

## The Metal Composition, Proximate Properties and the Effect of Refining on the Physico-Chemical Characterization of *Baphia nitida* and *Gliricidia sepium* Seed and Seed Oil

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**Abstract:** The effect of refining methods on the physico-chemical properties of *Baphia nitida* (BN) and *Gliricidia sepium* (GS) were examined. The refining methods used include alkali refining, bleaching and degumming treatment. The seeds were also subjected to standard chemical analysis to evaluate their properties. Proximate analysis indicated low moisture content in the 2 seeds. The ash content of BN ( $2.17 \pm 0.10\%$ ) was low while that of GS ( $4.06 \pm 0.80\%$ ) was slightly higher than the range recommended for compounding of animal feed. The crude protein content ( $18.72\text{--}20.30\%$ ) and carbohydrate content ( $41.58\text{--}45.41\%$ ) were fairly high. The oil content was appreciably high and the physico-chemical properties showed the free fatty acid value to be  $1.50 \pm 0.20\%$  in BN which reduced to  $0.52 \pm 0.10\%$  after bleaching and in GS it was  $0.80 \pm 0.10\%$  which also reduced to  $0.40 \pm 0.50\%$  after bleaching. The peroxide values of all the alkali refined oils were higher than that of the crude which shows some level of degradation. Triglycerol was the dominant lipid species in the oils. The refining methods used increased the diacylglycerol, monoacylglycerol and triacylglycerol content of the oils while the polar and sterol content reduced. The concentration of the macronutrients were high with K ( $627.21 \pm 0.80$  ppm) being the highest in BN. The concentration of the trace metals also differs with Mn ( $32.30 \pm 0.39$  ppm) being the highest in GS. The seeds are good sources of oil and minerals and there is the possibility of considering them as feed supplements and their oils in industrial applications. Out of all the refining methods used, bleaching appeared to be the best technique for refining in terms of stability and improvement of physico-chemical properties of the oils.

**Key words:** *Baphia nitida*, *Gliricidia sepium*, bleaching, degumming, alkali refining, seed oil

### INTRODUCTION

Seed oils are important sources of edible oils of nutritional, industrial and pharmaceutical importance. 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 (Schneider, 2001). In the search for new sources of novel oils, a large number of plants have been surveyed and some of the most promising species have been cultivated as new oil crops.

Seed oils represent one of the largest key materials that can be obtained from biomass and cheaply processed for food supply to the increasing population. There are many seeds, which are underutilized due to lack of information on their compositions and utilization. Some of these underutilized include *Baphia nitida* and *Gliricidia sepium*.

*Baphia nitida* is a tree which is about 10 m high with trunk to about 45 cm diameter and slender branches forming an umbrella-shaped crown; usually an under

storey tree of wetter parts of the coastal area. The tree is often planted in the villages as an ornamental or shade and as a source of medicines and dye. *Gliricidia sepium* is a small bushy-topped tree, shorthole, to 6-8 m high. The tree is pinkish when in flower. It is quick growing and easily propagated by seed or cuttings and is commonly grown as an ornamental (Burkill, 1995).

Crude edible oils are known to contain extraneous substances which may impart objectionable properties to the oil (Weiss, 1983). These objectionable properties can be improved by refining in term of shelf life and nutritive status of the resultant oils (Haraldson, 1983).

A number of minerals are required by human body in order to maintain good health. Some of these essential minerals are accumulated in different parts of plants as it accumulates minerals essential for growth from the environment. It has also been reported that trace metals can be detected in plants and food stuffs (Wen-Hua *et al.*, 2005).

This study aims at the determination of the physico-chemical properties, lipid classes, proximate content, nutritionally valuable minerals and the effect of refining (bleaching, degumming and alkaline refining) on the physico-chemical properties and lipid classes of these underutilized tropical seed oils: *Baphia nitida* (BN) and *Gliricidia sepium* (GS).

## MATERIALS AND METHODS

**Sample preparation:** The seed samples were obtained from the environment of the University of Ibadan, Oyo state, Nigeria. They were identified at the herbarium unit, Botany Department University of Ibadan. They were subsequently ground in a laboratory mill and stored in a cellophane bag at 4°C prior to analysis.

**Physico-chemical analysis:** Oil was extracted from *Baphia nitida* (BN) and *Gliricidia sepium* (GS) using soxhlet extractor with petroleum ether (40-60°C) for 1 h (Ajayi *et al.*, 2004). The extracted oil were immediately analyzed for iodine value, peroxide value, saponification value, acid value and unsaponifiable matter described by Mohammed and Jorf-Thomas (2003). Estimation of the percentage free fatty acids as oleic acid was done following the method described by Oderinde *et al.* (1990). The refractive indices of the oils (at room temperature) were determined with Abbe refractometer (Oderinde and Ajayi, 2000) and the specific gravity measurements were also carried out at room temperature using gravity bottles. Visual inspection was used to note the state and colour of the oils at room temperature. The mean molecular mass was estimated from the relation  $(56/SV) \times 1000$  (Akintayo and Bayer, 2002). Where, SV is the saponification value.

**Proximate analysis:** Proximate analysis was carried out as described by the Association of Official Analytical Chemist (Oderinde *et al.*, 2008).

**Mineral determination:** Metals determined were lead, cadmium, copper, zinc, iron, magnesium, calcium, sodium, potassium and manganese. This was achieved by digesting the samples using 5 mL (2:1) of 69.40% w w<sup>-1</sup> nitric acid and 90.00% w w<sup>-1</sup> perchloric acid (Oderinde and Ajayi, 1998). These metals were analyzed by atomic absorption spectrophotometry (Perkin-Elmer).

**Lipid classes:** Lipid classes were separated on 0.75 mm plates (20×20) coated on silica gel (Merck). Plates were

developed vertically in a 80/20/1 volume mixture of petroleum ether: diethylether: acetic acid. They were developed according to method described by Oboh and Oderine (1998).

**Isolation of unsaponifiables:** Ten gram of the oil was dissolved in 200 mL of 2M ethanolic potassium hydroxide and refluxed for 1 h. The reaction mixture was later diluted to 400 mL with distilled water and transferred into a 1 L separating funnel. The unsaponifiable were then extracted 3 times with 100 mL diethylether. The ether extract was first washed with 100 mL aqueous solution of 0.5 M potassium hydroxide in order to remove any residual fatty acids. This was further washed and cleaned with 5×100 mL distilled water and dried over anhydrous sodium sulphate. The solution was filtered and dried.

**Separation of unsaponifiables:** A chloroform solution (50%) of the unsaponifiable matter (30 mg plate<sup>-1</sup>) was then applied uniformly along the line from the edge of the 20×20 cm plate coated with 0.55 mm layer of silica gel and developed 3 times with hexane/ethylacetate (6:1 v v<sup>-1</sup>) as mobile phase. The developed plates were dried and irradiated at 254 nm with ultraviolet radiation. Three zones corresponding to N-alkanes, triterpene alcohols and sterols were marked, carefully scrapped and extracted with petroleum ether (Kayode *et al.*, 2001).

**Alkali refining:** Thirty gram of the oil was heated to 50°C for 10 min, 2 mL 18 M potassium hydroxide was added with continuous agitation for 30 min. The resultant mixture was there after heated to 80°C in order to break the soap stock formed. The neutral oil was separated from the soap stock by centrifugation at 3000 rev min<sup>-1</sup>. The separated oil was washed with boiling water for 10 min. The oil was finally separated from the soap solution by centrifugation.

**Degumming:** To 45 g of the oil sample in a conical flask was added phosphoric acid (85%) 2% w w<sup>-1</sup>. The flask was corked tightly and the content was agitated for 30 min. The oil was separated from the contents by centrifugation at 3000 rev min<sup>-1</sup> for 5 min.

**Bleaching:** 1:1 mixture of activated carbon and clay (bentonite) was used for the bleaching. Thirty five gram of the oil was warmed up to 50°C and the bleaching mixture was gradually added. The flask was corked and the temperature increased to 120°C and maintained for 45 min. The oil was filtered while hot through a sintered glass funnel using a suction pump.

**Statistical analysis:** Data were analysed by one way Analysis of Variance (ANOVA). Means were compared by the Duncan (1955) multiple range tests. Significance was accepted at 5% level ( $p \leq 0.05$ ).

## RESULTS AND DISCUSSION

**Proximate composition:** The summary of the proximate composition of *Baphia nitida* (BN) and *Gliricidia sepium* (GS) is presented in Table 1. The oil content of the seeds are quite high which are  $27.14 \pm 0.20\%$  for BN and  $24.70 \pm 2.00\%$  for GS, these compares favorably with oil-rich seeds such as *Monodora tenuifolia* (Adedire *et al.*, 2003). This high oil content shows that the exploitation of these oils for industrial or edible purposes will be economical. The moisture content of the seeds is low indicating a good shelf life characteristic. The moisture content of GS ( $4.01 \pm 0.20\%$ ) is lower than that of BN ( $6.80 \pm 0.30\%$ ) showing a longer shelf life of GS than BN. Generally, the moisture content of these seeds is lower than those of similar legumes (Vidivel and Janardhanan, 2001) which might be advantageous in keeping the qualities of the seeds.

The ash content of the seeds ranged from  $2.17 \pm 0.10$ – $4.06 \pm 0.80\%$ . BN showed much lower value than GS. Ash content is significant in food for various reasons. Among others, it is an index for the quality of feeding materials used for poultry and cattle feeding, already established by Pomeranz and Clifton (1981). The carbohydrate content of the seeds is fairly high. BN had a lower carbohydrate content ( $41.58 \pm 0.70\%$ ) than GS ( $45.41 \pm 0.80\%$ ). The crude fibre content of BN is lower than of GS and this result compared favorably with those reported for commonly cultivated pulses, such as chick pea and horse gram (Premakumari *et al.*, 1984). The presence of fairly high crude fibre in food material has been reported to decrease the dry matter digestibility in animals. These values suggest a good indication of nutritive value of feed material (Devendra, 1995). The values of the ash, crude fibre and carbohydrate contents of these seeds indicate their suitability in the compounding of animal feeds (Abighor *et al.*, 1997).

The crude protein is high,  $20.30 \pm 0.70\%$  for BN and  $18.72 \pm 1.30\%$  for GS. This value corresponds with those for similar seeds (Bressani, 2002). Therefore, based on the recommended average human protein intake of 23–50 g by the National Research Council (1974), these seeds could contribute to alleviating the problem of protein malnutrition in the 3rd world and developing countries. The values are higher than the range found for cereal seeds and protein animals (Heger and Eggum, 2000). They could be recommended as protein supplements, though,

Table 1: Proximate composition (%) of *Baphia nitida* and *Gliricidia sepium*

| Assay         | BN               | GS               |
|---------------|------------------|------------------|
| Crude fat     | $27.14 \pm 0.20$ | $24.70 \pm 0.50$ |
| Crude protein | $20.30 \pm 0.70$ | $18.70 \pm 1.30$ |
| Crude fibre   | $2.01 \pm 0.70$  | $3.01 \pm 0.10$  |
| Ash           | $2.17 \pm 0.10$  | $4.06 \pm 0.80$  |
| Moisture      | $6.80 \pm 0.30$  | $4.10 \pm 0.20$  |
| Carbohydrate  | $41.58 \pm 0.70$ | $45.41 \pm 0.80$ |

Table 2: Physico-chemical characteristics of oils from BN and GS

| Parameters                                          | BN                | GS                |
|-----------------------------------------------------|-------------------|-------------------|
| Colour                                              | Light green       | Orange            |
| Acid value ( $\text{MgKOH g}^{-1}$ )                | $2.24 \pm 0.01$   | $1.40 \pm 0.40$   |
| Free fatty acid (%)                                 | $1.50 \pm 0.20$   | $0.80 \pm 0.10$   |
| Saponification value ( $\text{MgKOH g}^{-1}$ )      | $194.50 \pm 0.20$ | $94.40 \pm 0.70$  |
| Iodine value ( $\text{Mg iodine g}^{-1}$ )          | $82.11 \pm 2.31$  | $87.60 \pm 3.01$  |
| Unsaponifiable matter (%)                           | $2.48 \pm 0.06$   | $1.00 \pm 0.20$   |
| Peroxide value ( $\text{MgO}_2 \text{ g}^{-1}$ Oil) | $1.10 \pm 0.40$   | $0.40 \pm 0.30$   |
| Mean molecular mass                                 | 287.92            | 593.22            |
| Refractive index ( $25^\circ\text{C}$ )             | 1.4810            | 1.4000            |
| Specific gravity ( $25^\circ\text{C}$ )             | $0.9215 \pm 0.02$ | $0.8760 \pm 0.05$ |
| State at room temperature                           | Liquid            | Liquid            |

Values are mean  $\pm$  standard deviation of triplicate determinations

the suitability of any plant material as food supplement depends on factors like the presence of antinutritional factors and digestibility of its nutrients.

**Physico-chemical properties:** The physico-chemical characteristics of the oils are shown in Table 2. BN is light green while GS is orange in colour. The acid values are low with that of GS ( $1.40 \pm 0.40 \text{ mgKOH g}^{-1}$ ) being lower than that of BN ( $2.24 \pm 0.10 \text{ mgKOH g}^{-1}$ ). The free fatty acids which stimulates oxidative deterioration of oils by enzymatic and chemical oxidation to form off-flavour components is also low. It falls within the desired limits ( $0.0$ – $3.0\%$ ) for cooking oil (Onyeika and Acheru, 2002). The saponification value suggests their use in the production of liquid soap and shampoo. The slightly high iodine values of  $82.11 \pm 2.31 \text{ mg iodine g}^{-1}$  (BN) and  $87.60 \pm 3.01 \text{ mg iodine g}^{-1}$  (GS) indicate the preponderance of unsaturated fatty acids. The unsaponifiable matters were found to be  $2.48 \pm 0.06\%$  for BN and  $1.00 \pm 0.20\%$  for GS. The peroxide values were obtained to be  $1.10 \pm 0.40 \text{ mgO}_2 \text{ g}^{-1}$  oil for BN and  $0.440 \pm 0.30 \text{ mgO}_2 \text{ g}^{-1}$  oil for GS these values are lower than values stipulated for rancid oil which ranges from  $20.00$ – $40.00 \text{ mgO}_2 \text{ goil}^{-1}$  (Pearson, 1976).

**Lipid classes and composition of the unsaponifiables matter of BN and GS:** The lipid classes of the studied oils are presented in Table 3 while the composition of the unsaponifiable matter is shown in Table 4. Triglyceride was the dominant lipid species in the oils (BN has  $84.70\%$  and GS has  $75.60\%$ ). The samples also contain varying concentration of hydrocarbons, free fatty acids, diacylglycerols, sterols, monoacylglycerols and polar lipids. Our studies show that the unsaponifiable matter

Table 3: Lipid classes of the oil from *Baphia nitida* and *Gliricidia sepium*

| Parameters        | BN (%)     | GS (%)     |
|-------------------|------------|------------|
| Polar lipids      | 5.90±0.60  | 7.50±0.20  |
| Sterols           | 2.00±0.40  | 2.70±0.10  |
| Diacylglycerols   | 2.20±0.50  | 5.30±0.80  |
| Monoacylglycerols | 1.30±0.03  | 4.60±0.20  |
| Triacylglycerols  | 84.70±1.00 | 75.60±1.20 |
| Hydrocarbons      | 2.70±0.20  | 1.90±0.10  |
| Free fatty acids  | 1.20±0.10  | 2.40±0.30  |

Table 4: Composition (%) of the unsaponifiables of the oil of BN and GS

| Composition         | BN         | GS         |
|---------------------|------------|------------|
| n-Alkanes           | 16.20±1.00 | 21.20±0.70 |
| Triterpene alcohols | 22.40±0.80 | 31.00±0.60 |
| Sterols             | 32.80±1.20 | 29.40±0.40 |
| Unidentified        | 28.60±0.60 | 18.40±1.00 |

Values are mean±standard deviation of triplicate determinations

Table 5: Nutritionally valuable and trace metals (ppm) of BN and GS

| Metal | Seed        |             | Oil         |             |
|-------|-------------|-------------|-------------|-------------|
|       | BN          | GS          | BN          | GS          |
| Na    | 389.83±0.30 | 342.57±0.04 | 298.78±0.30 | 281.10±0.08 |
| K     | 627.21±0.80 | 506.55±0.68 | 421.52±0.05 | 432.60±0.20 |
| Ca    | 251.63±0.05 | 293.10±0.02 | 230.10±0.20 | 271.10±0.10 |
| Mg    | 167.26±0.01 | 83.65±0.41  | 129.20±0.02 | 64.70±0.50  |
| Fe    | 140.20±1.26 | 128.11±0.01 | 98.54±1.00  | 102.00±0.04 |
| Cu    | 2.67±0.01   | 1.01±0.01   | 1.21±0.01   | 0.60±0.30   |
| Zn    | 30.21±0.01  | 21.78±0.01  | 22.30±0.02  | 16.80±0.01  |
| Mn    | 23.11±0.01  | 32.30±0.39  | 18.01±0.01  | 22.98±0.01  |
| Pb    | nd          | 0.85±0.01   | nd          | nd          |
| Cd    | 0.03±0.01   | nd          | nd          | nd          |

Average concentration±standard deviation of triplicate determinations (ppm) (mg kg<sup>-1</sup>), nd: not detected

consists mainly of n-alkane, triterpenoids and sterols. The fairly high unsaponifiable matter is an advantage for the use as natural insecticide. This is because unsaponifiable matter contains sterols and triterpene alcohols which are responsible for the insecticidal properties of fixed oils (Adebawale and Adedire, 2006).

#### Nutritional and trace metal composition of the seed and seed oil of BN and GS:

The results of the nutritionally valuable minerals and trace metals are presented in Table 5. Potassium was the most abundant mineral in the seeds and oils. BN has higher value (627.21±0.80 ppm) in the seed while GS has the higher value (432.60±0.20 ppm) in the oil. The concentration of copper and zinc has been reported to range from 4-15 ppm for copper and 15-200 ppm for zinc (Allaway, 1988). The values obtained from our studies falls within this range with BN having 2.67±0.01 and GS 1.01±0.01 ppm of copper in the seed and 1.21±0.01 ppm for BN and 0.60±0.30 ppm for GS in the oil. Lead was not detected in the seed of BN but was found to be 0.85±0.01 ppm in GS. Lead was not detected in the oils of the 2 seeds. Cadmium was only detected in the seed of BN as 0.03±0.01 ppm. The abundance of potassium,

sodium and calcium in the result of this analysis is in agreement with previous findings that these 3 metals represent the most abundant metal constituents of many plants.

#### Effect of refining on the physico-chemical properties and lipid profile of BN and GS:

This sharp increase might be due to the potassium hydroxide reacting with the double bonds of the unsaturated fatty acids in the triacylglycerol chain leading to the production of peroxides and hyperoxides which may eventually lead to oxidative rancidity and deterioration of the oils. This is also reflected in a gradual decrease in the iodine values of the alkali refined and degummed oils. The results are presented in Table 6 for BN and Table 7 for GS. There is significant difference in the saponification value of the alkali refined and the degummed. The specific gravity and the refractive index of the oil slightly reduced for the alkali refined.

On the other hand, the unsaponifiable matter was found to increase for the alkali refined. This might be due to the formation of some compounds which are unsaponifiable as a result of the deterioration of the oil. Table 8 shows the composition of the unsaponifiable after treatment. The hydrocarbons (N-alkane) content increased for the alkali refined oils where as there was a decrease in the case of the degummed and bleached. This might be due to the removal of impurities in the oil in the bleached oil as a result of the surface adsorption by the clay. The triterpene alcohol and sterol also reduced in all the treated oils except in GS where the value of sterol increased from 29.40-32.00%. Work is progressing on the identification of the fractions that were not identified.

Table 9 and 10 shows the lipid classes of the crude oils and the treated. The triacylglycerol of the oils increased in all the methods used. The removal of impurities and other substances from the oils might account for this (Haraldson, 1983). Degumming has also been reported to remove phospholipids, trace metals, pigments and carbohydrates in the oil while bleaching selectively removes pigments, oxidative products, trace metals and sulphur from the oil. Alkali refining on the other hand removes fatty acids, phospholipids, pigments, trace metals, sulphur, insoluble and water solubles in the oil (Jung *et al.*, 1989). The removal of these materials has led to the increase in the triacylglycerol content of the oil. The monoacylglycerol and the diacylglycerol content of these oils also increase in all the methods applied. The polar lipids of the oils decreased after the treatment with the bleached method having the highest reduction. It

Table 6: Effect of refining on the physico-chemical properties of BN

| Parameters                                            | Crude                    | Alkali refined           | Degummed                 | Bleached                 |
|-------------------------------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Free fatty acid (%)                                   | 1.50±0.20 <sup>a</sup>   | 0.70±0.10 <sup>b</sup>   | 1.02±0.40 <sup>a</sup>   | 0.52±0.10 <sup>c</sup>   |
| Acid value (MgKOH g <sup>-1</sup> )                   | 2.24±0.01 <sup>a</sup>   | 1.02±0.00 <sup>b</sup>   | 1.83±0.01 <sup>c</sup>   | 0.76±0.00 <sup>d</sup>   |
| Saponification value (MgKOH g <sup>-1</sup> )         | 194.50±0.20 <sup>a</sup> | 179.00±1.21 <sup>b</sup> | 153.00±2.11 <sup>c</sup> | 187.00±0.81 <sup>d</sup> |
| Iodine value (Mg iodine g <sup>-1</sup> )             | 82.11±2.31 <sup>a</sup>  | 78.67±4.11 <sup>b</sup>  | 80.77±0.60 <sup>a</sup>  | 86.01±1.25 <sup>a</sup>  |
| Peroxide value (MgO <sub>2</sub> g <sup>-1</sup> oil) | 1.10±0.40 <sup>a</sup>   | 6.21±0.10 <sup>b</sup>   | 0.85±0.20 <sup>a</sup>   | 0.61±0.20 <sup>c</sup>   |
| Specific gravity                                      | 0.9215±0.02 <sup>a</sup> | 0.8789±0.01 <sup>c</sup> | 0.9023±0.03 <sup>b</sup> | 0.8986±0.01 <sup>b</sup> |
| Refractive index                                      | 1.4810 <sup>a</sup>      | 1.4060 <sup>b</sup>      | 1.4870 <sup>a</sup>      | 1.4980 <sup>c</sup>      |
| Unsataponifiable matter (%)                           | 2.48±0.06 <sup>a</sup>   | 2.86±0.10 <sup>b</sup>   | 2.16±0.30 <sup>c</sup>   | 1.87±0.10 <sup>d</sup>   |

Table 7: Effect of refining on the physico-chemical properties of GS

| Parameters                                            | Crude                    | Alkali refined           | Degummed                 | Bleached                 |
|-------------------------------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Free fatty acid (%)                                   | 0.80±0.10 <sup>a</sup>   | 0.40±0.60 <sup>b</sup>   | 0.95±0.20 <sup>a</sup>   | 0.40±0.50 <sup>b</sup>   |
| Acid value (MgKOH g <sup>-1</sup> )                   | 1.40±0.40 <sup>a</sup>   | 1.10±0.20 <sup>a</sup>   | 0.80±0.40 <sup>c</sup>   | 0.60±0.10 <sup>b</sup>   |
| Saponification value (MgKOH g <sup>-1</sup> )         | 94.40±0.70 <sup>a</sup>  | 80.10±1.00 <sup>b</sup>  | 85.00±1.60 <sup>b</sup>  | 91.20±0.40 <sup>a</sup>  |
| Iodine value (Mg iodine g <sup>-1</sup> )             | 87.60±3.01 <sup>a</sup>  | 81.25±0.80 <sup>b</sup>  | 88.21±3.70 <sup>a</sup>  | 86.20±2.50 <sup>a</sup>  |
| Peroxide value (MgO <sub>2</sub> g <sup>-1</sup> oil) | 0.40±0.30 <sup>a</sup>   | 3.11±0.10 <sup>b</sup>   | 0.60±0.20 <sup>a</sup>   | 0.20±0.40 <sup>c</sup>   |
| Specific gravity                                      | 0.8760±0.05 <sup>a</sup> | 0.8523±0.03 <sup>b</sup> | 0.8720±0.05 <sup>a</sup> | 0.8660±0.01 <sup>b</sup> |
| Refractive index                                      | 1.4000 <sup>a</sup>      | 1.3890 <sup>b</sup>      | 1.4100 <sup>a</sup>      | 1.4610 <sup>c</sup>      |
| Unsataponifiable matter (%)                           | 1.00±0.20 <sup>a</sup>   | 1.60±1.20 <sup>b</sup>   | 1.10±0.50 <sup>c</sup>   | 0.80±0.70 <sup>d</sup>   |

Values are mean±standard deviation of triplicate determinations

Table 8: Effect of refining on the unsaponifiable composition (%) of BN and GS

| Method         | Triterpene alcohol      | Sterol                  | N-Alkane                | Unidentified            |
|----------------|-------------------------|-------------------------|-------------------------|-------------------------|
| <b>BN</b>      |                         |                         |                         |                         |
| Crude          | 22.40±0.60 <sup>a</sup> | 32.80±1.00 <sup>a</sup> | 16.20±0.20 <sup>a</sup> | 28.60±1.10 <sup>a</sup> |
| Alkali refined | 25.00±0.30 <sup>b</sup> | 31.20±0.50 <sup>a</sup> | 30.60±0.40 <sup>b</sup> | 13.20±0.50 <sup>b</sup> |
| Degummed       | 18.20±0.10 <sup>c</sup> | 28.00±0.10 <sup>b</sup> | 15.40±0.10 <sup>c</sup> | 38.40±0.70 <sup>c</sup> |
| Bleached       | 13.00±0.10 <sup>d</sup> | 25.40±0.30 <sup>c</sup> | 13.00±0.10 <sup>d</sup> | 48.60±0.40 <sup>d</sup> |
| <b>GS</b>      |                         |                         |                         |                         |
| Crude          | 31.00±0.10 <sup>a</sup> | 29.40±0.30 <sup>a</sup> | 21.20±0.10 <sup>a</sup> | 18.40±1.00 <sup>a</sup> |
| Alkali refined | 30.30±0.30 <sup>a</sup> | 32.00±0.10 <sup>b</sup> | 26.10±0.60 <sup>b</sup> | 11.60±0.40 <sup>b</sup> |
| Degummed       | 26.10±0.10 <sup>b</sup> | 25.10±0.10 <sup>c</sup> | 19.40±0.20 <sup>c</sup> | 29.40±0.80 <sup>c</sup> |
| Bleached       | 22.00±0.50 <sup>c</sup> | 27.70±0.40 <sup>d</sup> | 18.80±0.30 <sup>d</sup> | 31.50±1.00 <sup>d</sup> |

Table 9: Effect of refining on the lipid classes (%) of BN

| Parameters       | Crude                   | Alkali refined          | Degummed                | Bleached                |
|------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Polar lipid      | 5.90±0.50 <sup>a</sup>  | 5.10±1.00 <sup>b</sup>  | 4.70±0.70 <sup>c</sup>  | 3.50±0.20 <sup>d</sup>  |
| Sterol           | 2.00±0.30 <sup>a</sup>  | 1.50±0.80 <sup>b</sup>  | 1.80±0.60 <sup>a</sup>  | 1.20±0.10 <sup>c</sup>  |
| Diacylglycerol   | 2.20±0.20 <sup>a</sup>  | 2.60±0.10 <sup>c</sup>  | 2.90±0.50 <sup>c</sup>  | 3.00±0.80 <sup>c</sup>  |
| Monoacylglycerol | 1.30±0.50 <sup>a</sup>  | 1.80±1.50 <sup>b</sup>  | 2.00±0.50 <sup>b</sup>  | 2.30±1.00 <sup>b</sup>  |
| Triacylglycerol  | 84.70±0.80 <sup>a</sup> | 85.60±1.20 <sup>b</sup> | 85.50±1.00 <sup>b</sup> | 87.30±0.50 <sup>c</sup> |
| Hydrocarbon      | 2.70±0.30 <sup>a</sup>  | 3.40±0.10 <sup>b</sup>  | 2.10±0.40 <sup>c</sup>  | 1.90±0.40 <sup>c</sup>  |
| Free fatty acid  | 1.20±0.02 <sup>a</sup>  | nd                      | 1.00±0.50 <sup>b</sup>  | 0.80±0.03 <sup>c</sup>  |

Table 10: Effect of refining on the lipid classes (%) of GS

| Parameters       | Crude                   | Alkali refined          | Degummed                | Bleached                |
|------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Polar lipid      | 7.50±0.30 <sup>a</sup>  | 5.40±0.80 <sup>b</sup>  | 3.80±0.40 <sup>c</sup>  | 2.60±0.20 <sup>d</sup>  |
| Sterol           | 2.70±1.00 <sup>a</sup>  | 2.00±1.20 <sup>b</sup>  | 2.20±0.60 <sup>c</sup>  | 1.80±0.80 <sup>d</sup>  |
| Diacylglycerol   | 5.30±0.40 <sup>a</sup>  | 5.90±0.20 <sup>b</sup>  | 6.20±1.00 <sup>c</sup>  | 7.80±0.40 <sup>d</sup>  |
| Monoacylglycerol | 4.60±0.10 <sup>a</sup>  | 5.30±0.30 <sup>b</sup>  | 5.20±0.20 <sup>c</sup>  | 5.80±0.50 <sup>d</sup>  |
| Triacylglycerol  | 75.60±0.50 <sup>a</sup> | 78.80±0.50 <sup>b</sup> | 80.37±0.20 <sup>c</sup> | 80.40±0.80 <sup>d</sup> |
| Hydrocarbon      | 1.90±0.20 <sup>a</sup>  | 2.40±0.60 <sup>b</sup>  | 1.03±0.40 <sup>c</sup>  | 0.80±0.20 <sup>d</sup>  |
| Free fatty acid  | 2.40±0.10 <sup>a</sup>  | nd                      | 1.20±0.06 <sup>b</sup>  | 0.80±0.12 <sup>c</sup>  |

Values are mean±standard deviation of triplicate determinations

reduced from 5.90-3.50% in BN and 7.50-2.60% in GS. This reduction in the polar lipids and triacylglycerol content of these oils is of great importance. It is an indication of the possibility of the application of these oils in the

food industry and their safety in consumption. Hyperlipidemiads or high levels of serum triacylglycerol and cholesterol are a risk factor for premature atherosclerosis which is the underlying cause of heart attack, stroke and peripheral vascular diseases. These decreases can be viewed as a form of chronic inflammation that is induced and perturbed by lipid accumulation (Ruili *et al.*, 2006). A reduction in the polar lipid and glycerol (Tri, di and mono) of these oils after treatment shows the possibility of safety from these diseases. Free fatty acids were not detected in the alkali refined oils but its value reduced significantly in all the other treated oils.

## CONCLUSION

The present study has shown that the seed of BN and GS are good sources of protein, carbohydrate and mineral. They can be stored for a long time as a result of the low moisture content. In view of all the over all nutrient, physicochemical properties and proximate chemical composition, these seeds may be an economic and alternative protein, oil, mineral and carbohydrate source that could alleviate malnutrition in developing countries and improve overall nutritional status of functional food in the developed countries.

Out of all the methods used for the treatment of the oils, bleaching seems to be the best technique for the refining of oil in term of stability and improvement of the physicochemical properties of the oils.

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