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Original Research Article

Preliminary chemical Profile of *Telfairia occidentalis Hook. F* (Fluted pumpkin) Seed Shell

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Abstract

Despite being the largest morphological part of fluted pumpkin, the seed shell has not been put into use either by man or animal. In the present study fluted pumkin seed shell was subjected to proximate, phytochemicals, elemental and antinutritional analysis and results compared to standard values. Proximate composition showed crude fibre, (8.24±0.22); carbohydrates, (64.91±3.07); fat and oil, (2.271±0.31); crude proteins (5.23±1.63); moisture, (9.63±1.17) and ash content (11.63±0.56). Elemental analysis showed that apart from organic carbon (30.88±0.90) sodium, (23.2±0.72); iron, (7.94±1.56); copper, (8.1±2.40); zinc, (3.0±15) and chromium (0.71±0.01) were appreciable, suggesting that the mesocarp could be a source of mineral. Alkaloids, saponins, flavonoids, and tannins were present while toxicants in mg/100g showed oxalate, (17.81±1.60) phytate, (12.20±2.10) and hydrocyanide, (0.80±0.03). Results suggest possible utilisation of the fluted pumpkin seed shell as a food source, in medicine and as activated carbon precursor. However, antinutritional to nutrient ratios and other factors such as Na/K (38.8) could be the reason why the seed shell is not edible.

Keywords: Seed shell, Elemental, Antinutritional, Medicinal, Precursor.

INTRODUCTION

While other parts of fluted pumpkin shrub have been put into use by man, the fluted pumpkin seed shell (FPSS) which is the largest part of the fruit of fluted pumpkin has no reported use. According to Horsfall and Spiff, (2005) fluted pumpkin (*Telfairia, occidentalis Hook. F*) is a creeping vegetable shrub that spread low across the ground with large lobbed leaves and long twisting tendrils. Its habitat is the wet part of Nigeria and Africa in general. It belongs to the family cucurbitaceae (Ehiagbonare, 2008). Locally it is called 'ugu' by Ibos in Eastern part of Nigeria, Yoruba, 'egusi iroko' and Benin, 'uwmenkhen'. The young leaves are sliced and stored in a bottle to which coconut water and salt are added and used for the treatment of convulsion in ethno medicine (Ehiagbonare, 2008). The leaves contain essential oils, and vitamins, while root contains cucubitacine, sequisterpene, and lactones. The plant is mostly cultivated by women in the eastern part of Nigeria for its leafy shoots and edible seeds. The leaves are rich in iron and play a key role in the cure of anemia. The leaves are also noted for lactating properties and are in high demand for nursing mothers. The leafy vegetable is used mainly for soups, and salads to accompany main course (Hopkins, 2001; Agatemor, 2006). The roots are used as rodenticide and ordeal poison. Vegetables are very important food and highly beneficial for the maintenance of good health and prevention of diseases (Odiaka, 2008). The nutritional compositions of fluted pumpkin seeds has been reported (Fagbemi et al., 2005; Ganiyu, 2005; Fusuyi, 2008). Studies on fruit shells or mesocarps have been carried out with the view to assess shells' suitability as activated carbon precursor. Karthikeyen et al., (2008) reported synthesis and characterisation of activated carbon from seed shells of ferona, *jatropha curc*us and dolomix while Banerjee *et al.,* (1976) prepared activated carbon from coconut shells.

Fluted pumpkin is perhaps the largest consumed vegetable in Nigeria and thus provides one of the major agro wastes problems in market places and streets (Odiaka et al., 2008). Preliminary investigations showed that several tons of waste are produced each season but scarcely useful and therefore create an environmental nuisance. Despite the reported nutritional and medicinal potentials of fluted pumpkin seeds and leaves (Alabi et al*.,* 2005; Ejidike, 2007; Mensah, 2008) there is no analytical information on the chemical profile of the seed shell. The composition of the raw material influences the surface chemistry and offers a cost effective method for adjusting the properties of activated carbons (Gergova et al., 1994). The composition of seed shell will equally provide information on the supposed medicinal applications of the plant. However no mention is made in literature about the medicinal use of fluted pumpkin monocarps. This is not surprising given that most fruit shells have not been put into use especially here in Africa where the concept of sustainable developing and recycling are just being embraced. Also most cultures consider fruit shells as waste in the strictest sense and therefore avoid their use even in earthnomedicine (Verla *et al.,* 2012).

Therefore the objective of this study was to explore the composition of this waste. This will form baseline data for further study. The chemical composition is being discussed with regards to uses such as medicine, possibility of food and preparation of activated carbon.

MATERIALS AND METHODS

Preparation of fluted pumpkin seed shell

Mature fruits of fluted pumpkin were collected from a garden at Madonna University Elele campus, Nigeria, in October 2010. The seed shell was separated from the seeds with a knife and washed thoroughly with water, cut into smaller sizes and rinsed with distilled water; air dried for 16 days and later oven dried in an Astell Heavson model oven at 60 ^{o}C for 12 hours. The samples were blended in a hand milling kitchen blender and stored in a desiccators prior to analysis. Proximate composition of samples was carried to determine fat, moisture, crude protein, fibre, ash and dry matter (DM) content using methods of Oyoyede, 2005). Carbohydrate was estimated by method of difference (Mohammed et al., 2011). Hydrocyanic acid was estimated by the alkaline titration method; phytic acid and oxalate content were determined according to method of Mensah, (2008).

Elemental analysis

5g of dry milled samples were ashed in a furnace at 550^{o}C for 12hours. The ash was allowed to cool in desiccators and then weighed. 2mls of concentrated hydrochloric (HCl) acid were added to dissolve the ash, alongside few drops of nitric acid (A.O.A.C, 1990). The solution was evaporated to almost dryness in a boiling water bath. The content was transferred to 100ml volumetric flash and diluted to mark with deionised water. A Pye Unicam atomic adsorption spectrophotometer with acetylene flame was used to analyse calcium, magnesium, zinc; iron and copper as described in A.O.A.C (1990). Sodium and potassium were determined with a flame photometer, (Gallenkamp). Standard metallic ions solutions of all metals as Emark concentrated solutions were used for calibration.

Quantitative phytochemical analysis

Alkaloid content was determined by method of Awah and Verla, (2010) with slight modifications. 5 grams of sample was weighed into a 250ml beaker and 200ml of 20% acetic acid in ethanol was added and covered to stand for 4 hours. This was filtered and filtrate concentrated to one quarter its original volume using a hot water-bath. Concentrated ammonium hydroxide was added drop wise until the precipitate was complete. The entire solution was allowed to settle and then filtered the precipitate was dried and weighed and expressed as alkaloid content percent of weight of sample (Obomanu et al., 2005)**.** Saponin content was determined according to method of Mensah, 2008. Tanin was determined by Follin Dennis Colorimeter method as used by Awah and Verla, (2010) while flavonoids and phenols content were determined by the A.O.A.C, 1990 method.

RESULTS AND DISCUSSION

Analytical Quality Assurance (AQA)

All reagents used in this work were analytical grade chemicals, purchased from Fin lab limited Owerri, Imo State, Nigeria. Standard solutions of the various metals were prepared from Emark stock solutions for the calibration curves for all the metals. The stability of calibration was checked periodically by analyzing a standard solution. Blank samples made from only reagents without sample were analyzed to get rid of any background concentration metals in the system. The equations of the calibration curves of the metals are shown on table 1.

Table 1. Calibration curves for metals

Metal	Equation of Curve	Regression value (R^2)
Cr	Y = 0.0372X	0.9932
Fe	Y = 0.0283X	0.9989
Co	Y = 0.0294X	0.9998
Ni	Y = 0.0486X	0.9997
Cu	Y = 0.0239X	0.9960
Zn	Y = 0.0374X	0.9955

Table 2. Proximate composition of FPSS

Parameter	Fat	Moisture	Protein	Carbohydrate	Crude fibre	Ash
Conc. (mg/100g)	2.27±0.31	9.63±1.17	5.23±1.63	64.91±3.07	8.24±0.22	11.63±0.56

Table 3. Toxicant composition of FPSS

Toxicant	Oxalate	Phytic acid	Hydrocyanide	Nitrate
Con.(mg/100g DM)	17.81±1.60	12.20±2.10	0.80±0.03	0.56 ±0.02
Lethal dose	2.5g/kg	50mg/kg	30mg/kg	200mg/kg

Table 4. Toxicant to nutrient molar ratio and critical values

Toxicant to nutrient ratio	Calculated values	Critical values
[Oxalate]/[Ca]	7.8	2.5
[Oxalate]/[Ca +Mg]	5.8	2.5
[Ca][Phytate]/[Zn]	0.60	0.5
[Phytate]/[Zn]	3.5	1.5
[Phytate]/[Ca]	5.4	0.2
[Phytate]/[Fe]	1.6	0.4

Table 5. Elemental composition and organic matter of FPSS

Element	Mean ± SEM (mg/100g)
Sodium (Na)	23.2 ± 0.72
Potassium (K)	0.60 ± 0.05
Calcium (Ca)	2.27 ± 0.31
Magnesium (Mg)	0.80 ± 0.03
Iron (Fe)	7.64 ± 1.56
Copper (Cu)	8.10 ± 2.40
Zinc (Zn)	3.15 ± 1.03
Chromium (Cr)	0.70 ± 0.06
TTA (H^+)	0.29 ± 0.08
SO^-_4 (S)	0.23 ± 0.03
Org. Carbon (OC)	30.88 ± 0.90
Org. Matter (OM)	52.20 ± 2.48
Phosphorus (P)	0.75 ± 0.02
Nitrogen (N_2)	3.60±0.27
Cobalt (Co)	1.2± 0.02
Nickel (Ni)	0.08± 0.02

Table 6. Some phytochemicals of fluted pumpkin seed shell.

Phytochemical	Alkaloids	Flavonoids	Phenols	Saponins	Tanins
Mean ± SEM (mg/100g)	0.52 ± 0.03	0.03 ± 0.006	4.52 ± 0.31	6.41 ± 1.05	0.10 ± 0.02

All the curves gave regressions more than 0.9, an indication of the reliability of the curves.

Values obtained have been recorded in tables 2, 3, 4 and 5 as mean values of triplicate values for concentrations together with standard deviations.

Table 2 shows the proximate compositions (mg/100g) of FPSS. The fat content (2.27±0.31mg/100g DM) was the lowest value amongst the proximate parameters. As noted by Okoye and Verla, 2006, fruits are not a very good source of fat and are usually recommended as part of weight reducing diet since low fat foods reduces cholesterol and obesity. The moisture content (9.63±1.1mg/100g DM) was high when compared with other components. The moisture level of food is usually a measure of stability and susceptibility to microbial contamination (Amaerteifio and Mosase, 2006). High moisture content suggest high microbial activity, hence spoilage especially in the warm tropical region and consequent problems of storage (Olaofe *et al*., 2008). The crude protein content (5.23±1.63 mg/100g DM) in the seed shell was quite high compared to its fat content (Table 2). This does not conform to expected results because fruit mesocarps are usually not rich in nitrogenous components (Umar et al., 2008). This value is even higher than values for seeds and pulp of *gardenia, aqualla* fruits reported (Mohammad et al., 2011). When compared to some common consumed plant protein fruits in Nigeria; 24% for *Amaranthus vividis,* 20.72% for *Moringa, oleifera* and15% for *Lasianthera Africana,* (Bello et al., 2008) the seed shell of fluted pumpkin does not qualify as a protein source. However considering the increasing demand for protein driven by the population explosion and skyrocketing prices of conventional protein foods, fluted pumpkin seed shell could be considered a potential protein source. FPSS may be considered as carbohydrate source even though its available carbohydrate content (64.91±3.07mg/100g DM) does not compare well with conventional carbohydrate sources like cereals with72-90g/100 g samples. The energy value (489.72 kcal) calculated using method of Onyeike and Omubo-Dede, 2002 was strangely higher than that for cereals 388-389 kcal per 100g sample. Fluted pumpkin seed shell could be a good source of energy. The FPSS may also serve as a raw material for production of activated carbon given it carbon content (30.88±0.90% organic carbon). The crude fibre level was 8.24±2.22 mg/100g DM). Thus fluted pumpkin seed shell may be an average source of dietary fibre, which is important for reducing cholesterol levels in the body hence minimize risks of cardiovascular diseases caused by high plasma cholesterol. Food rich in crude fibre is restricted from children as this could lead to colon problems but women are encouraged as increase fibre intake has been linked to reduce risk of all cancers and diabetes (Mensah *et al.,* 2008). The ash content of the fluted pumpkin mesocarp was high in the present study and partly reflected the mineral composition (Oyoyede, 2005). The ash content (11.63±0.56mg/100g DM) was almost ten times the range reported for commonly consumed fruits. This could speed up metabolic processes and improve growth (Oluyemi et al., 2006). According to Gergova *et al.,* (1994), qualities of good activated carbon raw material include: high carbon content, low inorganic reflected in low ash content, sufficient volatile matter and a cheap and abundant source. The plant has also been reported to contain considerable amount of antinutrients (Mensah et al., 2006).

Table 3 shows values of toxicants determined in FPSS. Amongst the antinutrients, the hydrocyanide (HCN) content was 0.80 ± 0.03mg/100g. This is very low compared to 36 mg/100g DM considered to be lethal to man. This shows that the level of the acid in the samples is within the acceptable range for human consumption. Only plants with more than 200 mg of hydrocyanic acid equivalent per 100 mg fresh weight are considered dangerous. The oxalate level was also low and unlikely to pose toxicity problems since it is far below the toxic levels, (2.5g/kg) (Adewoye *et al.,* 1996). Oxalate is a concern because of its negative effect on mineral availability, causes irritation in the mouth and interfere with absorption of divalent minerals particularly calcium by forming insoluble salts. High oxalate diet can increase the risk of renal calcium absorption and has been implicated as a source of kidney stones. Excess consumption of oxalic acid can cause corrosive gastroenteritis (Ari *et al.,* 2012). Phytate in food can bind some essential mineral in the digestive tract and can result in mineral deficiencies. Phytate composition of the seed shell was 12.20±2.10 mg/100g. This is lower and might not pose any health hazard when compared to a phytate diet of 10-60mg/g. If consumed over a long period of time, phytate has been reported to decrease bioavailability of minerals in monogastric animals. The low phytate level of FPSS may not cause adverse effects on digestibility considering the lethal dose of 50mg/kg(IMFNB, 1999). The concentration of nitrate in the seed shell was 0.56 ±0.02mg/100g. This value is within the acceptable daily intake of 3.7 mg/kg body weight. Higher concentration of nitrate in the food can lead to a disease called methemoglobinaemia which is known to reduce the ability of red blood cells to carry oxygen. Nitrate concentration was significant considering the lethal dose of 300mg/kg. Nitrates are generally found in vegetables and have been linked to DNA mutation due to possible conversion to nitrosamines at various conditions. Even though the levels of toxicants were not considered relatively high their presence is possibly the main reason why both man and animal do not consume fluted pumpkin seed shell.

The results of the mineral contents (Table 4) shows that *T. occidentalis* seed shell may be a rich source of some mineral elements notably Na, Mg, Fe, Ca, Cu Zn and N_2 but not others like, K, Cr and P. Sulphate (sulphur) 0.23±0.03 was lowest while organic carbon 30.88±0.90 was highest. The sodium and potassium contents of the seed shell were 23.2 ± 0.72mg/100g and 0.60mg/100g respectively and are considered to be very low. These two metals are responsible for maintaining osmotic balance of the body fluids, the pH of the body to regulate muscles and nervous irritability, control glucose adsorption and enhance normal retention of protein during growth. The sodium to potassium (Na/K) ratio of less than 1 is recommended for preventing high blood pressure. However the (Na/K) ratio of fluted pumpkin seed shell was 38.8, suggesting that this plant seed shell is absolutely not edible. The calcium content of the seed shell 2.27 ± 0.31mg/100g was very low compared to major calcium sources such *Abelmoschus esculantus* with values of 170.6mg/100g (Aremu *et al*., 2012). Calcium is required in muscle contraction, in children, pregnant women and lactating mothers for bone and teeth growth. Phosphorous and calcium function together, contributing to blood formation processes and other supportive structures of the body. Some artificially and genetically produced foods rich in animal proteins and phosphorous have the tendency to promote the lost of calcium in urine. Therefore if the calcium to phosphorous ratio (Ca/P) is low the concentration of calcium will be low, since high phosphorous intake leads to loss of calcium in urine. Any food with Ca/P >1 is considered good, Ca/P <0.5 is poor, while Ca/P >2 will help to increase calcium adsorption in the small intestine. The Ca/P was 3.2 implying that calcium adsorption could be very high in consumers of fluted pumpkin seed shell. The phosphorous content of the seed shell was 0.75 ± 0.02mg/100g, a value considered very small compared to higher values reported for banana, 40mg/100g (Bello et al., 2008) and even insignificant when considering recommended dietary allowance (RDA) of 800mg phosphorous per day. The magnesium content of fluted pumpkin seed shell was 0.80 ± 0.03mg/100g. Compared to RDA of 420mg/day, the value of magnesium in the seed shell is nothing. Magnesium ions regulate over 300 biochemical reactions such as DNA and RNA synthesis, cell growth and cell reproduction in the body through their role as enzyme co-factors. Magnesium helps the body process fat and protein, and is important for the so that deficiency can affect virtually every organ system of the body. Magnesium helps the body process fat and protein, and is important for the proper functioning of the heart and the hormonal balance of the body. The ideal ratio for most people's needs is equal amounts of calcium and magnesium. If calcium consumption is high, then magnesium intake also needs to be high. Since calcium and magnesium depend on each other to be assimilated into your body, they should never be taken alone. The calcium to magnesium ratio of the seed shell was 2.8. This implies that consumers of the seed shell may experience magnesium depletion related disorders (Saris et al., 2000).

Anti nutrients to nutrients ratios were calculated (Table 5) so as to predict the bioavailability of some divalent elements (i.e. Ca, Mg, Fe and Zn). It was found that [Oxalate]/[Ca] ratio (7.8) and [Oxalate]/[Ca + Mg] ratio (5.8) were both far above the critical ratios of 2.5 at which there is impaired calcium bioavailability. Therefore the consumption of these samples can hinder calcium bioavailability.

To predict the effect of phytate on the bioavailability of Ca, Fe, and Zn, phytate to nutrients ratios were calculated (Ari et al., 2012). The [phytate]/[Ca] ratio in the seed was above the critical level of 0.2, likewise the ratio of [phytate]/[Zn] (3.5), was above the critical level of 1.5. Therefore the consumption of this seed shell can hinder zinc bioavailability. Though the calculated [Ca]/[phytate]/[Zn] ratio was (0.6) many times above critical level of 0.05, it is considered no better measure of zinc bioavailability than [phytate]/[Zn] ratio. This shows that the bioavailabity of Zn may not be hindered by the phytate content of fluted pumpkin seed shell. The [phytate]/[Fe] and [phytate]/[Ca] ratios in the seed shell are above the critical level of 0.4 and 0.2 respectively. This indicates that the consumption of the fruit pulp can hinder iron and calcium bioavailability. Amongst the mineral elements determine here are metals generally regarded as essential for human health in trace amounts. These include iron, zinc, copper, and nickel. The primary role of such elements is as a catalyst, and only trace amounts are necessary for cellular function. These metals are widely found in nature, particularly in various mineral deposits and soils, meaning that they are available to be taken up by plants and animals that serve as food sources for humans. Criteria for essentiality for human health are that withdrawal or absence of the metal from the diet produces either functional or structural abnormalities, and that the abnormalities are related to, or are a consequence of, specific biochemical changes that can be reversed by the presence of the essential metal. When in excess these essential metals Fe, Zn, Cu and Ni can be harmful to animals [Saris et al., 2000]. On the other hand, when available in desired levels, they contribute immensely to good health. The recommended dietary allowances (RDA) for iron, zinc, copper and nickel are 18, 15, 2 and 3 mg d^{-1} $person^{-1}$, respectively (USDA, 2001). Using the expression the toxicity index (TI) was calculated according to (1).

$$TI = \frac{\frac{[Fe]}{PL} + \frac{[Zn]}{PL} + \frac{[Cu]}{PL} + \frac{Ni]}{PL}}{4} \quad ....(1)$$

The permissible levels (PL) for used in this study were those of USDA, 2001, and 4 is the number of supposed toxic metals. TI of less than 1 show no multi-element contamination while above 1, it shows multi- element contamination. Results from equation...(1) gave a TI of 1.25. This implies that the consumption of the seed shell can cause multi-element contamination arising from the four essential metals.

The concentration of chromium was found to be 0.70 ± 0.06mg/100g. This is high compared to chromium which is widely distributed in most foods but provide only small amounts (less than 2 micrograms [µg] per serving). The high content of chromium in the seed shell may not be unconnected to adsorption from eroded chromium containing rocks in the soil on which the plant was cultivated or some anthropogenic activity releasing chromium in the environment. Although mechanisms of action in the body and the amounts needed for optimal health are not well defined, humans require chromium in trace amounts. Its trivalent (chromium 3+) form, is biologically active and found in food, whereas the hexavalent (chromium 6+), a toxic form results from industrial pollution. Chromium is known to enhance the action of insulin (Dattilo and Miguel, 2003), a hormone critical to the metabolism and storage of carbohydrate, fat, and protein in the body. Studies on a compound in brewers' yeast fingered Chromium as the active ingredient in this so-called "glucose tolerance factor" in 1959 (Porte et al*.,* 2003). Sulphur is the third most abundant mineral in your body, after calcium and phosphorous. It's an important mineral element that you get almost wholly through dietary proteins. Sulphur is the mineral that helps fight fatigue, stress, pain, cancer, and wrinkles. A shortage of sulphur likely contributes to these age-related problems. It is required for proper synthesis and biological activity of proteins and enzymes, and plays an important role in your body's electron transport system; vitamin conversion; synthesizing metabolic intermediates such as glutathione; detoxification; joint health; and proper insulin function (Fasuyi, 2006).

Phytochemical analysis of FPSS showed the presence of alkaloids, flavonoids, phenols, saponins, with tannins (0.10±0.02) as lowest and saponins (6.41±1.05) as highest. Analysis reveals that FPSS is rich in bases such as phenols and saponins but poor in tannins, flavonoids and alkaloids. However these secondary metabolites are known to have antimicrobial activity (Elemo *et al.,* 2002), suggesting the possible uses of the FPSS for medicinal purposes (Alimor, 2008).

Phenols are a varying group of compounds which include salicylic acid (Willow) and rosmarinic acid. Phenols are usually strongly antibacterial, antioxidant, anti-inflammatory, anti-septic and anti-viral, and are found in plants such as basil, cinnamon, thyme, wintergreen, rosemary and the mints. It is believed that phenols are present in plants in order for the plant to ward off infection or attack by insects. In skin care products, phenol-containing herbs are often used as contributors to natural preservative systems. The phenol content of FPSS was 4.52 ± 0.31 mg/100g.

Tannins content was 0.10 ± 0.02mg/100g. Tannins are polyphenolic compounds that are astringent, making them useful for tightening up loose tissue, such as that which is found in varicose veins, and for drying up secretions. Flavonoids are polyphenolic compounds found in a wide variety of plants. They impart a yellow or white pigment to flowers and fruit and have a wide range of activity. Flavonoids are particularly useful for maintaining healthy circulation and some are antioxidant, while others are anti-inflammatory, anti-viral or capillary strengthening (Ejidike and Ajileye, 2007). Flavonoids content of the seedshell was 0.03 ± 0.006 mg/100g. This value is insignificant compared to values in *Citrus limon* (Muhammad *et al.,* 2011).

The saponin content in the present studies was 6.41 ± 1.05mg/100g. Many key medicinal plants contain Saponins as their main ingredient. As the name suggests, saponins lather, like soap, when mixed with water. One useful type of saponin for skin care is a triterpenoid saponin, which can be found in herbs such as Licorice root (Glycyrrhiza glabra). This particular saponin can aid the absorption of nutrients. High level saponin has been associated with gastroenteritis manifested by diarrhea and dysentery, but it was reported that saponin reduces body cholesterol by preventing its re-absorption and suppresses rumen protozoan by reacting with cholesterol in the protozoan cell membrane there by dissolving it (Ganiyu, 2005).

CONCLUSION

Results of this study have revealed the nutritional potentials of FPSS majorly as a source of dietary fibre and some minerals. However, proper processing is advised so as to reduce the concentration of toxicants. Presences of secondary metabolites lend credence to local practices in which the plants parts are constituents of concoctions and decoctions for treatment of diseases. The high carbon content and low ash of FPSS are advantages to its possible use as activated carbon precursor. These potentials could lead to a drastic reduc- tion of the nuisance associated with this waste in places where the plant is grown and consumed.

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