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Effect of Processing Methods on Sodium Glutamate Seasoning Production from Milk Bush (*Thevetia peruviana*) Seed Collected at Lafe Area, Akure, Ondo State, Nigeria

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ABSTRACT: Sodium glutamate is a major component for the production of seasoning. Studies have revealed that *Thevetia peruviana* seed contained high amount of glutamic acid which makes it essential for industrial applications. Hence, the objective of this paper was to evaluate the effect of oven-dried, air-dried and sun-dried processing methods on sodium glutamate seasoning production from milk bush (*Thevetia peruviana*) seed collected at Lafe Area, Akure, Ondo State, Nigeria using appropriate standard procedures. The result for the qualitative determination of sodium glutamate ((seasoning) (oven, air and sun-dried kernel 0.792, 0.692 and 0.875 respectively against the control 0.750, 0.654 and 0.830) and quantitative analysis confirmed that the sample is sodium-glutamate (seasoning). The result revealed that there was presence of sufficient protein in the produced salt which can serve as a good source of protein, also the result showed that the processing technique does not affect the parameters evaluated. It was concluded that the production of seasoning from the kernel of *Thevetia peruviana* is an alternative source of producing seasoning which is biological, biodegradable, available in large quantity by planting and can replace the existing chemically produced seasoning.

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Thevetia peruviana (Pers.) K. Schum is an evergreen ornamental dicotyledonous shrub which belong to Apocyanaceae family (Ibiyemi *et al.*, 2002). The plant is commonly known as yellow oleander, lucky-nut, be- still tree and milk bush (Ibiyemi *et al.*, 2002). It is called Olomi-Ojo in Yoruba dialect. The plant has a wide geographical distribution such as tropical America, tropical Africa and out of 250,000 species of plant, *Thevetia peruviana* is occupied with seed oil, proteins and biologically active compound which can be used for nutrition, biofuel and medical purpose (Odihiambo *et al.*, 2012). Its habitat is the tropical and sub-tropical land areas of the world and has been found in Mexico, West-Africa, Central and South America. (Odihiambo *et al.*, 2012). The height of the plant is between 10 – 18 feet with straight leaves of 13 – 15 cm in length (Garima and Amla, 2011). The plant is of two types, the yellow oleander and nerium oleander. The fruit contains green fleshy mesocarp of 4 – 5 cm diameter. This becomes black on ripening. The fruit produce between 1 – 4 seeds in its kernel and the plants has creamy juice in all its organs. Several studies Akintelu *et al.* (2020); Eddleston *et al.* (2000); Bandara *et al.* (2010); Kareru *et al.*

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(2010); Gupta *et al.* (2011); Akintelu and Amoo (2017) have shown the significant importance of *Thevetia peruviana seed*. Typically, Akintelu *et al*. (2020) established that the seed contained high fat and protein which makes it useful for feed and food formulation. Similarly, studies by Eddleston *et al.* (2000); Bandara *et al*. (2010); Kareru *et al.* (2010); Gupta *et al.* (2011); Akintelu and Amoo (2017) revealed that *T. peruviana* plant can be used as a heart stimulant. Although, some studies found out that *T. peruviana* is toxic in its natural form due to the presence of cardiac glycosides called Thevetin A and Thevetin B (Ibiyemi *et al*., 2002; Bandara *et al*., 2010; Sharma *et al.*, 2022; Akintelu and Amoo, 2017). Sharma *et al.* (2022) established that *T. peruviana* seed requires adequate processing to remove its toxic agent and therefore be used as protein supplement in rabbits and other livestock feeds. The report of El-sawi *et al*. (2020) also confirmed that *T. peruviana* is a safe cytotoxic agent. *T. peruviana* has been used to traditionally treat many ailments including intestinal and inflammatory diseases, heart failures and cancer of the skin (Oji and Okafor, 2000; Alonso-Castro *et al.*, 2011; Ramos-Silva *et al.*, 2017).

Commercially, seasonings are produced from chemically synthesized glutamic acid which are not biodegradable, not environmentally friendly and may be toxic on consumption by mammals. Seeds that contain high amount of glutamic acid are good sources of seasoning production that are biological oriented. To mention a few, Ibrahim *et al.* (2011) produced seasoning (Dawadawa Botso) from the seeds of Roselle (*Hibiscus sabdariffa*) while Kantachote *et al.* (2016) produced meat seasoning powder from mature coconut water using *Pediococcus pentosaceus*. Lawal *et al.* (2011) also produced L-glutamic acid (seasoning) from three (3) different sources Dawadawa, Ugba, Ogiri (fermented vegetable protein) by *Bacillus spp.* However, there is no documented fact where seasoning is produced from the seed of *T. peruviana* despite the fact that the seed contained high content of glutamic acid (Akintelu *et al.*, 2020). *T. peruviana* seeds have been discovered to contain several nutrients such as protein, fibre, minerals, fat which makes it useful for food formulation Usman *et al.*, (2009). It can also serve as a good source of energy and contained several medicinal properties which can be used to treat malaria, rheumatism, fever among others (Buvaneswari *et al*., 2011; Akintelu and Amoo, 2016; Akintelu *et al*., 2020). However, this study tends to produce seasoning through biological sources which is expected to be biodegradable, environmentally friendly and available in large quantity by planting. Hence, the objective of this paper is to evaluate the effect of oven-dried, air-dried and sun-dried processing methods on sodium glutamate seasoning production from milk bush (*Thevetia peruviana*) seed collected at Lafe Area, Akure, Ondo State, Nigeria.

MATERIALS AND METHODS

Sample Collection and Preparation: The *Thevetia peruviana* fresh fruit samples used in this study were obtained from Lafe area Akure, Ondo State, Nigeria and identified at the Department of Crop, Pest and Soil Management, Federal University of Technology, Akure. The fruits were broken to remove its kernel which was further subjected to oven-dried, airdried and sun-dried processing method. The processed sample were further ground to powder, packed in an airtight plastic sample and preserved in a refrigerator at 4ºC separately prior to the analysis.

Where A = Pulp; B = Shell; C = Kernel

Fig.1:*Thevetia peruviana* pulp with shell and kernels

Reagent/Chemical/Apparatus Used: All the reagents and apparatus were of analytical grade and are available in Chemistry Department FUTA, others were obtained from commercial vendors in Lagos, Nigeria.

Production of Monosodium-glutamate (seasoning) from Thevetia perviana seed: The production of monosodium-glutamate from *Thevetia perviana* seed was done using a natural fermentation. In this study, a 100 g of each sample (sun, air and oven-dried) was weighed into 250 ml conical flask. A 150 ml of potassium phosphate buffer (87 g of K_2HPO_4 and 68g of KH_2PO_4) was added to the samples, covered with foil paper and transferred into an electric shaker incubator machine (VWR TM international) for 8 days at 37 ºC. After 8 days of fermentation, the samples were filtered using a clean white cloth to get the extract (liquid) and the residue. Subsequently, the extract (liquid) which is sodium-glutamate was freeze-dried to powder using the lyophilized machine (Christ alpha 1-2 LD plus 55 lyophilizer machine). Similarly, 1 ml of the extract (liquid) sample was transferred into the centrifuge (sigma 4-16K) at 25 ºC and 4100 rpm for 30 min, its forms into two layers (the supernatant and the residue) and then the supernatant was poured into PET (polyethylene terephthalate) bottle for estimation of protein and glutamate determination using the *Ultraviolet*-visible spectrophotometer (UV-6300PC double beam spectrophotometer VMR TM international).

Fig.2: Production of Monosodium-glutamate (seasoning) from Thevetia perviana seed
A= Raw ground sample; B= Sample + buffer; C= Sample during fermentation; D= Fermented sample; E= Filtrate sample; F= Sample produced and lyophilised to powder; G= Final product (sodium-glutamate)

Protein Determination of Sodium-glutamate: The protein determination of sodium-glutamate extract was carried out using Lowry *et al*., (1951) method. A 200 µl of each sample in triplicate was measured into the test tube and 800 µl of distilled water were added. Then 5 ml of solution C (50 ml of A ($NaCO_3$ + NaOH) and (1 ml of B [$CuSO_4$+$C_4H_4KNaO_6$.$4H_2O$ (Sodium Potassium tartarate]) was pipetted and transferred into each sample in the test tube. It was allowed to stay for 10 mins, thereafter 500 µl of folin c was pipetted and poured into each sample and was allowed to stay for 30 mins. The samples settled and form into layers showing reaction that there is presence of protein which gradually changes to blue. Then after 30 mins the sample were introduced into the UV spectrophotometer at 660 nm and the absorbance was read out. The protein content in the sample was determined by reference to a standard curve of known concentration. The same procedure was carried out for blank made by substituting the extract with distilled water and used as the control to set the absorbance to zero.

Qualitative determination of L-glutamic acid: Twenty microliters of the centrifuged portion from the fermentation medium by the test isolate were chromatographed by spotting on the thin layer chromatographic plates (TLC) and developing it in solvent mixtures of n-butanol. Acetic acid and water (4:1:1 v/v) in ascending direction for 6 hours. The developed chromatogram was dried, sprayed with 0.2% (w/v) ninhydrin in ethanol, and then heated at 110^0C for 30 minutes. Spots of same Rf value as the standard glutamic acid were compared for intensity (Kinoshita *et al.* (1956); Brenner and Nieser (1967); Ogbadu *et al.*, 1990).

Retention factor= Rf

$$Rf = \frac{Distance\ moved\ by\ the\ solute}{Distance\ moved\ by\ the\ solvent\ front} \quad \ldots 1$$

Quantitative determination of L-glutamic acid: The method of Rosen (1957) and Lawal *et al.* (2011) were used. It was estimated by ninhydrin color reaction and the absorbance was measured at 570 nm. 1ml of the sample extract was added to 0.5 ml cyanide-acetate buffer (pH 5.4) and 0.3% w/vol Ninhydrin in methyl cellulose was added. The mixture was heated in a boiling water bath at 100 ºC for 25 mins, immediately after 25 mins, the mixture was removed from the water bath; 5.0 ml Isopropyl alcohol to water mixture (ratio 1:1) was added as diluent and the mixture was mixed by shaken vigorously, cooled to room temperature (25 ºC). The amino acid profile was estimated by determining the optical density at 570 nm wavelength using UV spectrophotometer. The blank was similarly treated same as sample above and used as the control to set the absorbance to zero.

RESULTS AND DISCUSSION

Protein Content of Sodium-glutamate from Air-Dried (AD), Oven-Dried (OD) and Sun-Dried (SD) sample of Thevetia peruviana seed @ 660 nm: Table 1: presents protein determination of sodium-glutamate Air-Dried (AD), Oven-Dried (OD) and Sun-Dried (SD) sample of *Thevetia peruviana* seed at 660 nm for 8 days. The result reveals that air-dried sample had the highest (43247.83) protein value followed by sun-dried sample (35942.99) and the least among them oven-dried with 25092.42 for the supernatant at 8 days of fermentation. Also, for the crude extract, the air-dried sample still had the highest value (30382.96) of protein content, followed by the sun-dried sample with 28196.61 and the least among them is oven-dried sample with 25938.04 which is an indication that air-dried sample is a good source of protein. The result of Malviya *et al.* (2011) established a higher (68000) protein concentration from the seed extract of *Jatropha curcas* seed than the result in this study.

Qualitative determination of Sodium-glutamate from Air-Dried (AD), Oven-Dried (OD) and Sun-Dried (SD) sample of Thevetia peruviana seed using Thin layer Chromatography (TLC): Table 2: presents the result of Thin layer chromatogram of the three (3) samples and standard monosodium-glutamate (MSG). The identification of MSG in the samples from TLC chromatogram was achieved by comparing the R_f value of the spots in the samples with the R_f value of standard MSG which was taken to be within 0.83-0.65 in n-butanol. Acetic acid and water ratio (4:1:1 v/v) mobile phase. As a result, all the samples which were spotted on TLC plate showed that they contained MSG. The R_f value obtained is in conformity with the work done by George *et al.* (2013) for stem bark extract of *Blighia unijugata* (*Sapindaceae*) on monosodium-glutamate induced uterine leiomyoma in Sprague- Dawley rats. Also, the R_f values result of Ambusaidi *et al.* (2020) for monosodium-glutamate occurrence in food products are in agreement with the work done in this study.

Table 1: Protein Content of Sodium-glutamate from Air-Dried (AD), Oven-Dried (OD) and Sun-Dried (SD) sample of Thevetia peruviana pulp @ 660 nm

Parameters	AD(µg/ml)	AD(%)	OD(µg/ml)	OD(%)	SD(µg/ml)	SD(%)	Control (Maggi) in %
Supernatant @8days	43247.83	0.432	25092.42	0.251	35942.99	0.359	10.623
Crude extract @8days	30382.96	0.304	25938.04	0.259	28196.61	0.282	

***Key**: OD.S = Oven-dried kernel sample, AD.S=Air-dried kernel sample, SD.S=Sun-dried kernel sample*

Table 2: Qualitative determination of Sodium-glutamate from Air-Dried (AD), Oven-Dried (OD) and Sun-Dried (SD) sample of Thevetia peruviana seed using Thin layer Chromatography (TLC)

Coded Sample	**Solute front Sample**	**Solute front Standard**	**Dist. Travelled By solvent**	**R_f value Sample**	**R_f value Standard**
OD	9.5	9.0	12	0.7916	0.75
AD	9.0	8.5	13	0.692	0.6538
SD	6.3	6.0	7.2	0.875	0.83

Solvent system: n-butanol: acetic acid: water (4:1:1 v/v)

Quantitative determination of Sodium-glutamate from Air-Dried (AD), Oven-Dried (OD) and Sun-Dried (SD) sample of Thevetia peruviana kernel @ 570 nm: Table 3: presents the quantitative determination of Air-Dried (AD), Oven-Dried (OD) and Sun-Dried (SD) sample of *Thevetia peruviana* seed at 570nm. The result reveals that sun-dried sample had (0.301%) the highest percentage followed by oven-dried sample with 0.296% and the least among them air-dried with 0.191% for the supernatant at 8 days of fermentation. While for the crude extract of the oven-dried sample had the highest percentage (0.382%) of glutamic acid content, followed by the sun-dried sample with 0.295% and the least among them is air-dried sample with 0.273%. The result indicate that sun-dried sample contain high percentage of glutamic acid for the supernatant and oven-dried sample contain high percentage of glutamic acid for the crude extract which can be used as source for seasoning. The result is higher than the work of Zehra *et al.* (2017) for

monosodium glutamate concentration on common foods such as onions (0.118%), garlic (0.128%), potato (0.102%), carrot (0.054%), egg (0.023%).

Table 3: Quantitative determination of Sodium-glutamate from Air-Dried (AD), Oven-Dried (OD) and Sun-Dried (SD) sample of Thevetia peruviana seed @ 570nm

Parameters	AD(µg/ml)	AD (%)	OD(µg/ml)	OD (%)	SD(µg/ml)	SD (%)
Supernatant @8days	191.38	0.191	296.13	0.296	300.59	0.301
Crude extract @8days	272.58	0.273	382.71	0.382	295.32	0.295

Key: OD.S = Oven dried sample, AD.S=Air dried sample, SD.S=Sun dried sample

Conclusion: It was concluded that the processing techniques used in the study does not affect the production of seasoning produced. It was also concluded that *Thevetia peruviana* is an alternative source of producing seasoning which is biological, biodegradable, and available in large quantity by planting and can replace the existing chemically produced seasoning. Because of the nutritional quality of the kernel of *Thevetia peruviana*, it can be recommended that the kernel of *Thevetia peruviana* be used in food supplement especially in animal feeds and also serve as a good source of raw material for the production of seasoning More studies should be done on the molecular mechanism of glutamic acid in exerting its therapeutic effect as an antioxidative amino acid. Clinical trials could also be carried out to check the effect of this sample on the human system.

Declaration of Conflict of Interest: The authors declare that there is no conflict of Interest.

Data Availability Statement: The authors declare that data for this research are available upon reasonable request from the corresponding author.

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