

Research Article

Phytochemical Composition of Indigenous Vegetable Sauces with Anti-Diabetic Potentials

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Abstract: Chemical and mineral bioavailability of indigenous vegetable sauces: implication for type 2 diabetes mellitus was evaluated. Vegetables and ingredients for sauce preparation were purchased from Relief market Owerri, Imo State. The vegetables were used in preparation of okra sauce, African spinach sauce and lettuce sauce. It was oven-dried at 50°C for 14 hours. The sauces were analyzed for proximate, minerals, vitamin, antinutrient and mineral to antinutrient ratios was calculated using standard methods. Statistical analysis using Statistical Product for Service Solution (SPSS) was used to determine one way Analysis of variance (ANOVA) to separate the means while Turkey test model was used to test significant difference. P-value at 0.05 was considered significant. Significant difference was observed only in dietary fibre (p<0.05). Mineral composition showed significant (p<0.05) differences in African spinach sauce for sodium, calcium, magnesium, potassium, iron, zinc and manganese and only highest in phosphorus for lettuce sauce. Tannin was significantly highest in lettuce sauce and African spinach sauce, for phytate and oxalate in African spinach sauce. all the minerals studied showed high bioavailability. Vitamin composition was significantly (p<0.05) different among the vegetable sauces in all the water soluble vitamin studied but was only significant (p<0.05) for vitamin D for all the fat soluble vitamins in okra sauce. Consumption of these vegetable sauces should be encouraged especially the type 2 diabetic subjects.

Keywords: Vegetable Sauces, Indigenous, Type 2 diabetes Mellitus, Mineral Bioavailability.

INTRODUCTION

Diabetes mellitus is a consequence of carbohydrate, protein and lipid metabolism caused by lack/ reduced insulin production (Type 1DM) or resistance to insulin action (Type 2DM) (Gaikwad *et al.*, 2014). Insulin is a hormone secreted by the Beta cells of the pancreas of Langerhans which controls many processes in the body including the uptake of glucose into the body cells, synthesis of fatty acid in the liver and adipose tissue and also promotes protein synthesis in different tissues. In some people, their cells may stop responding to insulin which is a condition referred to as insulin resistance. Synthetic medicine which is used for the treatment of diabetes increases diverse secondary complications and side effects (Mohammed *et al.*, 2013) while phytochemical in foods are more preferable due to its rich availability, efficacy and fewer side effects especially in human

populations (Atanasov *et al.*, 2015 and Change *et al.* 2013).

Phytochemicals in diet have been reported to have therapeutic benefit that prevent, manage and fight against diet-related chronic diseases like hypertension, cardiovascular diseases and metabolic diseases such as diabetes (Gothai *et al.*, 2016; Patel *et al.*, 2012). Bioactive compounds or phytochemicals in fruits, vegetables, nuts and grains exert protective effects against the pathology of diseases through the attenuation of inflammatory mediators (Mursu *et al.*, 2014). observational and interventional studies that used plant-based diet shows that the presence of biologically active compounds or phytochemicals can reduce the risk of diet-related non-Communicable diseases (DRNCs) such as diabetes, cardiovascular diseases and cancer (Upadhyay and Dixit, 2015). Regular consumption of dietary polyphenols.

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anthocyanins, flavonoid, and isoflavones may improve insulin sensitivity, reduce insulin levels, total cholesterol, High Density Lipoprotein Cholesterol (HDL-C) ratio and lower Low Density Lipoprotein Cholesterol (LDL-C) and Type 2 Diabetes Mellitus risk (Hanhineva *et al.*, 2010; and Curtis *et al.*, 2012). The protective role of bioactive food and phytochemicals has been associated with their antioxidant activity because of the excess production of reactive oxygen species and reactive nitrogen species (oxidants) in human population which causes an imbalance and oxidative damage to large biomolecules such as lipids, DNA, and proteins. This effect leads to the pathogenesis of some chronic diseases such as CVD. Some cancers, ageing and diabetes (Pouiose *et al.*, 2014; Singh *et al.*, 2014).

Vegetables are succulent herbaceous plants that are harvested and eaten whole or in part, raw or cooked as part of a main dish or salad (Uzo, 1989). Vegetables are generally low in calories and protein, but high in vitamins, minerals, fibre and phytochemicals and bioactive compounds which help in the prevention and management of diabetes (Udentia *et al.*, 2014). In Nigeria, vegetable consumption is low despite its recognition as a very important food item to reduce micronutrient deficiencies from inadequate intake of minerals and vitamins. Consumption of vegetable has been reported to be beneficial in blood cholesterol reduction, prevents bowel diseases and improves glucose intolerance due to its dietary fibre (Ashaye, 2010).

MATERIALS AND METHODS

Procurement of Materials

Vegetables and ingredients for sauce preparation were purchased from Relief market Owerri, Imo State. The vegetables were identified at Crop Department, Faculty of Agriculture and Veterinary Medicine, Imo State University, Nigeria.

Recipe for Vegetable Sauces

Recipe for the vegetable sauces was designed to accommodate the same ingredients and method of cooking. Preparation was carried out at Food Laboratory, Nutrition and Dietetics Department Imo State University.

Okra Sauce Preparation

Ingredients	Quantity
Okra pods	1000g
Sliced onion	200g
Palm oil	15g
Milled crayfish	150g
Milled fresh pepper	15g
Salt	2 level teaspoon
Bouillon cube (knorr cube)	1cube
Sliced tomatoes	200g
Water	1500ml

METHOD OF COOKING OKRA SAUCE

Okra pods were washed and sliced into small size and was prepared separately. The pot was allowed to dry. The oil and onion was added immediately (it should not be allowed to heat before adding the onion so that the oil will not be hydrogenated). The sliced tomatoes was be added and was allowed to steam for 3 minutes. Crayfish, pepper, salt, knorr cube and water was added and allowed to stem for 2minutes. Okra and water was added and allowed to steam for 5minutes. It was removed from heat and allowed to cool. The okra sauce was oven dried at 50°C for 14 hours.

African Spinach sauce preparation

Ingredients	Quantity
African Spinach leaves	1000g
Sliced onion	200g
Palm oil	15g
Milled crayfish	150g
Milled fresh pepper	15g
Salt	2 level teaspoon
Bouillon cube (Knorr cube)	1 cube
Sliced tomatoes	200g
Water	1500ml

Method of Cooking African Spinach Sauce

African spinach leaves were washed and sliced into small sizes and was prepared separately. The pot was allowed to dry, the oil and onion was added immediately (it should not be allowed to heat before adding the onion so that the oil will not be hydrogenated). The sliced tomatoes was be added and was allowed to steam for 3 minutes. Crayfish, pepper, salt, knorr cube and water was added and allowed to stem for 2minutes. African spinach vegetables and water was added and allowed to steam for 5minutes. It was removed from heat and allowed to cool. The African spinach sauce was oven dried at 50°C for 14 hours.

Lettuce Sauce Preparation

Ingredients	Quantity
Lettuce leaves	1000g
Sliced onion	200g
Palm oil	15g
Milled crayfish	150g
Milled fresh pepper	15g
Salt	2 level teaspoon
Bouillon cube (knorr cube)	1 cube
Sliced tomatoes	200g
Water	150ml

Method of Cooking Lettuce Sauce

Lettuce leaves were washed and sliced into small sizes and was prepared separately. The pot was allowed to dry, the oil and onion was added immediately (it should not be allowed to heat before adding the onion so that the oil will not be hydrogenated). The sliced tomatoes was be added and was allowed to steam for 3 minutes. Crayfish,

pepper, salt, knorr cube and water was added and allowed to steam for 2 minutes. Lettuce leaves were added and allowed to steam for 5 seconds. It was removed from heat and allowed to cool. The lettuce sauce was oven dried at 50°C for 14 hours respectively.

Quantitative and Qualitative Characterization of Phytochemical Profile Preparation of Aqueous Extract of the Vegetable Sauces

Preparation of sample for characterization of phytochemical was carried out according to the method described by Ikekwuchi *et al.* (2016). The vegetable sauce was oven dried at 55°C, and ground into powder. The powder was soaked in boiled distilled water for 12 hrs. after which the resultant mixture was filtered and the filtrate was evaporated to dryness. The percentage recovery of the crude extract was recorded. The residue obtained from the crude aqueous extract was subjected to phytochemical analysis using gas chromatography.

(A): Determination of the Flavonoids

Extraction:

Flavonoid determination was carried out according to the method described by Millogo-Kone *et al.* (2009). The dried extract of ethanolic and aqueous extraction was sampled and made free of water by ensuring constant weight for a period of time. 1.0g of the pulverized vegetable and vegetable sauces respectively were weighed into the 250ml capacity conical flask with addition of 100ml of distilled water and boiled for 10 min. Flavonoid extract was obtained by pouring 100ml of the boiling methanol (70:30) v:v into the materials. The mixture was allowed to macerate for about 2hr and then filtered with Whatman filter paper (N01). The filtrate concentrated to 5 ml for the gas chromatographic analysis.

GC Conditions for the Analysis:

Gas Chromatographic analyses were carried out on an HP 6890 (Hewlett Packard, Wilmington, DE, USA), GC apparatus, fitted with a flame ionization detector (FID) (range scanned: 220-500 nm), and powered with HP Chemstation Rev A 09.01 (1206) software, to quantify and identify compounds. The column was a ZP-5 Column (30 m x 0.32 mm x 0.25 µm film thickness, carrier gas nitrogen, oven temperature program initial temperature at 50°C, first ramping at 8°C per min for 20 min, maintained for 4 min. second ramping at 12°C/min for 4 min. Detector temperature was 320°C.

(B): Determination of Phenolic Acid Composition

Extraction: The extraction was carried out by modification of the method of Andary *et al.*

(2013) two stage extraction procedure were followed for the effective removal of the phenolic compound.

Stage 1: Fifty gram of the pulverized vegetable and vegetable sauces respectively were extracted with 5ml of 1M NaOH for 16hr on a shaker at ambient temperature. After extraction, the sample was centrifuged at 5000 rpm, rinsed with water, centrifuged again, and the supernatant was combined and placed in a disposable glass test tube and heated at 90°C for 2hrs to release the conjugated phenolic compounds. The heated extracts were cooled, titrated with 4M HCl of pH < 2.0 diluted to 10ml, with deionized water, and centrifuged to remove the precipitate. The supernatant was saved for subsequent purification and the residue was extracted for further use in stage 2.

Stage 2: The residue from stage one above was extracted with 5ml of 4M NaOH, heated to 160°C in Teflon. After cooling, the mixture was filtered. Supernatant was collected and the residue washed with deionized water. The supernatant was combined and adjusted to pH < 2.0 with 4M HCl. The filtrates will be combined for further purification.

Preparation for GC: Two ml of the concentrated extract was transferred to 5.0 ml glass vial. The extract was saturated with NaCl salt before the addition to a 250.0µL of ethyl acetate. The mixture was agitated manually for 10mins at room temperature and later centrifuged for 15mins at 2500rpm. The organic phase was removed to a 1ml vial. The extraction was repeated twice and the organic phases were mixed together. 50.0µL of the N, O-BLs (trimethylsilyl) trifluoroacetamide was added and the mixture was manually agitated for 2min at room temperature for derivatization.

GC condition for analysis of phenolic compounds: Column type: **HP-1**; column dimension 30m x 0.25mm x 0.25µm film thickness; injection temperature 250°C; Detector temperature 320°C; temperature program initial temperature 60°C for 5mins first ramping at 15°C/min for 15min and maintained for 1min; second ramping at 10°C/min for 4mins, carrier gas nitrogen; nitrogen pressure.

(c): Determination

Extraction: The carotenoids extraction was carried out by a modification of the method of Rodriguex-Amaya and Kimura (2004). Exactly 5.0g of the pulverized sample was homogenized in 75ml of acetone and kept at room temperature for 1 hr in the dark. The homogenate was filtered through filter paper by suction. Extraction was repeated 3 times with the same volume of acetone. The

extracts was combined and evaporated under reduced pressure and the residue will be re-extracted by mixture of diethyl ether and petroleum ether in equal ratio. The extract was poured into the round bottom flask of the rotator evaporator arrangement. It was concentrated by evaporation. Then the concentrated extract was dried by using the anhydrous sodium sulphate before gas chromatographic analysis.

GC conditions for analysis: Gas Chromatogram HP 6890 powered with HP Chemstation Rev.09.01 [1206] software; injection temperature : split injection and split ratio 20:1; carrier gas nitrogen; inlet temperature 250°C; capillary type AC-5, column dimensions 30m x 0.25mm x 0.25µm ;oven program: initial temperature at 60°C for 5min, first ramping at 10°C/min for 20 min maintained for 2 min, second ramping at 15°C/min for 4min maintained for 4 min ;Detector: Flame ionized detector and detector temperature 320°C ;carrier gas nitrogen ;compressed air pressure 40psi.

Table 1: Quantitative phytochemical evaluation of vegetable sauces

Phytochemicals(mg/100g)	Lettuce sauce	Okra sauce	African spinach sauce
Carotenoids	4.52 ^b ±0.03	6.24 ^a ±0.04	1.40 ^c ±0.02
Flavonoid	51.17 ^c ±0.12	71.82 ^a ±0.21	45.79 ^c ±0.01
Phenolic acids	111.50 ^a ±0.23	67.77 ^b ±0.31	90.15 ^b ±0.23

Values are means ± SD (Standard) of triplicate determination. Mean values of different superscript in the same row are significant are p<0.05.

Qualitative Phytochemical Profile of Vegetable Sauces

Carotenoids profile of the vegetable sauces revealed that the vegetable sauces were low in carotenoids but alpha-carotene < lutein < zeaxanthin were significantly higher (p<0.05) in African spinach (Table 2). Flavonoid profile of the vegetable sauces showed that catechin (28.00±0.02mg/100g) and epicatechin (22.23±0.33mg/100g) were significantly (p<0.05) higher in okra sauces (Table 3). Narigenin (3.57±0.22mg/100g), luteolin (8.15±0.24mg/100g) and kaemferol (21.30±0.25mg/100g) were significantly (p<0.05) higher in lettuce while quercetin (22.05±0.32mg/100g) and isohamnetin (9.164±0.25mg/100g) were higher in African

Statistical Analysis

Statistical Product for Service Solution (IBM SPSS Inc., Chicago Ill) version 23.0 was used in statistical analysis. Descriptive statistics was used in analyzing the means and standard deviation of mineral and antinutrient vegetable sauces. One way analysis of variance (ANOVA) was used to test the significant difference of the different sauces while turkey test model. All statistical analysis was tested at 95% confidence level (p<0.05).

Quantitative phytochemical evaluation of vegetable sauces

Quantitative evaluation of the vegetable sauces shows that okra was significantly (p<0.05) higher in carotenoids (6.24±0.04mg/100g) and flavonoid (71.82±0.21mg/100g) while lettuce was significantly higher in phenolic acids (111.50±0.23mg/100g).

spinach.

The dominate phenolic acid in the vegetable sauces were salicylic acid (8.48±0.03mg/100g), syringic acid (12.22±0.1mg/100g), sinapinic acid (32.18±0.30mg/100g), ellagic acid (4.55±0.12mg/100g) and chlorogenic acid (8.08±0.01mg/100g) (Table 4). Gentistic (5.80±0.01mg/100g), vanillic acid (4.87±0.01mg/100g), phydroxybenzoic acid (33.47±0.03mg/100g), gallic acid and ferrulic acid (21.98±0.01mg/100g) were significantly (p<0.05) higher in African spinach while protocatechuic acid (38.26±0.01mg/100g) was significantly (p<0.05) higher in okra sauces.

Table 2: Qualitative carotenoids profile of vegetable sauces

Carotenoids (mg/100g)	Lettuce Sauce	Okra sauce	African spinach sauce
Beta-cryptoxanthin	0.00132 ^b ±0.02	0.00281 ^b ± 0.01	0.01968 ^a ±0.02
Alpha-carotene	0.000000269 ^c ±0.01	0.00085 ^b ±0.02	0.1173 ^a ±0,05
Lycopene	0.000000269 ^c ±0.02	0.00000043 2 ^b ±0.04	0.00025 a±0.03
Beta-carotene	0.001419 ^b ±0.03	0.00213 ^b ±0.03	0.13966 a±0.01
Lutein	0.0084 ^c ±0.02	0.01147 ^b ±0.03	0.237 ^a ±0.02
Luteoxanthin	0.000144±0.0	0.00988±0.03	0.01482±0.03
Zeaxanthin	0.00729 ^c ±0.02	0.00988±0.03	0.86294 ^a ±0.04
Xanthophyll	0.00712 ^b ±0.04	0.01061 ^a ±0.01	0.00000069 a±0.02
Neoxanthin	0.00565 ^{ab} ±0.02	0.00468 ^b ±0.03	0.00955 ^a ±0.012
Asta-xanthin	0.00055 1 ^a ±0.02	0.00070 ^a ±0.01	0.00000475 ^b ±0.02

Values are means ± SD (Standard) of triplicate determination. Mean values of different superscript in the same row are significant are p<0.05.

Table 3: Qualitative flavonoid profile of vegetable sauces

Flavonoid (mg/100g)	Lettuce sauce	Okra sauce	African spinach sauce
Catechin	1.13 ^b ±0.01	28.0026 ^a ±0.02	0.0089 ^c ±0.02
Resveratrol	0.00000126 ^b ±0.12	0.0000101 ^a ±0.01	0.00000091±0.01
Genistein	0.00000121 ^a ±0.23	0.00000963 ^a ±0.11	0.00000087 ^b ±0.12
Daidzein	0.00000133 ^b ±0.012	0.0000106 ^a ±0.02	0.00000094 ^a ±0.11
Apigenin	0.020 ^a ±0.11	0.00302 ^b ±0.01	0.00014 ^c ±0.01
Butein	0.00000248 ^b ±0.13	0.0000166 ^a ±0.01	0.0000016 ^b ±0.03
Biochanin	0.00000452 ^b ±0.12	0.0000351 ^a ±0.02	0.0000029 ^b ±0.02
Naringenin	3.565 ^a ±0.22	0.0278 ^b ±0.01	0.0022 ^c ±0.01
Luteolin	8.145 ^a ±0.24	0.00628 ^c ±0.12	5.84 ^b ±0.23
Kaemferol	21,300 ^a ±0.25	1.8870 ^c ±0.21	14.45 ^b ±0.33
Epicatechin	0.0004 ^b ±0.11	22.2299 ^a ±0.33	0.00021 ^b ±0.01
Epigallocatechin	0.00024 ^b ±0.10	0.4439 ^a ±0.01	0.00032 ^b ±0.01
Galocatechin	0.00014 ^b ±0.11	0.7137 ^a ±0.01	0.0000074 ^c ±0.02
Quercetin	6.25 ^c ±0.21	16.7756 ^b ±0.43	22.05 ^a ±0.32
Epicatechin-3-gallate	0.000046 ^b ±0.02	0.4439 ^a ±0.01	0.000037 ^b ±0.11
Epigallocatechin-3-gallate	0.000054 ^a ±0.02	0.000445 ^a ±0.01	0.000021 ^a ±0.02
Isorhamnetin	0.00000067 ^c ±0.03	1.7419 ^b ±0.11	9.164 ^a ±0.25
Robinetin	0.0000013 ^b ±0.01	0.0000204 ^a ±0.01	0.0000012 ^b ±0.11
Myricetin	10.46 ^a ±0.21	0.006 ^b ±0.01	0.00052 ^c ±0.02
Baicalein	0.0000015 ^{ab} ±0.02	0.000013 ^a ±0.01	0.00000072 ^b ±0.01
Nobiletin	0.00000079 ^b ±0.01	0.00000733 ^a ±0.01	0.00000036 ^c ±0.11
Baicalin	0.0000019 ^a ±0.03	0.00000051 ^b ±0.02	0.00000072 ^c ±0.02
Isoquercetin	0.01 ^a ±0.01	0.00749 ^b ±0.11	0.00033 ^b ±0.01
Tageretin	0.00000064 ^b ±0.11	0.00000659 ^a ±0.02	0.0000002 ^c ±0.11
Artemetin	0.0000008 ^b ±0.01	0.00000427 ^a ±0.02	0.00000017 ^c ±0.12
Silymarin	0.0000011 ^a ±0.02	0.00000491 ^b ±0.01	0.00000019 ^c ±0.01
Hesperidin	0.00000056 ^b ±0.03	0.000010 ^a ±0.01	0.00000039 ^b ±0.02

Values are means ± SD (Standard) of triplicate determination. Mean values of different superscript in the same row are significant are p<0.05.

Table 4: Qualitative phenolic acid profile of okra, African spinach and lettuce sauces

Phenolic acid (mg/100g)	Lettuce sauce	Okra sauce	African spinach sauce
Salicylic acid	8.48 ^V 0.03	5.561 ^b ±0.01	2.88 ^c ±0.01
Gentistic acid	0.0000021 ^c ±0.01	0.000098 ^b ±0.01	7.94 ^a ±0.01
Protocatechuic acid	19.66 ^b ±0.15	38.26 ^a ±0.01	1.52 ^c ±0.01
Vannilic acid	0.0235 ^b ±0.01	0.0453 ^b ±0.01	4.87 ^a ±0.01
p-hydroxybenzoic acid	13.47 ^b ±0.03	0.2761 ^c ±0.03	33.47 ^V 0.03
Gallic acid	2.34 ^b ±0.01	0.058 ^c ±0.01	5.80 ^V 0.01
Ferrulic acid	16.32 ^b ±0.31	0.137 ^c ±0.01	21.98 ^V 0.01
Syringic acid	12.22 ^V 0.11	0.0156 ^c ±0.01	5.51 ^b t-0.01
Piperic acid	0.0813 ^b ±0.01	0.000053 ^c ±0.01	4.48 ^V 0.01
Sinapinic acid	32.18 ^a ±0.30	0.00254 ^c ±0.01	23.01 ^V 0.01
Ellagic acid	4.55 ^a ±0.12	0.00167 ^c ±0.01	3.88 ^b ±0.01
Chlorogenic acid	8.08 ^a ±0.01	0.2614 ^V 0.01	3.83 ^V 0.01

Values are means ± SD (Standard) of triplicate determination. Mean values of different superscript in the same row are significant are p<0.05.

DISCUSSION

Carotenoids content in the vegetable sauces was significantly (p<0.05) highest in okra sauce (6.24mg/100g) while African spinach sauce had the lowest carotenoids content (1.40mg/100g). Prominent carotenoid content in the vegetable sauces were of (3-carotene, lycopene, lutein, and zeaxanthin). The implication of the study was that the vegetable sauces

especially okra sauce may help in modulating the chronic complication of retinopathy in diabetic subjects. This study was consistent with Kowluru *et al.* (2013). Carotenoids have antioxidant potentials which inhibit oxidative stress. This is in tandem with She *et al.* (2017). Also, the vegetable sauces may help in preventing atherosclerosis. This is in line with Valero *et al.* (2011). Flavonoid content was very high and

significantly ($p < 0.05$) highest in okra sauce (71.82mg/100g). The implication of the study was that the vegetable sauces will exert some antioxidant and antidiabetic benefits. The mechanism of action is that it inhibits insulin resistance and hyperglycemia. Also, the free radical scavenging activities of flavonoid in the vegetable sauces may help to ameliorate oxidative stress and inflammation in diabetic condition. This agrees with Liu *et al.* (2014) and Ding *et al.* (2014).

Prominent flavonoids in the vegetable sauces are quercetin, catechin, naringenin, luteolin, kaemferol, and isorhamnetin. Quercetin, catechin, naringenin, luteolin, kaemferol, and isorhamnetin were the prominent flavonoids in the sauces. Flavonoid compounds act by partly enhancing hepatic glycolysis and glycogen concentration or by lowering hepatic gluconeogenesis thereby preventing the progression of hyperglycemia. Phenolic content was very high ranging from 90.10mg/100g (African spinach sauce) to 111.50mg/100g (lettuce sauce). Consumption of these vegetable sauces among the diabetic subjects will improve glucose and lipid metabolism which will reduce blood glucose. Salicylic acid, protocatechuic acid, ferrulic acid, chlorogenic acid, ellagic acid, sinapinic acid, p-hydroxybenzoic acid was the dominant phenolic content in the vegetable sauces, except ellagic acid, syringic acid, ferrulic acid and gallic acid that were in small quantity in okra spinach. Previous studies showed that chlorogenic acid inhibits hepatic glucose-6-phosphate translocase, which lowers fasting blood glucose in mice, stimulates glucose transport in skeletal muscle and improve glucose and lipid metabolism (Meng *et al.*, 2013). In conclusion, the vegetable sauces contain phytochemicals that are capable of lowering Type 2 diabetes mellitus.

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