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Optimization of mixed spices from scent leaf, curry and African Black Pepper

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Abstract

D-optimal mixture design and the numerical optimization technique were used to optimize mixed spices produced from three leafy vegetables Scent leaf (*Ocimum grastissimum*), Curry (*Murray koemigii*) and African black pepper (*Piper guineense*), as the factors. The spices were blended according to the mixture design combinations generated based on the three factors and modelled with three sensory attributes (flavour, appearance and general acceptability) as the responses. Results indicated that 80:10:10 (scent leaf:curry:African black pepper leaves) mixed spice which was outstanding in sensory evaluation with scores of 8 (flavor), 7 (appearance) and 8 (general acceptability), was selected as the optimized product. Further evaluation of the optimized product showed a mineral profile of 1.462 (zinc), 0.326 (copper), 489.476 (calcium), 6.418 (magnesium), and 6.169 mg/100g (iron). Phytochemical profile showed the presence of phenol (0.15), tannin (0.72), phytase (0.56), flavonoid (0.73), sterol (0.14), alkaloid (0.95), oxalate (0.53) and saponin (0.94 mg/100g). Meanwhile the heavy metal screening showed that lead and cadmium were not detected. These results show that local spices, besides possessing excellent nutraceutical properties, can be processed into highly acceptable novel flavor enhancing products.

Key Words: Optimization, mixed spices, response surface methodology, *Ocimum grastissimum*

INTRODUCTION

Spices are dried, fragrant, aromatic, or pungent vegetative or plant substances in whole, broken or ground forms that are used to flavour food (Hussain *et al*., 2019). Spice is often used interchangeably with herb though they have specific meanings in botany. However, according to FAO (2005), herb is a subset of spice and refers to plants with aromatic leaves. Spices consist of rhizomes, roots, stems, bulbs, barks, stigmas, fruits, seeds and leaves (Chima, 2002; https://www.fs.fed.us, 2020). They are rich in essential oils and oleoresins which impact flavouring, antiseptic and preservative properties hence their use in food preparation and preservation for ages in areas where the plants are native. The essential oils and oleoresins extracts of raw spices as well as dried spices are some of the available product options of spices. Mixed spice which is not uncommon in cuisines around the world presents a unique variety. Nigerian indigenous foods such as *suya*, *kilishi*, and *kunu* are well known for the utilization of mixed spices which gives these products their unique flavour and attraction.

Spices and condiments constitute a huge component of trade in areas such as India, China, Indonesia, East and West Africa, and West Indies (Pathasarathy *et al*., 2008). Throughout the ages, the opening of trade routes and changing immigration patterns have affected the way the world eat. Today, there is a new revolution in eating patterns and the use of spices. Nigerian palates are becoming more adventurous seeking variety and something new. They want food with more intense flavor, hotter spicer profiles, perception of natural and more convenient to prepare and provide satisfaction. Also consumers are seeking natural ways of preventing ailments, and spices which possess medicinal properties such as antioxidant and antimicrobial are being sought for. Food professionals therefore must continue to search for new and unique spice flavouring because of the growing global demand for authentic ethnic and cross cultural style of cooking.

Scent leaf (*Ocimum grastissimum*) and African black pepper (*Piper guineense*) are natives to South-East Nigeria where they are commonly consumed. Curry leaves (*Murray koemigii*) on the other hand grows well in this area and has become part of the recipe of most dishes including soups, stews, and various fish and meat products etc. It has been shown that they are rich in nutrients and bioactive compounds (Ndulaka *et. al*., 2016). Dishes are often prepared from a variety of these spices which are often added individually suggesting the desire for mixed spice flavours. This therefore stresses the need to work towards the development of a novel product that provides a ready mix of these spices. The mixture response surface methodology which is increasingly being used for research in food mixtures is deemed appropriate for this investigation since it is a mixture experiment (spice mix). Mixture experiments consider the independent variables as proportions of the non-negative components which must sum to one or 100%, and the response depends only on the relative proportions of these components (Li *et al*., 2017). Empirical equation models of these relationships are then established which are important in predicting the optimized mixture and in determining influences within the blending system.

The objective of this work was to optimize mixed spices produced from blends of scent leaf (*Ocimum grastissimum*), curry (*Murray koemigii*) and African black pepper (*Piper guineense*) and evaluate the properties of the optimized product.

MATERIALS AND METHODS

Raw material collection and processing

Curry, scent leaf and African black pepper leaves were purchased from a local market in Umuahia, Abia State, South East Nigeria. The spices were washed, sun-dried at 33℃ for three days then milled. The milled spices were sieved using a 300μm mesh size to fine particles and stored in air tight plastic containers.

Experimental design

A 3-variable D-optimal RSM-mixture design was adopted for the optimization studies. The three variables, scent leaf -X_1, curry-X_2 and African black pepper-X_3, were the mixture ingredients which summed up to 100% (Li *et al.*, 2017), and were also combined to generate fourteen runs (ratios) as shown in Table 1. The second order canonical model (Eqn. 1) was used to approximate the unknown function.

$$Y = \sum_{i=1} \beta_i x_i + \sum_{i=1} \sum_{i<j} \beta_{ij x_i x_j} \qquad \text{Eqn. 1}$$

Where Y is the response, β_i and β_{ij} are the coefficients of the linear (x_i) and quadratic ($x_i x_j$) effects respectively (where i = 1-3, j =1-3 and $i \neq j$).

Sensory evaluation

A 25-man sensory panel consisting of men and women drawn from Michael Okpara University of Agriculture, Umudike community was used for the organoleptic evaluation of flavour, appearance and general acceptability. The mixed spices alongside a control sample (scent leaf) were used to prepare pepper soups and presented in identical containers coded 3-digit random numbers to the panelists in a randomized order. The evaluation was based on a nine-point Hedonic scale with the highest rating (9) representing "like extremely" while the lowest rating (1) represented "dislike extremely" (Onwuka, 2018).

Proximate analysis

Proximate analysis was carried out using the methods described by James (1995) as follows. Crude fibre and moisture were determined gravimetrically using the Weendy method while fat content was by the continuous solvent extraction method. Crude protein analysis was carried out using the Kjedahl method. Carbohydrate was estimated by difference as the nitrogen free extract by subtracting the weight (in grams) of protein, fat, water, ash and dietary fibre from 100g of the spices.

Phytochemical determination

The Folin-Denis spectrophotometric method was used for the determination of tannin (Pearson, 1976) while the methods described by AOAC (1995) was used for the determination of saponin. Alkaloid, steroid and flavonoid analyses were carried out using the gravimetric method of Harborn (1973). Oxalate was estimated by the method described by Ukpabi and Ejidoh (1989) while phytic acid was determined using the method described by Oberlease (1973).

Mineral analysis

Samples (1g each) were digested with 20ml of acid mixture containing concentrated nitric acid (650ml), perchoric acid (80ml) and sulphuric acid (20ml). Aliquots of the diluted clear digest were then taken and used for the Atomic Absorption Spectrophotometer reading. Detection limits had previously been determined using the methods of Techtron (1975) as 0.005 (cu), 0.005 (Zn), 0.02 (Fe), 0.002 (Mg), 0.04 (Ca), 0.03 (Pb), 0.02 ppm (Cd) all for aqueous solutions.

Statistical analysis

The Response Surface Methodology used for the modelling and optimization of the mixed spices was carried out using the Design-Expert (version 6.0.8) software while the Independent-Samples T test used for the determination of the significance ($p<0.05$) of difference between the treatment (optimized mixed spice and scent leaf) means was calculated using the SPSS (version 16.0) computer statistical program.

RESULTS AND DISCUSSION

The result of the sensory evaluation of the spices (Table 1) showed that 80:10:10 scent leaf:curry:African black pepper mixed spice had the highest values for flavour (8), appearance (7) and general acceptability (8). This implies that scent leaf, the highest in the ratio, contributed significantly to the

Table 1: Factors and responses for the mixed spices

Runs	Factors			Responses		
	Scent leaf	Curry	African black pepper	Flavour	Appearance	General acceptability
1	45.00	45.00	10.00	6	6	6
2	10.00	45.00	45.00	4	3	3
3	10.00	10.00	80.00	2	3	3
4	10.00	80.00	10.00	5	5	6
5	21.67	21.67	56.67	5	4	4
6	56.67	21.67	21.67	5	5	5
7	45.00	10.00	45.00	4	5	4
8	45.00	45.00	10.00	6	5	5
9	10.00	10.00	80.00	4	4	5
10	10.00	80.00	10.00	5	3	4
11	33.33	33.33	33.33	5	5	4
12	21.67	56.67	21.67	4	4	4
13	80.00	10.00	10.00	8	7	8
14	80.00	10.00	10.00	8	7	8

sensory properties of the mixed spices.

Moreover, the mixed spices with very

low scent leaf had very low scores for the sensory attributes. The 10:45:45 scent leaf:curry:African black pepper spice for instance scored 4 – slightly dislike for flavour, and 3 – dislike moderately for appearance and general acceptability; meanwhile 10:10:80 scent leaf:curry:African black pepper spice scored 2 – dislike very much for flavour, 3 – dislike moderately for appearance and 3 – dislike moderately for general acceptability.

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The non-significance ($p>0.05$) of the lack of fit as well as the adequacy of the diagnostic values such as the high R-squared, low press, high adequate precision (Table 2) show that the models were adequate. Whereas all the models and their linear mixtures (pure blends) studied were significant ($p<0.05$), only AC (scent leaf:curry) and BC (curry:African black pepper) binary blends of the general acceptability model were significant ($p<0.05$) (Table 3)

Table 2: Diagnostic values of the models

Diagnostic values	Flavour	Appearance	Geneneral acceptability
Std. Dev.	0.63	0.65	0.51
Mean	5.01	4.666	4.87
C.V.	12.61	13.89	10.37
Press	9.58	9.44	6.96
R-Squared	0.8723	0.7895	0.9150
Adj R-Squared	0.7925	0.7512	0.8618
Pred R-Squared	0.6165	0.5686	0.7104
Adeq Precision	10.204	11.760	12.953

A = Scent leaf, B = Curry, C = African black pepper

Table 3: P- values

Parameters	Flavour	Appearance	Gen. Accept.
Model	0.0020	0.0002	0.0004
Linear Mixture	0.0003	0.0002	0.0001
AB	0.2413	-	0.1188
AC	0.2214	-	0.0332
BC	0.6945	-	0.0451
Lack of Fit	0.4034	0.9530	0.8949

A = Scent leaf , B = Curry, C = African black pepper; Level of significance: $p<0.05$

The predictive models after the removal of all the non-significant terms are given as follows:

$$\text{Flavour} = 7.5A + 4.71B + 3.34C \quad \ldots\ldots\ldots\ldots \quad (1)$$

$$\text{Appearance} = 6.66A + 4.03 + 3.14C \quad \ldots\ldots\ldots\ldots \quad (2)$$

$$\text{General Acceptability} = 7.50A + 4.74B + 4.05C - 5.11AC - 4.72B \quad \ldots\ldots\ldots\ldots \quad (3)$$

The predictive models (Equation 1 – 3) show that all the linear variables had positive effects on the sensory attributes. While scent leaf (A) had a higher linear positive effect on general acceptability and flavour than appearance, curry (B) had positive linear effect on all the sensory attributes examined with the general acceptability being the highest. Also, the highest positive linear effect of African black pepper (C) was on the general acceptability. The positive linear effect implies that the affected factors increased with increasing sensory attributes as depicted in the relevant surface plots (figures 1, 2 and 3) meaning that those spices (factors) contributed significantly ($p<0.05$) to the relevant sensory attributes of the mixed spices.

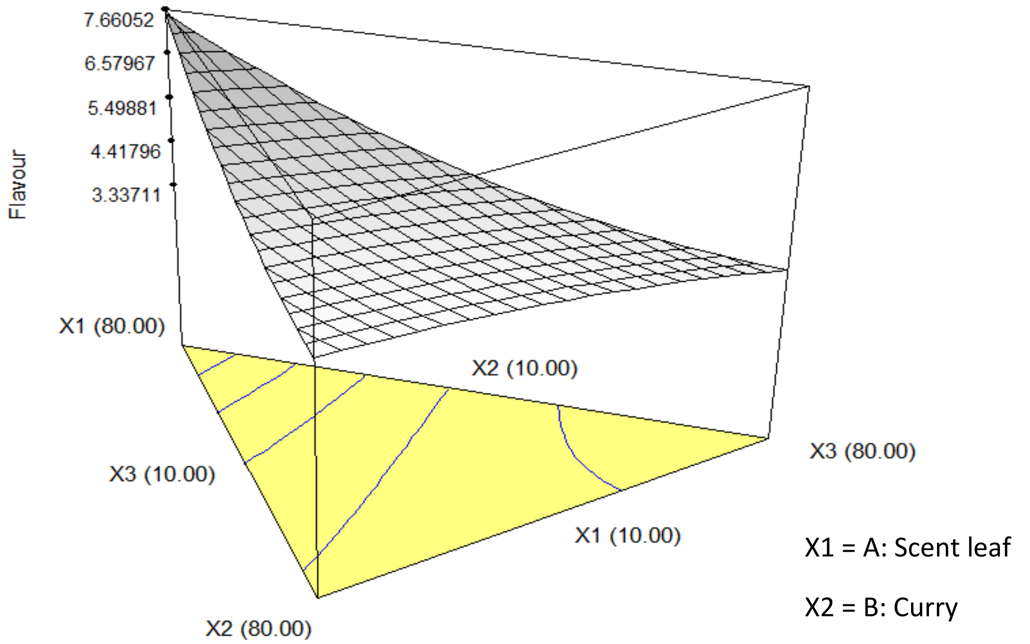

Fig. 1 Response surface plot for flavour

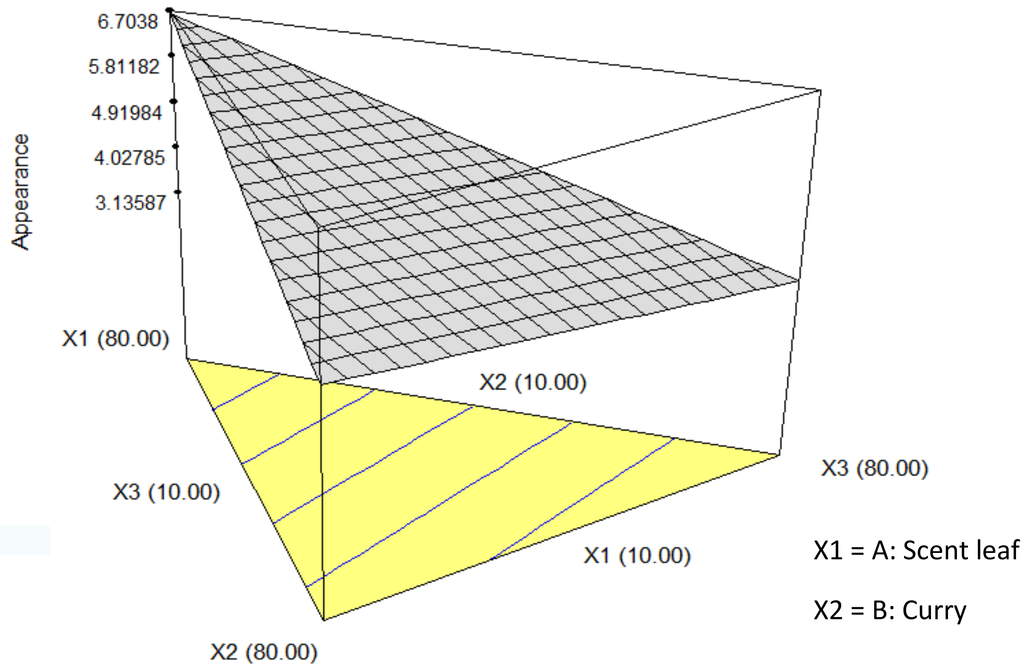

Fig. 2 Response surface plot for appearance

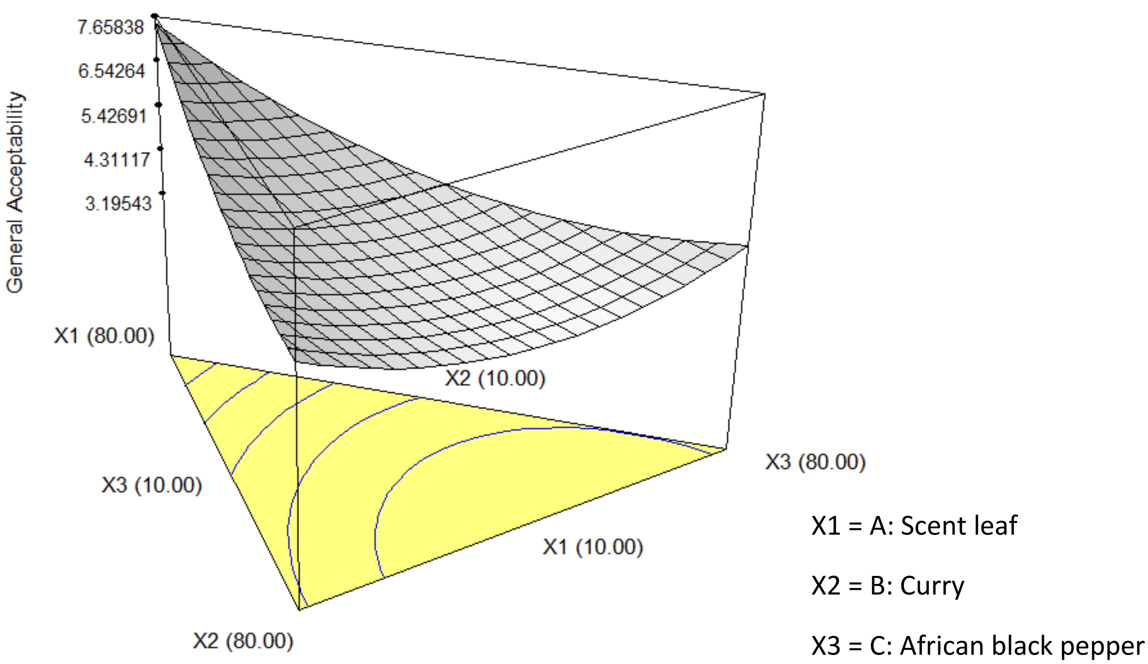

Fig. 3 Response surface plot of general acceptability

The models were successful in indicating the direction in which to change variables in order to maximize the formulation. The criteria of maximum overall acceptability include maximizing formulations with the variables kept in range to achieve the best product. The result of the optimization of the responses shows that 80:10:10 scent leaf:curry:African black pepper mixed spice with maximum values of 8-like very much (flavour), 7-like moderately (appearance) and 8-like extremely (general acceptability) and desirability of 1 was selected (optimized) (Table 4). This implies that the 80% scent leaf, 10% curry and 10% African black pepper were the values of optimum mixture parameters.

Table 4: Optimized mixed spice

Number	Scent leaf	Curry	African black pepper	Flavour	Appearance	General acceptability	Desirability	
1	80.00	10.00	10.00	7.56	6.66	7.50	1.00	Selected

The response surface plots for the chosen model equations show that scent leaf had more effect on the sensory properties than African black pepper and curry, meanwhile the general acceptability surface plot indicates a steady increase with increasing higher values of curry in the mixture.

Table 5 presents the proximate composition of the mixed spice alongside a

Table 5: Proximate analysis of the optimized mixed spice and Scent leaf

Spices	Moisture (%)	Dry matter (%)	Ash (%)	Crude fibre (%)	Ether extract (%)	Crude protein (%)	Carbohydrate (%)
80:10:10 Spice	10.890[a] ± 0.00	89.100[a] ±1.41	11.680[a] ±1.42	10.740[a] ±0.36	16.540[a] ±1.47	20.930[a] ±1.31	31.750[a] ±0.77
Scent leaf	10.730[b] ±0.042	89.680[a] ±0.028	13.700[a] ±1.27	10.500[a] ±0.56	20.380[a] ±1.24	20.820[a] ±1.44	23.530[b] ±1.59

Means with the same superscripts within each column are not significantly different (p>0.005).

control (100% scent leaf). Scent leaf had higher ash content (13.70%), dry matter (89.68%) and ether extract (20.38%), while 80:10:10 (scent leaf:curry:African black pepper) had higher moisture content (10.89%), crude fibre (10.74%), crude protein (20.93%) and carbohydrate (31.75%).This shows that besides its function as a spice, it can also be depended upon as a good source of protein. Moreover, plant foods have been shown to be good sources of protein (Sa *et al.*, 2020).

The phytochemical profile (Table 6) shows that there was no statistical difference (p>0.05) between the mixed spice and scent leaf in the values of all the phytochemicals determined exception of oxalate and alkaloid. While the presence of alkaloid in the leaves is beneficial as it poses important pharmacological property, that of oxalate is a cause for alarm, as antinutrient, it reduces the bioavailability of metals.

Table 6: Phytochemical composition of the optimized mixed spice and Scent leaf

Spices	Phenol mg/100g	Tannin mg/100g	Phytate mg/100g	Flavonoid mg/100g	Steroid mg/100g	Alkaloid mg/100g	Oxalate mg/100g	Saponin mg/100g
80:10:10 Spice	0.150^{a} ±.04	0.720^{a} ±.084	0.560^{a} ±.155	0.730^{a} ±.056	0.140^{a} ±.028	0.950^{b} ±.070	0.530^{b} ±.042	0.940^{a} ±.113
Scent leaf	0.230^{a} ±.0424	1.040^{a} ±.2262	0.660^{a} ±.084	1.140^{a} ±.197	0.240^{a} ±.056	1.690^{a} ±.127	0.940^{a} ±.056	1.380^{a} ±.113

Means with the same superscripts within each column are not significantly different (p>0.005).

The presence of tannin suggests the ability of the spice to play major roles as antifungal and antioxidant agent. Saponin has also been reported to have analgesic, anti-inflammatory and cardio-protective properties (Alemu *et al*, 2018). Flavonoid is also an antioxidant.

Table 7 shows that the mixed spice had lower (p<0.05) concentration of iron, magnesium, and zinc than the scent leaf. Calcium and copper, meanwhile, were not significantly (p>0.05) different. Iron was the highest in the spices which is quite important as a crucial factor in hematopoiesis, control of infection and cell mediated immunity.

Table 7: Mineral profile of the optimized mixed spice and Scent leaf

Spices	Iron mg/100g	Calcium mg/100g	Magnesium mg/100g	Zinc mg/100g	Copper mg/100g
80:10:10 Spice	6.1690[b] ±1.626	0.0004[a] ±.013	6.4180[b] ±2.77	1.4620[b] ±.820	0.3260[a] ±.084
Scent leaf	7.4480[a] ±1.30	0.0006[a] ±.01	7.5340[a] ±1.25	3.2940[a] ±.36	0.4790[a] ±.52

Means with the same superscripts within each column are not significantly different (p>0.005).

The levels of zinc in both spices were moderately high, although higher in scent leaf, the values were similar to those of Asaolu and Asaolu (2010) and Ayoola *et al*. (2010). Calcium an important mineral needed for healthy bones was not significantly (p>0.05) different in the spices. Calcium is essential in maintaining phosphorus-calcium balance whose deficiency results in osteoporosis (Elbossaty, 2017).

The non-detection of the heavy metals (Table 8) in the spices portends safety of the spices. The maximum permissible concentration of 10 and 0.3 mg/kg lead and cadmium respectively are recommended by FAO/WHO (WHO, 2005; 2006).

Table 8: Heavy metal profile of the optimized mixed spice and Scent leaf

Spices	Lead mg/100g	Cadmium mg/100g
80:10:10 Spice	Not Detected	Not Detected
Scent leaf	Not Detected	Not Detected

Lead and cadmium are quite toxic in foods. Lead complexes with many biomolecules and adversely affects the reproductive, immune, renal, cardiovascular, skeletal and muscular system as well as the developmental processes (Dghaim *et al.*, 2015).

CONCLUSION

The optimization studies indicated the possibility of producing acceptable mixed spice from scent leaf, curry and African black pepper at the ratio of 80:10:10. It also showed that besides functioning as a good flavouring agent, the spices possess nutraceutical properties due to its content of phytochemicals such as flavonoids, alkaloids, steroids and minerals such as iron, magnesium, calcium, zinc. Local spices should therefore be processed into useful products and used for their various nutritional, health and economic benefits.

REFERENCES

Alemu, A., Tamiru, W., Nedi, T. and Shibeshi, W. (2018). Analgesic and anti-inflammatory effects of 80% methanol extract of *Leonotis ocymifolia* (Burm. f.) Iwarsson leaves in rodent models. Evidence - Based Complementary and Alternative Medicine. Article ID 1614793, 8 pages https://doi.org/10.1155/2018/1614793.

AOAC (1995). Official Methods of Analysis. Association of Official Analytical Chemists. 16th Edition. Washington D. C., USA.

Asaolu, S. S. and Asaolu, M. F. (2010). Trace metal distribution in Nigerian leafy vegetables. Pakistan Journal of Nutrition 9(1): 91 – 92.

Ayoola, P. B., Adeyeye, I. and Onawumi, O. O. (2010). Trace elements and major minerals evaluation of *Spondias mombin*, *Verornia amydalina* and *Mmordica charatia* leaves. Pakistan Journal of Nutrition 9(8):755 – 756.

Chima, M. (2002). *Uziza* (*Piper guineense*) – local food condiments in Nigeria. Journal of African Medicinal Plants 18:114-119.

Dghaim, R., Khatib, S. A., Rasool, H. and Khan, M. A. (2015). Determination of heavy metals concentration in traditional herbs commonly consumed in the United Arab Emirate. Journal of Environment and Public Health. Article ID 973878, 6 pages http://dx.doi.org/10.1155/2015/973878.

Elbossaty, W. F. (2017). Mineralization of bones in osteoporosis and osteomalacia. Annals of Clinical Laboratory Research 5(4):201. DOI:10.21767/2386-5180.1000201

Harborn, J. B. (1973). Textbook of Phytochemical Methods. Chapman and Hall Ltd, London pp. 49-188.

https://www.fs.fed.us/wildflowers/ethnobotany/food/spices.shtml

(14th May, 2020) U.S. Forest Service, United States Department of Agriculture (USDA).

Husain, N, Pandey, B., Trak, T. H. and Chauhan, D. (2019). Medical virtues and phytochemical constituents of some of the important Indian spices. World Journal of Pharmaceutical Research 8(11): 909-919.

James, C. S. (1995). Experimental Methods of Analytical Chemistry of Food. Chapman and Hall, New York pp. 6:75 – 84.

Li, Y., Raghavarao, D., Chervoneva, I. (2017). Extensions of D-optimal minimal designs for symmetric mixture models. Journal Communications in Statistics – Theory and Methods 46:5, 2542-2558, DOI: 10.1080/03610926.2014.988258

Oberlease, D. (1973). Phytases. In: toxicant occurring naturally in foods. National Academy of Science, Washington, D. C. 363 – 371.

Onwuka, G. I. (2018). Food Analysis and Instrumentation – Theory and Practice. 2nd Edition. Naphthali Prints, Lagos pp. 414-441.

Pathasarathy, V. A., Chempakam, B. and Zachariah, T. J. (2008). Chemistry of Spices. Biddles Ltd, UK.

Pearson, D. (1976). Chemical Analysis of Food, 7th Edition. Churchhill, London pp 7-11.

Sa, A. G. A., Moreno, Y. M. F., Carciofi, B. A. M. (2020). Plant proteins as high-quality nutritional source for human diet. Trends in Food Science and Technology 97:170 – 184.

Techtron, V. (1975). Basic Atomic Absorption Spectroscopy a Modern Introduction. Dominican Press, Victoria, Australia, pp. 104-106.

Ukpabi, A. and Ejidoh, E. O. (1989). Experimental Procedures of Food and Water Analysis. San Press Publishers, Enugu, Nigeria pp. 89.

World Health Organization (WHO) (2005). Quality Control Methods for Medicinal Plant Materials, World Health Organization, Geneva, Switzerland.

World Health Organization (WHO) (2006). WHO Guidelines for Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues, World Health Organization, Geneva, Switzerland.