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# Evaluation of proximate and functional properties of rubber (*Hevea brasiliensis*) seed meals

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**With the ever-growing demand for food and food-feed competition because of population growth, the nutritional and functional properties of rubber seed meal were evaluated. Rubber seeds were processed as raw, cooked, and fermented seed meals and analysed for proximate, bulk density, water absorption capacity, oil absorption capacity, emulsion capacity, and least gelation concentration. Results showed significant differences ($P < 0.05$) in the proximate and functional properties of the seed meals. Fermented rubber seed meal (FRSM) had higher ash (4.04%), crude fat (54.17%), and crude protein (22.25%) but with the least content in carbohydrates (11.58%). Cooked rubber seed meal (CRSM) and FRSM had improved oil absorption capacity (OAC), bulk density (BD), and water absorption capacity (WAC). The seed meals showed good thickening and gelling properties with WAC of 138 to 174% and least gelation concentration (LGC) of 5 to 6%. Rubber seed has an appreciable amount of food nutrients and good functional properties and therefore has a great potential for use in food and food product formulations.**

**Key words:** Rubber seed, functional properties, proximate, food formulation.

## INTRODUCTION

In developing countries such as Nigeria, with impeding population growth and high level of inflation, there has been increased demand for food and food-feed competition on the available grain seeds accepted for consumption, which has led to shortage and high cost of protein rich sources. There is therefore need to exploit the less utilized seeds abundant in the region such as rubber seeds.

The rubber tree (*Hevea brasiliensis*) is of commercial value in Nigeria for its latex production but its seeds are not utilised as a food material (Iyayi et al., 2008). Rubber tree yields about 150 to 250 kg of seeds per hectare (Yusup and Khan, 2010). The yield depends on the soil nutrients, ecosystem of the located area, type of planting materials adopted and crop density (Lukman et al., 2018). The seeds are underutilized except when used as sources of seedlings (Lukman et al., 2018).

Some researchers have reported that rubber seed meal has considerable amounts of absorbable nutrients than many conventional seed meals and exhibits high essential nutritive value as a better alternative for protein supplements in livestock diets (Aguihe et al., 2017; Udo

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et al., 2018). The oil content in dried kernel varies from 35 to 45% (George et al., 2000). According to Aigbodion and Bakare (2005), rubber seed oil has good fatty acid constituents and a higher percentage of linolenic acid; an omega 3 fatty acid important in human diet. Rubber seed is processed and consumed in some regions of Indonesia as part of their staple diet (Lukman et al., 2018). In Jerantut, Pahang (Malaysia), rubber seed has been in use as one of their daily dishes known as '*asamrong*'; a native dish in which the seeds are served with sambal and curries (Siti et al., 2013). Notwithstanding, there has continued to be hindrances to the utilization of rubber seeds as food or feed product.

To eradicate hunger and minimize waste, there is need for continuous discovery, investigation and documentation of unharnessed potential food sources. This research is designed to access the proximate composition and functionality of rubber seed meal in a bid to increase its acceptability and awareness as a high nutrient dense seed and suggest possible methods of utilizations for human consumption or at least for livestock feed formulation.

## MATERIALS AND METHODS

### Source of material

Mature rubber seeds were collected from Nigerian Rubber Institute, Akwete, Abia State. The sample preparation was carried out in Food Science and Technology Laboratory, Federal University of Technology, Owerri, Imo State. All reagents used were of analytical grade.

### Sample preparation

Dirt and foreign materials were sorted out from the seeds. The cleaned seeds were divided into three treatment groups.

Treatment group 1 was oven dried at 60°C and pulverised in a blender (KenWood BL330) into fine meal. Treatment group 2 was boiled in a stainless steel vessel for 2 h at 100°C after which it was oven dried at 60°C and then blended into fine meal. Treatment group 3 was boiled for 2 h; boiled seeds were wrapped in blanched plantain leaves and put in a basket to ferment for 3 days at room temperature, then oven dried at 60°C and ground into fine meal. The processing treatments are as presented in Figure 1. Multipurpose dryer operating at 60°C owned and fabricated at the Nigerian Stored Products Research Institute (NSPRI), Port Harcourt station was used and samples were dried for duration of 24 h. The meal samples were stored in properly labelled air tight glass containers.

### Determination of proximate composition

#### *Determination of moisture content*

The moisture content of the rubber seed was analysed using the method of AOAC (2000). An evaporating dish was weighed in 5 g of the sample which was dried in an oven for 3 h at 105°C, after which, it was cooled in a dessicator and weighed. The process was repeated continuously every 30 min until constant weight.

The moisture content was then calculated as follows with Equation 1:

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1 - W_0} X\,100 \qquad (1)$$

where $W_0$= weight of Petri dish in grams, $W_1$ = weight of Petri dish in grams and sample before drying, and $W_2$ = weight of Petri dish in grams and sample after drying.

#### *Total ash determination*

Ash content was analysed using the method of AOAC (2005). Weighed out 5 g of sample was incinerated in a muffle furnance at 550°C in duplicate crucibles till constant weight and light grey ash was obtained. Ash was then cooled in a dessicator and weighed.

Percentage ash was calculated using Equation 2:

$$Ash(\%) = \frac{W_3 - W_1}{W_2 - W_1} X\,100 \qquad (2)$$

where $W_2$= weight of food sample and dish; $W_1$= weight of crucible; $W_3$ = weight of crucible and ash.

#### *Crude protein determination*

The protein of the samples was analysed using Micro-Kjeldahl method as described by Chang (2003). Measured 0.5 g of the sample was digested till a clear solution was obtained; the sample was mixed with 10 ml of concentrated $H_2SO_4$ in a Kjeldahl apparatus and heated in a fume chamber. Then in a Kjeldahl distillation apparatus, 10 ml of the digest was mixed with 45% NaOH and distilled. A solution containing 10 ml of 4% boric acid solution with 3 drops of mixed methyl red and bromocresol green indicator was used to collect the distillate. 50 ml of the distillate was then titrated against 0.02N $H_2SO_4$. A reagent blank was also digested and titrated.

Percentage crude protein was calculated using Equation 3:

$$Crude\ protein\,(\%) = \left[\frac{100}{W} X \frac{NX14}{1000} X \frac{V_f}{V_a} X\,T\,\right] X\,6.25 \qquad (3)$$

where W=weight of sample analysed; N=concentration of $H_2SO_4$ titrant; $V_f$=total volume of digest; $V_a$=volume of digest distilled; T=titre value- blank.

#### *Fat content determination*

Fat content was determined using the method of AOAC (2005). Measured 5 g of the sample was wrapped in a filter paper which was then transferred to already cleaned, oven dried and cooled extraction thimble. Fat was extracted by addition of 25 ml petroleum ether solvent after which solvent was evaporated by oven drying. The flask was cooled in a desiccator and weighed. The percentage fat content was calculated using Equation 4:

$$Fat\,(\%) = \frac{Weight\ of\ the\ extracted}{Weight\ of\ sample} X\,100 \qquad (4)$$

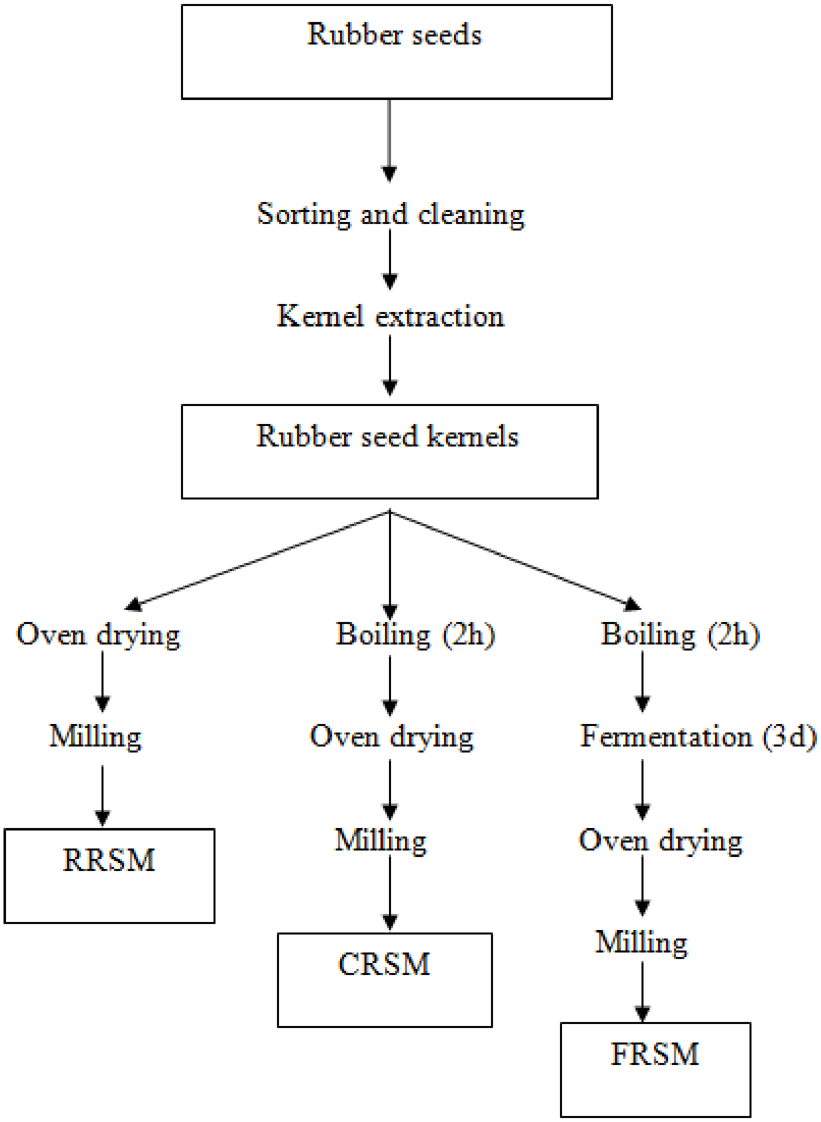

**Figure 1.** Flowchart for sample preparation. FRSM: Fermented rubber seed meal; CRSM: cooked rubber seed meal; RRSM: raw rubber seed meal.

### *Crude fibre determination*

Fibre content was analysed using the method of AOAC (2005). Five grams of the defatted sample was boiled under reflux in 200 ml of 1.25% $H_2SO_4$ for 30 min and then washed with boiling water trapping the particles with a 2 fold muslin cloth. Collected particles were again boiled under reflux in 200 ml of 1.25% $H_2SO_4$ for 30 min and washed with hot water as earlier. Then, sample particles were collected in weighed porcelain crucibles and oven dried for 3 h at 105°C. It was then cooled in a dessicator and reweighed (W2) and then ashed in a muffle furnace at 550°C for 2 h. It was cooled and reweighed.

The crude fibre content of each sample was calculated gravimetrically using Equation 5:

$$\text{Crude fibre (\%)} = \frac{W_2 - W_3}{Weight\ of\ sample} X\,100 \qquad (5)$$

where $W_2$=weight of crucible + sample after washing and drying in oven; $W_3$=weight of crucible + sample ash.

### *Carbohydrate determination*

The carbohydrate content was calculated by difference as the nitrogen free extract (NFE). The nitrogen free extractive will be calculated using Equation 6:

$$\text{NFE (\%)} = 100\text{-\% }(a+b+c+d+e) \qquad (6)$$

where a=protein; b=fat; c=fibre; d=ash; e=moisture.

### Functional properties

These were carried out according to methods described by Onwuka (2018).

### *Bulk density (BD) (g/ml)*

The sample was gently filled into 10 ml capacity calibrated measuring cylinder. Bulk density was calculated as weight of the sample (g) divided by the volume of sample (ml).

### *Water absorption capacity (WAC) and oil absorption capacity (OAC) (g/g)*

One gram of sample was mixed with 10 ml distilled water (WAC) or oil (OAC) in a conical calibrated centrifuge tube for 30 s. The mixture was allowed to stand at room temperature for 30 min and then centrifuged for 30 min at 5000 rpm. The volume of the supernatant was read from the calibrated centrifuge tube. The absorption capacity was expressed as grams of water and oil absorbed per gram of sample for WAC and OAC, respectively.

### *Emulsification capacity (EC) (%)*

Two grams sample was mixed with 25 ml distilled water in a blender for 30 s at 1600 rpm. 25 ml vegetable oil was added slowing into the mixture and blended for another 30 s. Mixture was then centrifuged at 1600 rpm for 5 min. The volume of oil separated from the mixture after centrifuging was read directly from the tube. Emulsification capacity was calculated as the amount of oil emulsified per gram of sample expressed in percentage.

### *Least gelation concentration (LGC) (%)*

Sample suspension preparations of 2 to 20% (W/V) in 5 ml distilled water in test tubes were prepared and heated for 1 h in a boiling water bath followed by rapid cooling under running cold tap water. Then, further cooled at 4°C for 2 h. The least gelation concentration is determined as the concentration when the sample from the inverted test tube will not fall.

### Statistical analysis

The data generated from the study was analyzed by one way analysis of variance (ANOVA) using statistical package of social sciences software (SPSS) version 20 for windows. The results were expressed as mean ± standard deviation. A level of P value less than 0.05 was considered to be significant.

## RESULTS AND DISCUSSION

The results of the analysis carried out on the proximate and functional properties are presented in Tables 1 and 2, respectively.

### Proximate composition

The proximate composition of the rubber seed meals is presented in Table 1. The cooked seed meal with moisture content of 9.92% had the highest moisture content and differed significantly from the other samples, which could be due to starch hydrolysis with consequent higher moisture retention. However, the moisture percentage is lower than 16% as reported by Sharma et al. (2014) and 14.30% as reported by Hossain et al. (2015). The moisture contents of the raw (4.05%) and fermented (3.75%) seed meal are generally low, in agreement with literature (Eka et al., 2010; Aguihe et al., 2017), and therefore will be more shelf stable. The fermented seed meal had the lowest moisture content, which is in line with increased shelf stability after fermentation as reported by Chaves et al. (2014).

Ash content gives an estimation of quantity of minerals in the sample (Oyekunle and Omode, 2008). The cooked meal had the lowest ash percentage, which could be because of some losses due to cooking. The fermented seed meal with ash percentage of 4.04% had the highest ash percentage, differed significantly from the raw (3.47%), and cooked (3.10%) seed meals. This could be attributed to the effect of the fermentation in reducing antinutrients thereby making more minerals available (Kumar et al., 2010). The results of the ash percentage in rubber seed meal agreed with values of 3.10 to 5.90% as reported in previous researches (Mmereole, 2008; Suprayudi et al., 2015; Udo et al., 2018). The values compare favourably with the ash contents of other oil seeds like melon seed (3.30%), groundnut seed (3.08%) as reported by Atasie et al. (2009) and soybean seed (5.1%) as reported by Oladimeji and Kolapo (2008).

The crude fat content of the seed meals are in the range of 43.52 to 54.17% and is in good comparism with earlier studies; 42.50% as reported by Onwurah et al. (2010), 45.50% as reported by Lalabe et al. (2017) and 49.30% as reported by Suprayudi et al. (2015). However, it is lower than 68.53% reported by Eka et al. (2010). This could be due to differences in varieties of the rubber tree as well as the method of seed meal preparations. The results obtained can also be compared to the crude fat content of well know oil seeds like soybean, 28.20% (Ogbemudia et al., 2018) and groundnut 46.10% (Ayoola et al., 2012). It could therefore be said that rubber seed with respect to oil yield is a good substitute for these seeds. The fermented seed meal had the highest value (54.17%) which could be as a result of reduced carbohydrates used up by the enzymes during fermentation as carbohydrates are the preferred nutrient choice of fermentation microorganisms (Nkhata et al., 2018).

The crude fibre ranged from 3.83 to 4.63% and is comparable to earlier reports; 3.19% reported by Suprayudi et al. (2015), 4.50% (Lalabe et al., 2017) and 5.61% (Aguihe et al., 2017). The cooked rubber seed meal sample had the highest value of 4.63% and is in unison with earlier studies. A study by Vasishtha and Srivastava (2013) reported an overall increase in dietary fibre of chickpeas when cooked; cellulose, lignin and pectin concentrations increased while hemicelluloses decreased. The study by Dhingra et al. (2012) also observed that domestic cooking causes a reduction in amount of *in-vitro* digestible starch and therefore, increases both the resistant starch and water-insoluble

**Table 1.** Mean values for the proximate composition (%) of *Hevea brasiliensis* seed meal sample.

| Parameter | RRSM | CRSM | FRSM |
|---|---|---|---|
| Moisture | $4.05^{a} \pm 0.22$ | $9.92^{b} \pm 0.40$ | $3.75^{a} \pm 0.09$ |
| Ash | $3.47^{a} \pm 0.13$ | $3.10^{a} \pm 0.01$ | $4.04^{b} \pm 0.24$ |
| Crude fat | $43.52^{a} \pm 0.21$ | $47.60^{b} \pm 0.29$ | $54.17^{c} \pm 0.13$ |
| Crude fibre | $3.83^{a} \pm 0.11$ | $4.63^{b} \pm 0.13$ | $4.20^{c} \pm 0.00$ |
| Crude Protein | $19.95^{a} \pm 0.13$ | $17.89^{b} \pm 0.01$ | $22.25^{c} \pm 0.15$ |
| Carbohydrate | $25.19^{a} \pm 0.04$ | $16.86^{b} \pm 0.04$ | $11.58^{c} \pm 0.07$ |

FRSM: Fermented rubber seed meal; CRSM: Cooked rubber seed meal; RRSM: Raw rubber seed meal. Means with similar superscripts in the same row are not significantly different ($p>0.05$).

**Table 2.** Mean values for the functional properties of *H. brasiliensis* seed meal sample.

| Parameter | RRSM | CRSM | FRSM |
|---|---|---|---|
| BD (g/ml) | $0.45^{a} \pm 0.01$ | $0.43^{a} \pm 0.01$ | $0.39^{b} \pm 0.01$ |
| WAC (%) | $138.00^{b} \pm 25.06$ | $174.00^{a} \pm 5.29$ | $168.67^{a} \pm 4.16$ |
| OAC (%) | $135.00^{a} \pm 4.28$ | $142.50^{a} \pm 6.36$ | $130.50^{a} \pm 6.36$ |
| EC (%) | $59.00^{a} \pm 1.41$ | $57.00^{a} \pm 1.41$ | $51.50^{b} \pm 2.12$ |
| LGC (%) | $5.00^{a} \pm 0.00$ | $6.00^{a} \pm 0.00$ | $6.00^{a} \pm 0.00$ |

FRSM: Fermented rubber seed meal; CRSM: Cooked rubber seed meal; RRSM: Raw rubber seed meal; BD: Bulk density; WAC: Water absorption capacity; OAC: Oil absorption capacity; EC: Emulsification capacity; LGC: Least gelation concentration. Means with similar superscripts in the same row are not significantly different ($p>0.05$).

dietary fibre. The subsequent reduction in crude fibre with fermentation (4.20%) could be because of digestion by microbial activities.

The crude protein values ranged from 17.95 to 22.25%. The values compare favourably with earlier studies as reported; 17.41% (Eka et al., 2010), 19.40 (Lalabe et al., 2017), 21.87 (Suprayudi et al., 2015) and 22.30% (Onwurah et al., 2010). The protein content of groundnut is in the range of 22 to 30% (Mckevith, 2005), soybean ranges from 37 to 40% (Ogbemudia et al., 2018; Grieshop and Fahey, 2001), and castor bean (20.11%) and African oil bean (20.60%) (Enujiugha and Ayodele-Oni, 2008). Similar to these legumes, rubber seed can be said to be a good source of plant-based protein and can contribute to the daily protein need. The raw seed meal had a crude protein value of 19.95%, which was reduced to 17.89% in the cooked seed meal. Cooking is known for degradation and conversion of proteins in raw food samples into soluble forms but also is usually accompanied by some losses in quantity (Omenna et al., 2016). The fermented seed meal crude protein value of 22.25% could be explained by reduction of carbohydrate after fermentation (Pranoto et al., 2013).

The carbohydrate values ranged from 11.58 to 25.19%. The raw seed meal had the highest percentage of 25.19%, which was reduced to 16.86% by cooking. This was similar to the research done by Ikanone and Oyekan (2014) which recorded a considerable loss of low molecular weight carbohydrates into the processing water. The fermented seed meal had the least value of 11.58%. This is because, activities of microorganism lead to the breakdown of complex carbohydrates into simpler forms. Therefore, decrease could be because of breakdown and usage by microorganisms (Osman, 2011). The rubber seed meal carbohydrate values from the study compare favourably with 13.80% (Hossain et al., 2015) and 21% (Suprayudi et al., 2015) from earlier studies.

## Functional properties

The bulk density of the seed meals was 0.45 g/ml (raw seed meal), 0.43 g/ml (cooked seed meal) and 0.39 g/ml (fermented seed meal). The fermented seed meal had a significant reduction in bulk density from the cooked and raw seed meal, which is in line with the reports of Ogodo et al. (2016) which reported decreasing bulk density of maize flours with increasing fermentation period. Fermentation has also been a traditional tool for reducing bulk density of foods especially those intended for infant weaning meals.

The water absorption capacity of the seed meals was 138% (raw seed meal), 174% (cooked seed meal) and 168.67% (fermented seed meal). The processed seeds had a significant increase in water absorption. Although, no significant difference between the CRSM and FRSM was recorded, the cooked seed meal had the highest

WAC percentage. This is in line with the report of Obatolu et al. (2001) on the water absorption of African yam bean flours; the processed flours had significantly higher water absorption than the raw African yam bean flour. Water absorption is a measure of the optimum amount of water than can be added to flour before it becomes too sticky to process. Heat processing known to cause starch gelatinisation and protein denaturation increases its water absorption capacity (Ha-jung et al., 2019; Obatolu et al., 2001). High water absorption capacity is required in baking applications (Iwe et al., 2016). WAC range from 149.1 to 471.5% is critical in viscous foods as gravies and soups (Akinyede and Amoo, 2009). The high WAC of the flours suggests that it may have application as a thickening agent in food formulations.

Oil absorption capacity of the seed meals was 135% (raw seed meal), 142.5% (cooked seed meal) and 130.5% (fermented seed meal). CRSM had the highest OAC although no significant difference exists amongst the samples. High oil absorption is required in food formulations because of its relationship with mouth feel and flavour retention (Iwe et al., 2016). The result obtained is closely related to 128.8% reported for white melon seed flour, a well-known thickening ingredient used in soups, gravies and in the formulation of meat analogs (Ogunbusola et al., 2012). The result suggests that the seed meals have a good oil absorption capacity and therefore can function optimally in food formulations.

The emulsion capacity of the seed meals was 59% (raw seed meal), 57% (cooked seed meal) and 51.5% (fermented seed meal). FRSM had emulsion capacity that was significantly lower than that of CRSM and RRSM. It was also noted that the emulsion capacity decreased with processing. The report of Obatolu et al. (2001) on the effect of processing on emulsion capacity of African yam beans reported similar trend. The emulsion capacities of the seeds were lower than 85% reported for white melon (egusi) seeds (Ogunbusola et al., 2012). Processing by cooking (which is necessary for detoxification of antinutrients) will be more appropriate for use for rubber seed meal intended for use in systems requiring high emulsifying properties.

The least gelation concentration of the seed meals was 5% (raw seed meal), 6% (cooked seed meal) and 6% (fermented seed meal). There were no recorded significant differences on LGC due to processing. The processed seed meals however had higher LGC percent. The values were generally lower than 16% reported for white melon seeds (Ogunbusola et al., 2012). The lower the LGC, the better the gelling ability of the protein material (Chandra et al., 2015). The low LGC of the seed meals suggest its usefulness in food formulations that require thickening and gelling.

## Conclusion

Rubber seed has appreciable amount of food nutrients and showed high similarity with other oil seeds such as melon (*Egusi*) seed and groundnut seed. A protein content of up to 22% makes it a great potential raw material in the production of high protein food ingredients. The study on the functional properties showed that processing improved the bulk density, oil absorption and water absorption capacity of the seed meal. Rubber seed meal has great potentials for incorporation as a thickening agent in soups, gravies and food products. Further study is required on the behaviour of the seed meal in relation to other ingredients in a potential food product. The crude fat content of up to 54% also suggests a great potential in oil production, therefore, further study is required to ascertain its fatty acid profile and oil characteristics (Chaikil et al., 2017).

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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