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Comparative Assessment of Lipids and Physicochemical Properties of African Locust Beans and Shea Nut Oils

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Abstract
The lipid composition and the physico-chemical properties of two Nigerian grown seed oils of *Parkia biglobosa* and *Vitellaria paradoxa* were investigated. The seeds of *Parkia biglobosa* were boiled for 12hours, dehusked, sun-dried and grinded into fine powdery form for oil extraction while the fruits of Vitellaria paradoxa were depulped such that its seeds on drying were deshelled and grinded into fine powdery paste for oil extraction.The seeds consist of 34% and 25% (dry w/w) crude oil respectively. At room temperature, the seed oil of *Parkia biglobosa* is liquid while the seed oil of *Vitellaria paradoxa* is semi-solid. The physicochemical properties of these oils indicated that mean free fatty acid, saponification, peroxide, acid and iodine values were 4.77%, 175.32mgKOH/g, 3.7mgO_2/g, 9.48mgKOH/g, 82.40 for *Parkia biglobosa* and 5.93%, 63.77mgKOH/g, 3.0mgO_2/g, 11.7mgKOH/g, 56.35 for *Vitellaria paradoxa* respectively. The Specific Gravity (SG) of both oils were less than a unit, so they are less dense than water and will float on it. The moisture content is in the range 0.83 – 1.21%, which is generally low hence indicating that the seed oils could be stored for a long period. Both oils were found to have iodine values, which conveniently places them as non-drying oils. The acid values also showed that the oils may be sourced for edible purposes. The saponification value of *Parkia biglobosa* seed oil indicated that it is good for soap production. The High performance Liuid Chromatography (HPLC) results showed that the oils contain 63% and 54% of triacylglycerol for *Parkia biglobosa* and *Vitellaria paradoxa* respectively. Major fatty acid - oleic acid, lauric acid, myristic acid, palmitic acid, stearic acid were 12.5%, 9.0%, 6.7%, 5.0%, 5.3% and 13.1%, 9.5%, 7.0%, 5.3% and 5.2% for *Parkia biglobosa* and *Vitellaria paradoxa* respectively.The study indicated a marked significant difference in the lipid composition and physicochemical properties of *Parkia biglobosa* as compared with *Vitellaria paradoxa.*
Keywords: HPLC, Parkia biglobosa, Specific gravity, Triacylglycerol, Vitellaria bigloboxa,.

1.Introduction

Globally, natural vegetable oils and fats are increasingly becoming important in nutrition and commerce because they are sources of dietary energy, antioxidants, biofuels and raw materials for the manufacture of industrial products. They are used in food, cosmetic, pharmaceutical and chemical industries. Vegetable oils account for 80% of the world's natural oils and fat supply (*Okullo et al. 2010*).Vegetable oils are sourced from diverse varieties of leguminous plants, which are considered the major sources of dietary proteins. They are consumed worldwide, especially in developing and underdeveloped countries where consumption of animal protein may be limited as a result of economic, social, cultural or religious factors (Oluwole and Oluremi, 2012). Among the leguminous plants used by man particularly in some African countries, are the African locust bean tree (*Parkia biglobosa*) and Shea nut tree (*Vitellaria paradoxa*).

Parkia biglobosa, named after the famous Scottish botanist and surgeon, Mungo Park by Robert Brown has long been widely recognized as an important indigenous multipurpose fruit tree in many countries of the sub-saharan Africa. It is called the African locust bean tree. The tree is the source of a natural nutritious condiment which features frequently in the traditional diet of both rural and urban dwellers in at least seventeen West African countries including Nigeria(Hopkins and White, 1984). The estimated average consumption of the condiment per head per day for Nigeria, Togo and Ghana are 10g, 4 g and 2 g respectively(Campbell-Platt, 1980). Locust bean seed is the matured fruit seed that comes from the parkia tree. It is harvested and processed into a fermented product known as '*Iru*', *'Ogiri'* and '*Dadawa*' in Yoruba, Igbo and Hausa languages respectively in Nigeria (Odunfa, 1985). Alabi *et. al.*(2005) reported that locust bean is rich in lipid, protein, carbohydrate, soluble sugars and ascorbic acid. The cotyledon is very nutritious, has less fibre and ash contents. The oil content is suitable for consumption since it contains very low acid and iodine contents. The oil has very high saponification value and hence would be useful in the soap industry. It has essential acids and vitamins and serves as a protein supplement in the diet of poor families (Diawara *et al.*2000). *Dawadawa* is used in soups, sauces and stews to enhance or impart meatiness (Omafuvbe *et al.* 2004). In Nigeria, the production of fermented locust beans has remained a traditional family art practiced in homes, especially in the rural areas with rudimentary utensils (Audu *et al.*

2004). The methods used vary from one locality to another depending on the culture of the people, their beliefs, taste and the practice of the fore parents who were involved in the same vocation. These variations in the processing techniques in turn bring about variations in the quality of *Iru.* Many urban dwellers, according to field survey though are often cautious in consuming the fermented locust beans, being skeptical about the processing hygiene, they prefer the traditionally processed type to the industrially processed type called 'Dadawa cube' manufactured by Cadbury Nigeria PLC because of their belief that the traditionally processed type contains less addition of chemical preservatives than the other type. They also believe that the natural flavour and aroma in the traditional *Iru* are lost in Dadawa cube (Rhoda, 2004).

Shea nut (SN), however, is known as Kandayi, Osisi and Emi among the Hausa, Igbo and Yoruba people of Nigeria, respectively. It is obtained from the fruit of shea tree (*Vitellaria paradoxa*), which exists in the wild and grows in an uncultivated state in most parts of Africa. Shea fruit (SF) is made up of a green epicarp, a fleshy mesocarp (pulp) and a relatively hard shell (endocarp) which encloses the shea kernel (embryo). The kernel, according to Axtell et al (1993) contains about 60% edible fat (shea butter) and the residual product, from which the butter is extracted (shea cake), is an excellent ingredient for livestock feed production. Shea butter (SB) is good as table oil because of its high nutritive value. It is widely used locally for curing leprosy and other ailments. It also has various industrial uses that include soap making, cosmetics, lubricants and paints. According to Russo and Etherington (2001), shea products, such as solid fat (butter or stearin) and the liquid oil (olein) are ideal for use as raw materials for cooking oil, margarine, cosmetics, soap, detergents and candles.However, they have found their primary market as a substitute for cocoa butter in the chocolate and confectionary industry.According to the American Shea Butter Institute (ASBI 2004), 100% pure natural SB is an all-natural vitamin A cream. SB has been shown to be a superb moisturizer, with exceptional skin healing properties. ASBI (2004) compiled a list of skin conditions where 100% SB has been proved to be effective. These skin conditions are dry skin, skin rash, skin peeling after tanning, blemishes and wrinkles, itching skin, sunburn, shaving cream for a smooth silky shave, small skin wounds, skin cracks and tough or rough skin (on feet). Others are cold weather, frost bites, stretch mark prevention during pregnancy, insect bites, health skin, muscle fatigue, aches and tension,skin allergies such as poison ivy or poison oaks, eczema, dermatitis and skin damage from heat. It is because of these unique healing properties that the shea tree is called karite tree which means tree of life (ASBI 2004).

In view of the outstanding nutritional, industrial and traditional uses of *Parkia biglobosa* and *Vitellaria paradoxa,* the objective of this study is to investigate comparatively the lipids and physicochemical properties of the seed oils of these leguminous plants.

2. Materials and Method

2.1 Sample Preparation:

The seeds of African locust bean (*parkia biglobosa*) were purchased in the local market of Jettu, Auchi, Edo State and Shea nut (*vitellaria paradoxa*) purchased from village women in Bida, Niger State.The seeds of *parkia biglobosa* were boiled for 12 hours to facilitate the removal of the testa. After dehusking, the white cotyledon was then sun dried for two days. The dried sample was grounded into a fine and homogenous powdery form. The fine sample was then kept in a container for subsequent oil extraction to be performed. The fruits from shea tree are depulped to yield the pulp and seeds. The seeds was then dried before cracking and then deshelled. The shell was discarded and the remaining seed was grinded using a small pestle mortar to reduce the large seeds into smaller particles and consequently grounded using a manual blender into a fine powder.

2.2 Lipid Extraction and Determination:

Extraction of lipid in a suitable apparatus, with n-hexane as extracting solvent and weighing of the extract obtained was carried out. Apparatus used includes weighing balance, absorbent filter paper, the soxhlet extractor, rotary evaporator, oven and desiccator. The samples were then put into the extraction chamber of a Soxhlet extractor, after extraction, the hexane-lipid mixture is now transferred into a quick-fit round bottom flask and fixed to a rotary evaporator which is now used to separate the hexane and the lipids. The final oil (lipid) was then stored in the refrigerator until further analysis were to be carried out.

2.3 Physicochemical Analysis:

The extracted oil was immediately analysed for acid, peroxide, saponification and iodine value following the method described by the Association of Official Analytical Chemists (AOAC, 1984).Estimation of the percentage free fatty acids as oleic acid was done following the method described by the American Oil Chemists' Society (AOCS, 1997).All Reagents used for these analyses were of analytical grade and most of the equipments were products of Gallenkamp and Pyrex, England.The specific gravity measurement (also carried out at room temperature) using specific gravity bottle. The state and colour of the oil were noted using visual inspection and with the aid of a lovibond tintometer for the later at room temperature. The moisture content of the oil was also analysed.

2.3.1 Determination of Acidity

5g of sample was weighed into the flask and 50ml of the FFA solvent added. The flask was then placed on the sand bath and regulate the temperature to about 40°C. About 0.5ml of phenolphthalein was added. The sample mixture was then shaked gently while titrating with standard alkali by drop wise addition of 0.1N potassium hydroxide till faint, but permanent pink colour is obtained.

FFA% as palmitic acid (for palm oil and fractions) = $\frac{25.6 \times N \times V}{W}$

FFA% as lauric acid (for coconut oil, palm kernel oil and fractions) = $\frac{20.0 \times N \times V}{W}$

FFA% as oleic acid (for corn oil, soyabean oils and other liquid oils) = $\frac{28.2 \times N \times V}{W}$

Where : N = normality of KOH solution
V = volume of KOH solution used in ml
W = weight of sample

FFA is frequently expressed in terms of Acid Value instead of percentage free fatty acid.Acid Value is defined as the number of milligram of KOH or NaOH to neutralize 1g of oil sample.To convert FFA to Acid Value multiply FFA % by 1.99 for Oleic acid, 2.81 for Lauric acid and 2.19 for Palmitic acid.

2.3.2 Saponification Value:

2g of test portion was weighed into a conical flask and 25ml of the ethanolic potassium hydroxide solution and some boiling aids were added. The reflux condenser was connected and the mixture boiled gently for at least 60minutes, swirling the contents of the flask from time to time. The flask and condenser was allowed to cool slightly before washing the inside of the condenser with a little distilled water. 1ml of phenolphthalein solution was added and then titrated with the 0.5N hydrochloric acid until the pink colour of the indicator disappears.A blank determination was conducted simultaneously using the same procedure.

The saponification value is given by

$$SV = \frac{56.1\ N(V_b - Vs)}{W}$$

Where: V_b is the volume in milliliters of the hydrochloric acid solution used for the blank.
Vs is the volume, in milliliters of the hydrochloric acid for the determination of the sample.
N is the normality of the hydrochloric acid.
W is the weight, in grams of the test portion.

2.3.3 Peroxide Value

5.0g of test sample was weighed into the 250ml flask.30ml of the acetic acid –chloroform solution was added and the flask swirled until the sample is dissolved in the solution. 0.5ml of saturated potassium iodide was added with a graduated pipette. The solution was swirled for 1minute and then 30ml of distilled water added.It was then titrated with 0.01N sodium thiosulphate solution, by adding it gradually and with constant and vigorous shaking until the yellow iodine colour has almost disappeared. Then 0.5ml of starch indicator was added Addition of the thiosulphate solution dropwise continued until a blue colour just disappears.The blank test was carried out in parallel with the determination.

The peroxide value expressed in milliequivalents of active oxygen per kilogram of sample is:

$$PI = \frac{(Vs - V_b)\ N \times 1000}{W}$$

Where: Vs is the volume in milliliters of the sodium thiosulphate solution of normality N, used for the determination;
V_b is the volume, in milliliters of the sodium thiosulphate solution used for the blank test;
W is the weight, in grams of the test portion;
N is the normality of the sodium thiosulphate solution

2.3.4 Determination of Iodine Value (Wijs Methods)

0.4g of the sample was weighed in the glass weighing scoop or vial. The scoop was placed in a 500ml flask. 15ml of the carbon tetrachloride was added to dissolve the fat. 25ml of the Wijs solution was then added, the stopper was inserted, and the bottle then shaked gently and thereafter put in the dark for 1hour.After standing, add 20ml of the potassium iodide solution and 150ml of water was added after standing. The solution was then titrated with the sodium thiosulphate solution until the yellow colour due to iodine has almost disappeared. 1 – 2ml of the starch indicator solution was added and the titration continued until the blue colour just disappears after very vigorous shaking.Two determinations on the same test sample were carried out and Blank test too simultaneously under the same conditions.

Iodine value is given by

$$IV = \frac{12.69N\ (V_2 - V_1)}{W}$$

Where: N is the normality of the sodium thiosulphate solution used;
V_2 is the volume in milliliters of the sodium thiosulphate solution used for the blank test;
V_1 is the volume in millilitre of the sodium thiosulphate solution used for the determination;
W is the weight, in grams of the test portion.

2.3.5 Specific Gravity

A clean, dry Density/Specific gravity bottle was weighed and then filled with distilled water and the stopper inserted in such a way that the capillary portion is completely filled with water. The excess liquid at the top of the stopper was then wiped off. and then weighed.The test samples are poured into previously weighed clean and dry Specific gravity bottle. The stoppers are placed over the bottles so that any excess oil flow out of the capillary portion and are wiped off using tissue paper.The weight of the bottle and sample is then taken on an analytical balance.

Specific gravity is expressed as:

$$SG = \rho / \rho H_20$$

Where: SG = Specific Gravity , ρ = Density of fluid/oil (Kg/m^3)
ρH_20 = Density of water (Kg/m^3) also expressed as:

$$\frac{(W_1-W_0)}{(W_2-W_0)} = \frac{\text{Mass of the substance}}{\text{Mass of an equal volume of water.}}$$

2.3.6 Moisture Content

10g of the oil sample was weighed in a Petri dish that has been previously dried in an oven and weighed after cooling. The oil sample was spread evenly over the whole base of the dish and dried in the oven, which has previously been set to operate at 103 $\pm$ 2oC, for 4 hours.After 4 hours the dish was removed and immediately closed with lid and place in a desiccator to cool. After which it was now weighed.The moisture and volatile matter content as a percentage by mass of the samples as received is equal to:

$$\frac{w_1 - w_2}{w_1 - w_0} \times 100$$

where: w_0 is the weight in grams of the dish
w_1 is the weight in grams of the dish and test portion before drying
w_2 is the weight in grams of the dish and the test portion after drying

2.3.7 Colour and State

The sample was melted and homogenized at 50°C – 60°C before taking a test portion and pour into the cell.
Using the lovibond tintometer, the colour of the sample was immediately determined by achieving the best possible match with the standard colour slide.The results where expressed in terms of;
(a) the number of Red, Yellow, Neutral or Blue units needed to obtain the match and
(b) the cell used and the Lovibond Model Number.

2.5 Preparation of Methyl Esters of Fatty Acids:

10mg of oil sample was weighed into a volumetric conical test tube and 2ml of Methanolic Boron Triflouride was added and the test tube lid shut tight. The tube were then placed in a boiling bath for 20 minutes and allowed to cool. 3ml of Saturated Salt (Sodium Chloride) Solution was then added and the tube vortex and allowed to settle.1ml of Iso-Octane was then added and again vortexed and allowed to settle. The upper phase which was the Iso-Octane layer was then pipetted out ready to be injected into the HPLC vial for analysis.
20µL of the sample were injected into HPLC with a total running time of 10 minutes. The chromatograph then output the peaks that correlate to the concentration of the compound injected.This is also repeated for a series of the standard compound solution onto the HPLC for detection. The chromatograph of these known concentrations will give a series of peaks.The components were detected at 215 nm. Mono-, di- and tri- acylglycerols were identified by comparison of the retention times of the lipid components with those of standards analyzed under the same conditions.

3. Results and Discussion

Table 1: The proximate physicochemical compositions of the two seed oils.

Physicochemical parameters	African Locust Bean Seed oil (*Parkia biglobosa*)	Shea nut oil (*Vitellaria paradoxa*)
State at room temperature	Liquid	Semi Solid
Colour	30 Yellow, 10 Red - Brownish Yellow	30 Yellow, 7 Red - Dark Yellow
% Lipid Composition	33.97	25.02
Moisture content %	1.107	0.881
Specific gravity	0.890	0.897
% FFA	4.77	5.93
Acid Value (mgKOH/g)	9.48	11.79
Peroxide Value (mgO_2/g)	3.7	3.0
IodineValue Wij's	82.40	56.35
Saponification Value (mgKOH/g)	175.32	63.77

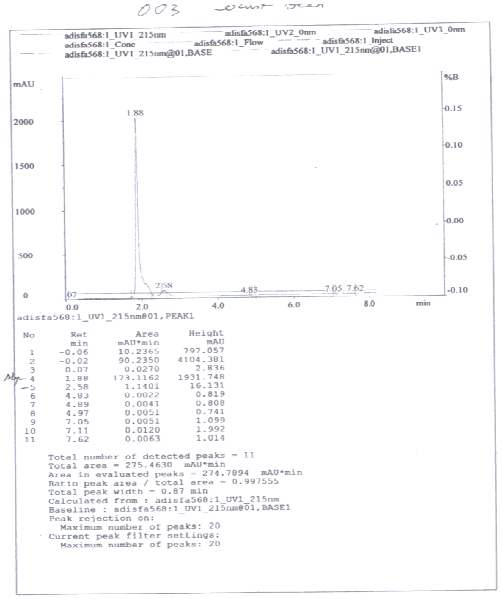

Fig 1:HPLC profile of *Parkia biglobosa* seed oil

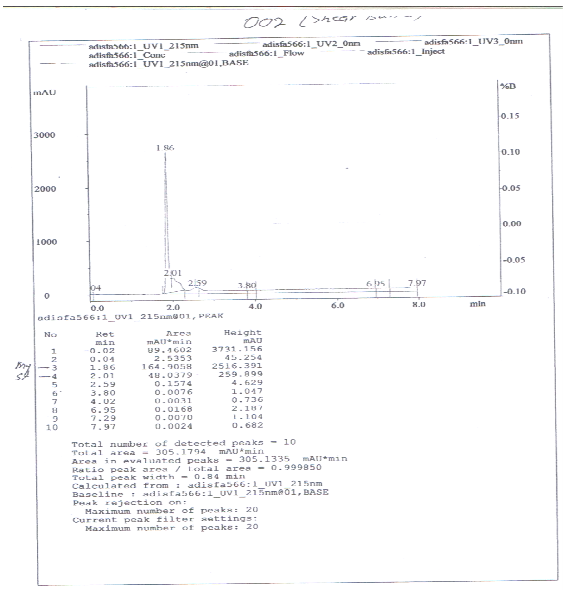

Fig 2: HPLC profile of V. paradoxa seed oi

Table 2: HPLC Analysis of the seed oils of both plants

Lipids/Fatty Acid Composition	African Locust Bean Seed oil in (%)	Shea nut oil in (%)
Triacylglycerol	63 ± 0.50	54 ± 0.21
Oleic	12.5	13.1
Lauric	9.0	9.5
Myristic	6.7	7.0
Palmitic	5.0	5.3
Stearic	5.3	5.2

The characteristics of oils from different sources depend mainly on their compositions, and no oil from a single source can be suitable for all purposes [Mohammed and Jorg, 2003]. At room temperature 27°C the seed oil of African locust bean (*Parkia biglobosa*) examined is liquid while that of shea nut (*Vitelleria paradoxa*) is semi-solid. The colours of the oils are brownish yellow and dark yellow for *Parkia biglobosa* and *Vitelleria paradoxa* respectively. The dark brown or brownish yellow can be improved upon by bleaching. The Specific Gravity (SG) of both oil are lesser than one *Parkia biglobosa* SG = 0.890 and *Vitelleria paradoxa* SG = 0.897), so they are less dense than water and will float in it.The moisture content is in the range 0.83 – 1.21%. *Parkia biglobosa* has the higher mean moisture content of 1.107% while *Vitelleria paradoxa* has the lower mean moisture content of

0.881%. The moisture content of the sample seed oils in this study are generally low indicating that the seed oils could be stored for a long period.The oil yields are in the range 24.04 – 34.96%. *Parkia biglobosa* has the higher mean oil yield of 33.97% while *Vitelleria paradoxa* has the lower mean oil yield of 25.02%. The oil yield in the above range could compare favourably with the oil yield reported for some commercial vegetable oils such as cotton seed (19.50%), soybeans (19.0%), palm oil (48.65%) and groundnut (49.0%). Therefore, the seed oils studied could be exploited economically. Acid value is an indicator for edibility of oil and suitability for industrial use. *Vitelleria paradoxa* has the higher acid value of 11.79 while *Parkia biglobosa* has the lower acid value of 9.48. This in essence suggests that the oils are suitable for edible purposes and also in accordance with the report of Akintayo et al.,(2004) and Akanni et al.,(2005) .

The iodine value which is useful in predicting the drying property of oils was found to be in the range 56.20 – 82.80. Oil with iodine value less than 100 indicates that the oil is not a drying oil. Both oils could be classified as a non-drying oils, since their iodine values are lower than 100 (Kirsehenbauer, 1965). The mean iodine value of *Parkia biglobosa* seed oil has the higher mean iodine value of 82.40 suggesting that they could be used as semi-drying oils while *Vitelleria paradoxa* seed oil has the lower mean iodine value of 56.35 suggesting that they are non drying oils. The high iodine value is evidence that the oil could be used in the manufacture of cosmetics, oil paints and vanishes and also serve edible purposes. The low iodine values recorded for the non-drying and semi-drying oils could be of significance in the manufacture of leather, dressing, candle lubricants and hydraulic brake fluids,as reported by Adelaja (2006). The iodine value is also an index for assessing the ability of oil to go rancid. The low iodine values in this study indicated that the oil contain low level of polysaturated fatty acid. Storage procedure used should ensure protection of oil from oxidative deterioration both oils.

The seed oils studied have saponification values between the ranges 56.00 – 176.72 with *Parkia biglobosa* seed oil having the higher mean saponification value (175.32) and *Vitelleria paradoxa* seed oil having the lower mean saponification value (63.77). The high saponification values recorded for the seed oils suggested that the oils contain high molecular weight fatty acids and low level of impurities. This is evidence that the oil could be used in soap making industry (Alander and Anderson, 2002; Akanni et al, 2005).Perioxide value of the seed oils studied is in the range 2.80 - 4.00. Seed oils having values below 10 characterize the majority of the conventional oils. Peroxide value is used as indicator of deterioration of oils. Fresh oils have values less than 10 mEq kg-1 values between 20 and 40 mEq kg-1 results to rancid taste (Adelaja, 2006).

From the HPLC profile (Fig 1&2) ,10 peaks were detected from *Vitelleria paradoxa's* lipid profile. Triacylglycerol being a major component of lipids was identified at peak with retention time of 1.86, and was found to be 54% present. 11 peaks were detected from the *Parkia biglobosa's* lipid profile and Triacylglycerol was identified at peak 1.88 and of 63% present in the seed oil.

Other peaks present are probably of free fatty acids, methyl esters, monoacylglycerols and diacylglycerols. Based on the Standards (oleic acid, lauric acid, myristic acid, palmitic acid, stearic acid) used for the extrapolation, the major Triaclyglyceride identified are oleic acid (13.1%), lauric acid (9.5%), myristic acid (7.0%), palmitic acid (5.3%), and stearic acid (5.2%) for *Vitelleria paradoxa* and oleic acid (12.5%), lauric acid (9.0%), myristic acid (6.7%), palmitic acid (5.0%), and stearic acid (5.3%) for *Parkia biglobosa*.The two oils contain similar fatty acids in almost the same ratio. Oleic acid is believed to be the most abundant fatty acid in both oil samples, with *Vitelleria paradoxa* having more of the saturated palmitic acid.

4. Conclusion

The study of these constituents is important for their effective uses. Seed oils are known to deteriorate when processed inadequately with the principal decomposition reaction being oxidation. However, the physico-chemical properties of the seed oils studied have close similarity with groundnut oil and other commercial seed oils such as palm oil, soybean and cotton seed. The results obtained from this study could be used as baseline data to develop these seed oils for both domestic and industrial purposes.

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