leaf. The ingredients were weighed according to the specified percentages for a 1kg batch and thoroughly blended in a mini planetary mixer (N-50, Hobart, Sweden) for 5

min. The prepared products were finally dried in a hot air drier at 40°C for 1 hour to remove any moisture absorbed during the earlier storage and subsequent blending operation. Five variations were formulated as shown in Table 1.

Determination of antioxidant activity:

Preparation of soup mix extracts: One gram of soup mix powdered samples with and without fresh scent leaf, with thickener, with fresh and dried tea leaf. Have a standard letter format for these words were extracted in 10ml of boiling distilled water and vortexed for 10 min. Samples were cooled and centrifuged at 3000 rpm for 15 min at room temperature (27±3°C) (Kumar *et al.*, 2006). The extracts were stored in the refrigerator at 4°C for subsequent evaluation.

Total phenol content: The total phenol content of samples was analyzed by Folin-Ciocalteu colorimetric method with slight modification (Singleton and Rossi, 1965). One milliliter of appropriate dilutions (10-1000 µg/mL) of sample extract was oxidized with 1ml of 10% Folin C reagent. After 3 minutes the reaction was neutralized with 1ml of saturated 15% sodium carbonate (Na₂CO₃) solution. The solution was made up to 5 mL with distilled water. The absorbance of the resulting blue color of the mixture was read at a wave length of 760 nm in a UV-visible spectrophotometer (Spectrum lab 752s). Sample procedure was repeated for catechin used as standard and the results were expressed as mg of catechin equivalent per gram of soup mix.

Total flavonoid content: Flavonoid content was determined colorimetrically using the method described by Jia *et al.* (1999) with some modifications. The extract was made up to 10-1000 µg/mL with distilled water. A definite volume of 75 µL of sodium nitrite was next added. After 5 min interval, 150 µL of Aluminum chloride was then added. This was followed by the addition of 500 µL of sodium hydroxide to the mixture and made up with 1.28 mL of distilled water. The absorbance of the mixture was measured at a wave length of 760 nm. Catechin was used as standard.

DPPH (2, 2-Di Phenyl-1-Picrylhydrazyl) radical scavenging activity:

The effect of the extracts on DPPH radical was estimated according to the method of Mensor *et al.* (2001). Extracts (40-2000 µg) in 4 mL of

Table 1: Soup mix formulations per 100 g

Sample	Scent leaf (%)	Ginger (%)	Black pepper (%)	African nutmeg (%)	Ashantipeppe (%)	Thickner (%)	Tealeaf (%)
SOUP A	15.0 _(f)	30.00	25.00	17.50	12.50	-	-
SOUP B	15.0 _(d)	30.00	25.00	17.50	12.50	-	-
SOUP C	13.5 _(d)	27.00	22.50	15.70	11.30	10	-
SOUP D	14.25 _(f)	28.50	23.75	16.60	11.90	-	5 _(f)
SOUP E	14.25 _(d)	28.50	23.75	16.60	11.90	-	5 _(d)

N.B: (%): percentage, (d): dried, (f): fresh; FOg: fresh *Ocimum gratissimum*; DOg: dried *Ocimum gratissimum*; FTL: fresh tea leaf; DTL: dried tea leaf; Soup A: 15% FOg: 85% soup spice; Soup B- 15% DOg: 85% soup spice; Soup C: 90% soup A: 10% soup thickener; Soup D: 95% soup; A: 5% FTL; Soup E: 95% soup A: 5% DTL

H₂O was added to the methanol solution of DPPH (1mg: 1 mL). The mixture was shaken and left to stand at room temperature for 30 min. Thereafter the absorbance reading was made at 517 nm. Catechin was used as standard.

Nitric oxide scavenging activity assay: The effect of the extracts on nitric oxide radical was estimated according to the method of Ebrahimzadeh *et al.* (2009). Sodium nitroprusside (5mM) in phosphate buffer saline was mixed with different concentrate of each extracts and incubated at room temperature for 150 min. After the incubation period, 0.5 mL of Griess reagent was added. The absorbance of the chromophore was read at 546nm. Catechin was used as control.

Hydrogen peroxide scavenging activity assay: The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Nabavi *et al.* (2008). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (0.1-1 mg/mL) in water was added to a hydrogen peroxide solution (0.6 mL, 40 mM). The absorbance of hydrogen peroxide (H₂O₂) at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without H₂O₂ scavenging by the extracts and standard compounds was calculated using the following equation:

$$\% \text{ scavenging (H}_2\text{O}_2) = \left[\frac{A_{ctrl} - A_{sample}}{A_{ctrl}} \right] \times 100$$

Proximate composition of the soup mix:

Moisture content: Percentage moisture content of soup samples was determined by a gravimetric method (Association of Official Analytical Chemists, 2000). One gram of each sample was weighed (W₁) into pre-weighed moisture can and placed in an oven at 105°C for 24 h. The sample was removed from the oven, cooled in a desiccators and reweighed (W₂). Moisture percentage was calculated according to the formula:

$$\text{Moisture (\%)} = (W_1 - W_2)/W_1 \times 100$$

Ash content: Total ash content was evaluated at 600°C (Association of Official Analytical Chemists, 2000). Two grams of each soup sample was weighed into a pre-weighed porcelain crucible and charred on an electric heater and then transferred to a muffle furnace at 600°C for 2 h. The crucible was removed from the muffle furnace, cooled in a desiccator and weighed. Ash content was calculated according to the following formula:

$$\text{Ash (\%)} = (\text{Final weight of sample/weight of sample}) \times 100$$

Crude fiber: It was determined according to method of Association of Official Analytical Chemists (AOAC)

(2000). One gram of each defatted sample was placed in a glass crucible (attached to the extraction unit) followed by digestion with the addition of 150 mLs of sulphuric acid solution (1.25%) for 30 min. Thereafter, the acid was filtered off and the residue washed with boiling distilled water. This resulting residue was again digested using 150ml of sodium hydroxide solution (1.25%). The crucible was removed from the extraction unit and oven dried at 105°C overnight. The sample was allowed to cool in a desiccator, weighed (W₁) and followed by ashing at 550°C in a muffle furnace for 2 h. It was cooled and reweighed (W₂). Extracted fibre was expressed as percentage of the original sample and calculated according to the formula:

$$\text{Crude fibre (\%)} = \frac{\text{Digested sample (W}_1\text{)} - \text{Ashed sample (W}_2\text{)}}{\text{Wt of sample}} \times 100$$

Fat content: It was carried out using a soxhlet apparatus. Three grams of sample was dried at 105°C for 3-4 h, cooled, wrapped with filter paper and placed in a thimble in the extraction unit with n- hexane as the extracting solvent.

$$\text{Crude fat (\%)} = \frac{\text{Extracted fat}}{\text{Sample weight}} \times 100$$

Crude protein: Crude protein was determined by the Kjeldahl method as described by Association of Official Analytical Chemists (2000). *Two grams of each Sample was placed in a Kjeldahl flask and 5g Na₂SO₄+1 g CuSO₄ and 25 ml conc. sulphuric acid added. The mix was allowed to stand for 1 hour to cool after which 20 mL de-ionized water was gradually poured in. A specific volume (25 mL) of 40% sodium hydroxide was next added followed by distillation. The ammonia liberated was collected in boric acid and titrated with 0.1M hydrochloric acid. A blank was prepared and treated in the same manner. Protein percentage was calculated according to the formula:*

$$\text{Crude protein (\%)} = \frac{\text{(sample titre-blank titre)} \times 14 \times 6.25 \times 100}{\text{sample weight}}$$

Where 14 is the molecular weight of nitrogen and 6.25 is the nitrogen factor.

Sensory evaluation: Sensory evaluation was carried out by rating each of the five soup-mixes by 30 panelists on a nine point hedonic scale for taste, color, taste, aroma, consistency and overall acceptability according to Ihekoronye and Ngoddy (1985). This was conducted in the sensory laboratory of the Department of Food Technology, University of Ibadan using panelists recruited from among staff and students. Their ages ranged from 22 to 45 years with male and female in equal ratio. The sensory evaluations took an average of 15 minutes per panelist.

Statistical analysis: The statistical significance of the observed differences among means of triplicate readings of experimental results of the soup mix samples was evaluated by Analysis of Variance (ANOVA). The means were separated using Duncan's multiple range tests. These analyses were carried out using SPSS version 17.0 Computer Software.

RESULTS AND DISCUSSION

Total phenol content: The amount of total phenol present in the soup mix is *as indicated* in Table 2. At 1000 µg there was no significant difference ($p>0.05$) between the fresh *Ocimum gratissimum* based soup mix (0.28) and dried *Ocimum gratissimum* based soup mix (0.27). The soup containing 10% cocoyam flour (thickener) (with an absorbance value of 0.12) was significantly different ($p<0.05$) from other soup samples. A significant difference ($p<0.05$) was also observed between catechin and the entire soup samples at all concentrations.

The phenolic content of the scent leaf enriched pepper soup mix and their variants were low (ostensibly due to poor solubility in water when compared to the soup mix containing *Anethum sowa* L (dill leaf) (Balentine *et al.*, 1999; Rekha *et al.*, 2010). The antioxidant activity of an extract cannot be predicted on the basis of its total phenolic content as their chemical structure is more crucial in this regard (Kahkonen *et al.*, 1999). Phenolic compounds possess diverse biological activities such as anti-inflammatory, anti-carcinogenic and anti-atherosclerotic activity (Chung *et al.*, 1998). The scavenging activity of phenolic compounds is due to its hydroxyl group (Padmanabhan and Jangle, 2012). The values of total phenols for soups A and B showed that they have a higher relative potential to reduce the

risk of oxidative stress induced pathological disease than soup C.

Flavonoid content: At 750 µg/mL flavonoid content (Table 3), *Ocimum gratissimum* based soup mix containing thickener exhibited higher absorbance (0.29) in contrast to *Ocimum gratissimum* based soup mix without thickener (0.13). The flavonoid content of aqueous extract of the soup mixes at all concentrations (10-1000 µg/mL) was not significantly different ($p>0.05$) amongst all the soup samples. These values were reduced when compared to catechin.

The flavonoids as reflected in Table 3 are low and apparently constitute a small fraction of the total phenol content. Processing method applied (such as drying, milling and boiling) variety of species used, storage duration and soup mix temperature may have had some bearing on the flavonoid content of the soup mix like a number of other food products (Bravo, 1998).

Flavonoids are a class of widely distributed phytochemicals with anti-oxidant and biological activity. They are constituents of plant foods that have been implicated in the reduction of cancer risk (Neuhouser, 2004). They have been reported to interfere with the activities of the enzymes involved in Reactive Oxygen Species (ROS) generation, quenching of free radicals, chelating transition metals and rendering them redox inactive in the fenton reactions (Omeregbe and Osagie, 2012). In addition to the vitamins and minerals present in vegetables and spices, phytochemicals such as flavonoids and other phenolics contribute to beneficially protective effects (Chun *et al.*, 2005).

Nitric oxide radical scavenging activity: A cursory look at Table 4 showed the percentage nitric oxide scavenging activity of the soup mixes to be

Table 2: Total phenol content of samples of soup mix

Sample	Concentration of soup extract (µg/mL)							
	0.00	10	50	100	250	500	750	1000
CATECHIN	0.03 ^b	0.15 ^a	0.61 ^a	1.18 ^a	0.65 ^a	1.77 ^a	2.02 ^a	2.06 ^a
SOUP A	0.02 ^b	0.04 ^b	0.07 ^b	0.07 ^b	0.09 ^c	0.14 ^b	0.18 ^b	0.27 ^b
SOUP B	0.04 ^a	0.04 ^b	0.05 ^{bc}	0.19 ^b	0.22 ^b	0.14 ^b	0.11 ^c	0.28 ^b
SOUP C	0.01 ^c	0.01 ^c	0.01 ^c	0.03 ^b	0.04 ^d	0.06 ^b	0.10 ^c	0.12 ^c

NB: Mean for each concentration of soup mix followed by the same letter are not significantly different at 5% level ($p>0.05$); Catechin- Standard used for the identification of phenolic acids present in the soup; Soup A: 15% FOg: 85% soup spice; Soup B- 15% DOg : 85% soup spice; Soup C: 90% soup A: 10% soup thickener; Where; FOg: fresh *Ocimum gratissimu* DOg: dried *Ocimum gratissimum*; FTL: fresh tea leaf; DTL: dried tea leaf; Soup spice (30% ginger, 25% black pepper, 17.5% African nutmeg and 12.5% Ashanti pepper)

Table 3: Flavonoid content of samples of soup mix

Sample	Concentration of soup extract (µg/mL)							
	0.00	10	50	100	250	500	750	1000
CATECHIN	0.01 ^a	0.08 ^a	0.35 ^a	1.64 ^a	1.76 ^a	1.93 ^a	1.95 ^a	1.95 ^a
SOUP A	0.01 ^a	0.00 ^a	0.00 ^b	0.00 ^b	0.00 ^b	0.01 ^c	0.00 ^d	0.01 ^d
SOUP B	0.01 ^a	0.00 ^a	0.01 ^b	0.03 ^b	0.07 ^b	0.09 ^b	0.13 ^c	0.15 ^c
SOUP C	0.01 ^a	0.01 ^a	0.02 ^b	0.01 ^b	0.05 ^b	0.11 ^b	0.29 ^b	0.19 ^b

NB: Mean for each concentration of soup mix followed by the same letter are not significantly different at 5% level ($p>0.05$); Catechin- Standard used for the identification of flavonoid acids present in the soup; Soup A: 15% FOg : 85% soup spice; Soup B- 15% DOg : 85% soup spice; Soup C: 90% soup A: 10% soup thickener; Where; FOg: fresh *Ocimum gratissimum*; DOg: dried *Ocimum gratissimum*; FTL: fresh tea leaf; DTL: dried tea leaf; Soup spice (30% ginger, 25% black pepper, 17.5% African nutmeg and 12.5% Ashanti pepper)

Table 4: Nitric oxide radical scavenging activity of samples of soup mix

Sample	Concentration of soup extract (µg/mL)								
	0	50	100	200	300	400	500	750	1000
CATECHIN	0.00	9.13 ^{ac}	0.68 ^b	0.30 ^c	14.2 ^a	8.2 ^b	11.5 ^b	37.8 ^{bc}	78.7 ^a
SOUP A	0.00	16.8 ^a	19.8 ^a	14.6 ^a	14.1 ^a	15.3 ^b	16.1 ^b	77.0 ^a	101 ^a
SOUP B	0.00	19.4 ^a	12.9 ^b	11.3 ^b	15.3 ^a	19.4 ^a	16.4 ^b	23.6 ^c	54.1 ^a
SOUP C	0.00	2.01 ^b	5.25 ^c	6.73 ^b	12.3 ^a	6.58 ^c	22.1 ^a	57.7 ^{ab}	85.8 ^a

NB: Mean for each concentration of soup mix followed by the same letter are not significantly different at 5% level (p>0.05); Catechin- Standard used for the identification of nitric oxide radical scavenging activity present in the soup; Soup A: 15% FOG : 85% soup spice; Soup B-15% DOg: 85% soup spice; Soup C: 90% soup A: 10% soup thickener; Where; FOG: fresh *Ocimum gratissimum*; DOg: dried *Ocimum gratissimum*; FTL: fresh tea leaf; DTL: dried tea leaf; Soup spice (30% ginger, 25% black pepper, 17.5% African nutmeg and 12.5% Ashanti pepper)

Table 5: Hydrogen peroxide radical scavenging activity of samples of soup mix

Sample	Concentration of soup extract (µg/mL)									
	0	10	50	100	250	500	750	1000	1250	
CATECHIN	0.00	4.65 ^c	2.61 ^c	9.49 ^c	6.87 ^c	8.17 ^c	8.80 ^b	9.03 ^c	9.45 ^d	
SOUP A	0.00	29.0 ^b	29.3 ^b	29.7 ^b	27.4 ^a	25.5 ^b	21.6 ^b	19.4 ^a	16.7 ^b	
SOUP B	0.00	42.0 ^a	34.8 ^a	33.3 ^b	33.2 ^a	30.3 ^a	26.2 ^b	24.5 ^a	25.5 ^a	
SOUP C	0.00	36.1 ^{ba}	33.0 ^a	31.7 ^b	17.6 ^b	6.58 ^c	17.4 ^c	17.0 ^b	11.8 ^c	

NB: Mean for each concentration of soup mix followed by the same letter are not significantly different at 5% level (p>0.05); Catechin-Standard used for the identification of hydrogen peroxide radical scavenging activity present in the soup; Soup A: 15% FOG : 85% soup spice; Soup B-15% DOg: 85% soup spice; Soup C: 90% soup A: 10% soup thickener; Where; FOG: fresh *Ocimum gratissimum*; DOg: dried *Ocimum gratissimum*; FTL: fresh tea leaf; DTL: dried tea leaf; Soup spice (30% ginger, 25% black pepper, 17.5% African nutmeg and 12.5% Ashanti pepper)

concentration dependent. At 100 µg/ml, *Ocimum gratissimum* based soup had the highest value 20% (approx.) compared to soup containing thickener (5.3%) and catechin (0.7%) There was no significant difference (p>0.05) between *Ocimum gratissimum* (dried) soup mix and *Ocimum gratissimum* (fresh) soup mix.

The percentage nitric oxide radical scavenging activity is remarkably high and is a reflection of the major ingredients utilised especially those rich in essential oils such as *O. gratissimum*. The values are higher than those reported (8.25 at 100 µg/mL) for the essential oil extract of *Anethum graveolens* (dill) (Dadashpour *et al.*, 2011). Essential oil from plants have been reported to possess an interesting spectrum of antioxidant properties (Dubey *et al.*, 2000).

Reactive Oxygen And Nitrogen Species (ROS/RNS) are continuously produced in living organisms during oxidation and controlled by endogenous enzymes such as superoxide dismutase, glutathione peroxidase and catalase in the human body (Oke *et al.*, 2004). These species like their externally generated oxidant counterpart are deleterious to macromolecules. Increased levels of nitric oxide in the body can be found in certain spasmodic conditions, for example, allergic rhinitis, adult respiratory distress syndrome and asthma (immediate and late phase) (Ashutosh, 2000). It is well known that nitric oxide plays an important role in various inflammatory processes and sustained levels of production of this radical are directly toxic to tissues and contribute to the vascular collapse associated with septic shock (Burney *et al.*, 1999). However the present study proved that the *Ocimum gratissimum* based soup mix had a more potent nitric oxide scavenging activity than the standard catechin. The soup can sufficiently serve as antidote against nitric oxide induced degenerating diseases.

Hydrogen peroxide radical scavenging activity: An evaluation of Table 5 indicates that the 50 µg/mL soup mix containing fresh *Ocimum gratissimum* had the highest value compared to other soups. There was no significant difference (p>0.05) among the soup samples at 100 µg/mL. The antioxidant activity of *Ocimum gratissimum* and that of catechin showed a significant difference at all levels of concentration.

Evidently, *O. gratissimum* soup mix is a good antidote against the adverse effect produced by hydrogen peroxide radicals. The higher value associated with soup B may be a function of increased concentration of active ingredients per unit weight in the dry leaves utilized in contrast to the fresh ones, all other conditions being the same. Figures obtained for soup C are lower owing to the 10% inclusion of the soup thickener. All soup samples A, B and C were better hydrogen peroxide scavengers than the catechin standard.

Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly; once inside the cell, it can react with Fe²⁺ and possibly Cu²⁺ ions to form hydroxyl radicals which may be the genesis of its toxic effect (Nabavi *et al.*, 2008).

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity: At 1000 µg/mL, the soup extract of soup A, B, C, D, E and catechin equivalent resulted in a DPPH radical scavenging activity of 15.45, 15.60, 14.85, 28.95, 47.64 and 57.72%, respectively (Table 6).

The low percentage trend (0.58-13.3%) of DPPH radical scavenging activity among *Ocimum gratissimum* based soup mixes at a concentration of 250

Table 6: DPPH radical scavenging activity of samples of soup mix

Sample	Concentration of soup extract ($\mu\text{g/mL}$)								
	0	40	100	250	500	750	1000	1500	2000
CATECHIN	0.00	27.5 ^a	40.2 ^a	48.8 ^a	57.3 ^a	59.0 ^a	57.7 ^a	64.2 ^a	58.5 ^a
SOUP A	0.00	6.80 ^b	2.94 ^{cd}	0.63 ^d	3.29 ^c	12.2 ^d	15.5 ^c	25.9 ^c	23.7 ^c
SOUP B	0.00	0.69 ^c	0.84 ^d	0.58 ^c	1.59 ^c	7.45 ^c	15.6 ^c	16.6 ^d	17.2 ^d
SOUP C	0.00	0.96 ^b	1.24 ^{bc}	6.39 ^c	9.13 ^d	13.8 ^d	14.9 ^c	17.1 ^d	14.4 ^c
SOUP D	0.00	6.11 ^b	6.96 ^b	7.96 ^c	16.6 ^c	18.7 ^c	29.0 ^b	19.8 ^d	21.4 ^c
SOUP E	0.00	3.75 ^b	6.94 ^b	13.3 ^b	28.8 ^b	40.6 ^b	47.6 ^a	33.1 ^b	44.5 ^b

NB: Mean for each concentration of soup mix followed by the same letter are not significantly different at 5% level ($p > 0.05$); Catechin-Standard used for the identification of DPPH radical scavenging activity present in the soup; Soup A: 15% FOg : 85% soup spice; Soup B- 15% DOg: 85% soup spice; Soup C: 90% soup A: 10% soup thickener; Soup D: 95% soup A: 5% FTL; Soup E: 95% soupA: 5% DTL; Where; FOg: fresh *Ocimum gratissimum*; DOg: dried *Ocimum gratissimum*; FTL: fresh tea leaf; DTL: dried tea leaf; Soup spice (30% ginger, 25% black pepper, 17.5% African nutmeg and 12.5% Ashanti pepper)

Table 7: Sensory evaluation results of samples of soup mix

Sample	Color	Aroma	Taste	Consistency	Overall acceptability
SOUP A	7.30 ^a	6.90 ^a	7.73 ^a	6.47 ^a	7.47 ^a
SOUP B	7.67 ^a	7.10 ^a	7.50 ^{ab}	6.63 ^a	7.70 ^a
SOUP C	6.10 ^c	0.17 ^b	7.07 ^{bc}	6.97 ^a	6.70 ^b
SOUP D	6.63 ^b	6.43 ^{ab}	7.07 ^{bc}	5.90 ^b	6.57 ^b
SOUP E	5.97 ^c	5.47 ^c	6.50 ^c	6.77 ^a	6.47 ^b

NB: Mean for each concentration of soup mix followed by the same letter are not significantly different at 5% level ($p > 0.05$); Soup A: 15% FOg: 85% soup spice; Soup B- 15% DOg : 85% soup spice; Soup C: 90% soup A: 10% soup thickener; Soup D: 95% soup A: 5% FTL; Soup E: 95% soup A: 5% DTL; Where; FOg: fresh *Ocimum gratissimum*; DOg: dried *Ocimum gratissimum*; FTL: fresh tea leaf; DTL: dried tea leaf; Soup spice (30% ginger, 25% black pepper, 17.5% African nutmeg and 12.5% Ashanti pepper)

$\mu\text{g/mL}$ (Table 6), as observed when compared to catechin standard. (48.8%) is noteworthy. It may be related to reduced concentration of their active compounds. The percentage 2, 2-Diphenyl-1-Picrylhydrazyl radical scavenging activity of methanolic extract of *Ocimum gratissimum* and gallic acid found to be 84.3% and 91.4 at 250 $\mu\text{g/mL}$, respectively underscores this assertion (Afolabi *et al.*, 2007). The DPPH radical scavenging activity of *Anethum sowa L* dry soup mix was reported to be 3.80% at 6 $\mu\text{g/mL}$, another low value compared to catechin (Rekha *et al.*, 2010). The DPPH assay has been widely used as a quick, reliable and reproducible in vitro parameter to determine the antioxidant activity of pure compounds as well as plant extracts (Goncalves *et al.*, 2005). The decrease in absorbance by the DPPH radical with increase in concentration of the extract manifested in the rapid discoloration of the purple DPPH suggest that the extract's antioxidant activity is due to its proton donating ability. The tendencies of electron or hydrogen donation are reliable indicators of anti-oxidant activity having to do with free radical scavenging (Hu *et al.*, 2000).

The 2, 2-Diphenyl-1-Picrylhydrazyl radical scavenging activity of ethanolic extract of green tea was reported to be 94.28% at 0.39 $\mu\text{g/mL}$ (Panchuti *et al.*, 2006). The blending of the soup mix with *Camellia sinensis* (tea leaf) a rich source of catechin was obviously a sound option.

Proximate composition of the soup mix: The moisture content of the soup mix ranges from 5.79-

7.55%. The soup mix were observed to be very high in inorganic residue after incineration ranging from 5.75-6.8%, implying that the soup mix is rich in mineral content. Soups A and E had the lowest and highest values respectively. The protein content of soup mix samples A, B, C, D, E were 11, 11.35, 11.84, 11.20 and 11.5%, respectively. Although the fat content (essential oils or oleoresins) of the soup mixes was highest in sample E (containing tea leaf), it was not significantly different ($p > 0.05$) from samples A and D. The crude fibre of the soup mixes determined ranged from 4.75-8.2. It was again observed that soups A and E had the lowest and highest value in this component respectively. The values for moisture fell within the range of standard moisture content of grounded spices and powdered foods, although figures higher than the 9.2% have been reported for dry soup mix (Rekha *et al.*, 2010). The protein content is significant though less than 12%. According to Pearson (1976), a plant food that provides more than 12% of its caloric value as protein is considered a good source of protein. Although the protein content of the soup (11-11.8%) is higher than that of *Anethum sowa L* dry soup mix and mushroom-whey soup powder, it falls short of protein food requirement. Consumption of animal protein with the soup would bridge this gap. The fat content of all the samples were indeed higher than the fat content of a reported soup mix and is a functions of the selected inputs and their inclusion levels (Rekha *et al.*, 2010). This proximate component enhanced the flavor and preservative properties of the soup mix. Essential oils from the plant have been reported to possess an interesting spectrum of antifungal and antioxidant properties (Dubey *et al.*, 2000).

Sensory evaluation: Soup B had the highest score in three of five attributes but it was also not significantly different to at least one of the other samples (Table 7). Soup C had the highest rating (6.97) in terms of consistency, though not significantly different ($p>0.05$) from soups A, B and E. The panelists noted the unique flavor of Soup E which must have been due to inclusion of dried tea leaf.

The use of the dried *O. gratissimum* leaf in the soup B alone with no inclusion of tea leaves may have accounted for its relative high sensory score. The drying unit operation may have enhanced the taste and volatile components of the leaf. Though contributed significantly to the DPPH scavenging activity in soups D and E, the astringent flavor associated with dried tea leaf made those soups less preferred.

CONCLUSION

The fresh *Ocimum gratissimum* leaf based pepper soup mix as presently consumed exhibits a high percentage Nitric Oxide radical scavenging activity but low in DPPH activity potential. The inclusions of fresh and dried tea leaves in the formulation and processing of the pepper soup mix further beneficially enhanced the DDPH antioxidant property of the pepper soup mix.

ACKNOWLEDGMENT

We wish to acknowledge the technical support of Miss Bukky Akanni of the department of Biochemistry, University of Ibadan, Ibadan.

REFERENCES

- Afolabi, C., E.O. Akinmoladun, E.A. Ibukun, E.M. Obuotor and E.O. Farombi, 2007. Phytochemical constituent and antioxidant activity of extract from leaves of *Ocimum gratissimum*. *Sci. Res. Essay*, 2(5): 163-166.
- Aprioku, J.S. and A.W. Obianime, 2008. Antioxidant activity of the aqueous crude extract of *Ocimum gratissimum* Linn. leaf on basal and cadmium-induced serum levels of phosphatases in male guinea-pigs. *J. Appl. Sci. Environ. Manage.*, 12(4): 33-39.
- Ashutosh, K., 2000. Nitric oxide and asthma: A review. *Curr. Opin. Pulm. Med.*, 6: 21-25.
- Association of Official Analytical Chemists (AOAC) 2000. Official Methods of Analysis. 17th Edn., Official Methods 925.09, 923.03, 979.09, 962.09, 4.5.1 and 923.05. Washington DC, USA.
- Balentine, D.A., A.S. Wiseman and L.C.M. Bouwens, 1999. The chemistry of tea flavonoids. *Crit. Rev. Food Sci.*, 37(8): 693-704.
- Bravo, L., 1998. Polyphenols chemistry: Dietary sources, metabolism and nutritional significance. *Nutr. Rev.*, 56: 317-333.
- Burney, S., J. Caulfield, J. Niles, J.S. Wishnok and S.R. Tannenbaum, 1999. The chemistry of DNA damage from nitric oxide and peroxyntirite. *Mutat. Res.*, 424: 37-39.
- Cao, D., H. Li, J. Yi, J. Zhang, H. Che *et al.*, 2011. Antioxidant properties of the mung bean flavonoids on alleviating heat stress. *PLoS One*, 6(6): e21071.
- Chun, O.K., D.O. Kim, N.L. Smith, D. Schroeder, T. Hanj and C.Y. Lee, 2005. Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. *J. Sci. Food Agr.*, 85: 1715-24.
- Chung, K.T., T.Y. Wong, T.Y. Huang and Y. Lin, 1998. Tannins and human health: A review. *Crit. Rev. Food Sci.*, 38: 421-464.
- Dadashpour, M., I. Rasooli, F. Sefidkon, M.B. Rezaei and A.A. Darvish, 2011. Lipid peroxidation inhibition, superoxide anion and nitric oxide radical scavenging properties of thymus daenensis and anethum graveolens essential oils. *Winter*, 10(37).
- Dubey, N.K., T.N. Tiwari, D. Mandin, H. Andriamboavonjy and J.P. Chaumont, 2000. Antifungal properties of *Ocimum gratissimum* essential oil (*Ethyl cinnamate chemotype*). *Fitoterapia*, 7(15): 567-569.
- Ebrahimzadeh, M.A., S.F. Nabari and S.M. Nabari, 2009. Antioxidant activities content of methanol extract of *Sambucus ebulus* L. flower. *Pak. J. Bio. Sci.*, 12(5): 447-450.
- Goncalves, C., T. Dinis and M.T. Batista, 2005. Antioxidant properties of proanthocyanidins of *Uncaria tomentosa* bark decoction: A mechanism for anti-inflammatory activity. *Photochemistry*, 66: 89-98.
- Hu, C., Y. Zhang and D.D. Kitts, 2000. Evaluation of antioxidant and prooxidant activities of bamboo *Phyllostachys nigra* var. Henonis leaf extract in vitro. *J. Agr. Food Chem.*, 48: 3170-3176.
- Ihekoronye, A.I. and P.O. Ngoddy, 1985. Integrated Food Science and Technology for the Tropics. Macmillan Publishers, London, U.K., pp: 386.
- Jia, Z.S., M.C. Tan and J.M. Wu, 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.*, 64: 555-559.
- Kahkonen, M.P., A.I. Hopia, H.J. Vuorela, J.P. Rauha, K. Pihlaja, T.S. Kujala and M. Heinonen, 1999. Antioxidant activity of plant extracts containing phenolic compounds. *J. Agr. Food Chem.*, 47: 3954-3962.
- Kumar, S., T.J. Stohlgrevn and G.W. Chway, 2006. Spatial heterogeneity influences native and non native plant species richness. *Ecology*, 89: 3186-3199.

- Mensor, L.I., F.S. Menezes, G.G. Leitao, A.S. Reis, T. Dos Santos, C.S. Coube and S.G. Leitao, 2001. Screening of Brazillian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother. Res.*, 15: 127-130.
- Nabavi, S.M., M.A. Ebrahimzadeh, S.F. Nabavi, A. Hamidinia and A.R. Bekhradnia, 2008. Determination of antioxidant activity, phenol and flavanoids content of *parrotia persica* Mey. *Pharmacologyonline*, 2: 560-567.
- Neuhouser, M.L., 2004. Dietary flavonoids and cancer risk: Evidence from human population studies. *Nutr. Cancer*, 50(1): 1-7
- Nweze, E.L., J.L. Okafor and O. Njoku, 2004. Antimicrobial activities of methanolic extracts of *trumequineesis (Scchum and Thorn)* and *morinda lucida* used in Nigeria herbal medicinal practice. *J. Biol. Res. Biotech.*, 2(1): 34-36.
- Oke, F., B. Aslim, S. Ozturk and S. Altundag, 2004. Essential oil composition, antimicrobial and antioxidant activities of *Satureja cunenifolia* Ten. *Food Chem.*, 112(4): 874-879.
- Omoregie, E.S. and A.U. Osagie, 2012. Antioxidant properties of methanolic extracts of some Nigerian plants on nutritionally-stressed rats. *Niger. J. Basic Appl. Sci.*, 20(1): 7-20.
- Padmanabhan, P. and S.N. Jangle, 2012. Evaluation of DPPH radical scavenging activity and reducing power of four selected medicinal plants and their combinations. *Int. J. Pharm. Sci. Drug Res.*, 4(2): 143-146.
- Panchuti, P., T. Masubon, Y. Rangrong and S. Panuwat, 2006. Antioxidant and radical scavenging activities of selected plant extracts and possible application in active packaging. *Proceeding of the 15th International Association of Packaging Research Institute, Tokyo, Japan.*
- Pearson, D., 1976. *The Chemical Analysis of Food.* Churchill, Livingstone, Edinburgh London, pp: 352-353.
- Proestos, C., I.S. Boziaris, G.J.E. Nychas and M. Komaitis, 2006. Analysis of flavonoids and phenolic acids in Greek aromatic plants: Investigation of their antioxidant capacity and antimicrobial activity. *Food Chem.*, 95: 664-671.
- Rekha, M.N., A. Ramesh, Y.S. Dharmesh, A.S. Chauhan and R.S. Ramteke, 2010. Evaluation of antioxidant properties of dry soup mix extracts containing dill (*Anethum sowa* L.) leaf. *Food Bioprocess Tech.*, 3: 441-449.
- Singleton, V.L. and J.A. Rossi, 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Viticult.*, 16: 144-158.
- Solomon, H.K. and W.W. Williams, 2003. *Encyclopedia of Food and Culture.* Scribner Publisher, New York, 3: 297.