



Full Length Research Paper

# Comparative study of phytochemical profiles and *In Vitro* multienzymes protein digestibility of two species of *Mallotus subulatus* --- A tropical underutilized hard-to-cook legume

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## ABSTRACT

Two varieties of *Mallotus subulatus* (white and brown), underutilized hard-to-cook legumes, were analysed for the nutrients, phytochemicals and *in vitro* multienzyme protein digestibility. The ash content of 4.38% and 4.25% were recorded for the white and brown varieties respectively. The two varieties are poor sources of fat. The protein content of the white variety (20.97%) was lower than that of brown variety (21.90). The crude fibre content of 9.68% for the white variety was significantly higher ( $p < 0.05$ ) than 3.93% recorded for the brown variety. The two varieties of *M. subulatus* are good sources of Ca, K, P, Zn, Mg. Although, the quantity of non-haem Fe was low. The phytic acid and trypsin inhibitor --- 71.31mg/g and 34.37mg/g, respectively were higher in white variety than brown variety --- 69.53mg/g and 30.44mg/g. The concentration of tannin in white and brown varieties were 28.10mg/g and 38.87mg/g, respectively. The percentage digestibility of the white variety was higher than that of the brown variety. The study showed that both varieties are good sources of critically important nutrients such as protein and their adaptation will not only strengthen dietary diversity but solve the wider problem of protein energy malnutrition (PEM) — a major nutritional syndrome in many parts of the world.

**Keywords:** *Mallotus subulatus*, underutilized, hard-to-cook legume, *in vitro* multienzyme protein digestibility.

## INTRODUCTION

Legumes are sources of protein which contribute substantially to the protein intake of a significant proportion of the world population, particularly in developing countries (Aletor, 1993). Legumes are worldwide in distribution and one or more of them are consumed regularly in some form practically in every country of the world (Gept, 2005). Although proteins from animal sources are considered superior in terms of quality compared to plant because of their better profile of essential amino acids, the use of animal protein cannot satisfy the needs of the world population. Animal protein is relatively expensive, scarce and not readily available to a large percentage of consumers particularly in less developed countries (Lewis *et al.*, 2005; Graham and

Vance, 2003). The need for more protein consumption by a large percentage of the world population particularly those of the developing countries has not been met, although many efforts have been made to provide the world population with protein rich diets. Current trends in population growth indicate that protein deficiency may continue to increase unless planned measures are put in place to tackle the situation. The continuing shortage of good biological protein causes serious physiological disorders. To meet the expanding protein needs of the world population, the consumption of plant foods that contain appreciable quantities of protein is of paramount importance (Lewis *et al.*, 2005).

Legumes are widely cultivated and available at affordable prices. There are varying concentrations of lipids, proteins, minerals, vitamins and carbohydrates in legumes. The crude protein content of most raw legume seeds varies between 20 and 40%. The fat content is generally low except for the oil seeds such as groundnut and soybean with fat content of 20-50% (Norman and Joseph, 2006). Generally, they are not rich sources of vitamins although some group of B vitamins (thiamine, riboflavin and nicotinic acid) are present in some (Marero *et al.*, 1991). Since legumes are known to be good sources of plant protein, one of the most valuable and practical approaches for tackling the problems of protein deficiency is the continued search for and utilization of protein rich plants. Hence, the use of lesser known underutilized legumes that contain appreciable quantity of protein becomes paramount for making more protein available for people at affordable prices.

*Mallotus subulatus* is one of the lesser known species of legumes grown in south west Nigeria. Two varieties are cultivated -- white and brown varieties. These varieties are cultivated and used by peasant farmers as alternative crops that are brought out for consumption especially during dry season when other crops are out of stock. A major limiting factor to their cultivation is their hard-to-cook nature which localizes their cultivation to certain areas in the country but they are easy to cultivate and are highly disease resistant. There is paucity of information on their nutritional and antinutritional properties. Therefore, provision of information about their components will enhance adaption of these local lesser known legumes, strengthen dietary diversity and thus make critically important nutrients available at affordable prices.

## MATERIALS AND METHOD

### Materials

Fresh matured legume seeds of *Mallotus subulatus* were obtained from peasant farmers in Saki (8.67° N, 3.40° E), Saki West Local Government Area of Oyo State, Nigeria. The white variety is locally called *pelpelupe funfun* and the brown variety is known as *pelpelupe pupa*. The legume seeds were dry-cleaned by removing extraneous particles such as stones, stalks, broken and immature seeds. They were then milled using Nulux (LA) Premium grinding mill. The milled samples were then sieved using 500µm sieve and packaged in labeled plastic containers prior to analysis.

### Proximate analysis

The procedures of AOAC (2005) were used. The moisture content was determined using air-oven method

at 105°C. Total ash determination was carried out by using muffle furnace (Carbolite 301) to incinerate at 550°C. Extraction apparatus of the soxhlet type was used to determine the crude lipid using petroleum ether (40°C). Crude fibre was determined after treating the sample with conc. H<sub>2</sub>SO<sub>4</sub>, NaOH and petroleum spirit. Macro Kjeldahl method (N × 6.25) was used to determine the protein content of the samples. The total carbohydrate content of the legume samples was determined by difference.

### Mineral elements determination

Analysis were carried out for some mineral elements: Ca, Zn, Na, Fe, Mg, P and K using atomic absorption spectrophotometer -- Buck 205 (AOAC, 2005). The pulverized ashed sample was dissolved in 100cm<sup>3</sup> HCl (10% v/v) which was subsequently used in mineral content determination. Atomic absorption spectrophotometer - Buck 205 model was used. Its hollow cathode lamp supplied resonance line radiation of each element. Standard calibrations were employed in the analysis.

### Determination of phytochemical components

The phytic acid content of the legume seed was determined by extraction and precipitation as described by Fagbemi *et al.* (2005). The tannin content of the legumes was determined by the procedure of Makkar (1994). The method described by Smith *et al.* (2000) was used to determine the trypsin inhibitor. The procedure of Makkar and Becker (1997) was used for the determination of saponin.

### *In vitro* multi enzymes protein digestibility (ivpd) determination

The IVPD of the seed flour was determined using the procedure of Hsu *et al.* (1977). Fresh multi enzyme solution containing trypsin, chymotrypsin and peptidase dissolved in distilled water was used. The IVPD was calculated using the equation:  $Y = 210.464 - 18.103x$   
Where Y = *in vitro* protein digestibility (%)

X = pH of sample suspension after 10min and 15min.

### Boiling and determination of boiling time

Boiling of each of the legumes (500g) was conducted at atmospheric pressure using a domestic cooker (Binatone PC 5001). Determination of cooking time was conducted by tactile method (Vindiolla *et al.*, 1996) in which the cooked legumes was squeezed between the fore finger

**Table 1.** Proximate composition of the two varieties of *Mallotus subulatus*

	White variety (%)	Brown variety (%)
Moisture	10.11a±0.40	10.10a±0.45
Ash	4.38a±0.2	4.25a±0.28
Ether extract	0.97a0.02	0.93a±0.01
Crude protein	20.97a±0.77	21.90b±1.04
Crude fibre	9.68a±0.45	3.93b±0.02
Total carbohydrate	53.89a±1.22	58.89b±0.97
Dry matter	88.89a±1.97	88.90a±2.11

Values with different subscript in the same row are significantly different ( $p < 0.05$ )

and thumb with moderate pressure. A seed was considered to be cooked when it could be squeezed by finger easily. After boiling, the legumes and the cooking water were cooled in plastic containers. Subsequently, the cooked legumes and cooking water were dried at 45-50°C using cabinet drier. The dried samples were stored in cellophane bags prior to digestibility determination.

### Statistical analysis

All the analysis were conducted in three replicates and expressed as mean data  $\pm$  standard deviation. Statistical analysis was performed using SAS (2005). The data were subjected to Analysis of Variance and Duncan multiple range tests were used to separate the means.

## RESULTS AND DISCUSSION

### Proximate composition

The proximate constituents of the two varieties of *M. subulatus* were presented in Table 1. There was no significant difference ( $p < 0.5$ ) in the moisture content between the two varieties. The moisture content of the white and brown varieties were 10.11 and 10.10% respectively. This was comparable to a value of 10.15% reported by Ojmelukwe (1991) for a variety of bambara groundnut. The percentages moisture content was comparable to 10% recommended for storage stability (Oyebode *et al.*, 2007). There was slight difference in the total ash content of the two varieties. Although legumes are not regarded as rich sources of mineral elements, the total ash content were 4.38% for white and 4.25% for brown. These results agree with a previous study carried out by Siddhuraju *et al.* (2002) in which seeds from different species of an unconventional legume, *Sesbania* were reported to have varying concentrations of ash. In

the study, *S. aculeata*, *S. rostrata* and *S. canabina* were found to contain 3.65, 6.07 and 3.77% of ash respectively. Similar work by Fasoyiro *et al.* (2006) showed that Lima bean and cowpea contained 4.31 and 3.80% ash respectively. The two varieties of *Mallotus subulatus* had fat contents of 0.97% for white and 0.93% for brown. Generally, legumes have been reported to be poor sources of fat. With the exception of soybean, groundnut and *Adenopus breviflorus*, the fat content of most legume seeds is low (Lewis *et al.*, 2005). There was significant difference ( $p < 0.5$ ) in the crude fibre contents of the two varieties. The white varieties had 9.68% while the brown variety had 3.93%. The values for the amount of crude protein were 20.97% for the white variety and 21.90% for the brown variety. Legumes have been reported to be good sources of protein. The percentage protein contents obtained for the two varieties studied compared favourably well with values reported by previous studies (Siddhuraji *et al.*, 2002) reported a higher crude protein content of 26.6% for *Vigna radiata* while a lower value of 17.80% was earlier reported for *Vigna subterranean* by Adewusi and Osuntogun (1991).

### Mineral element constituents

The mineral element profile of the legume seeds studied is shown in Table 2. The calcium content of the white and brown varieties was 128.81 and 126.78 mg/100g respectively. Calcium being a mineral element responsible for the formation and development of bones and teeth is therefore an element of interest in the nutritional composition of these underutilized legumes. Sodium, a common element in many food plant was present in the legumes at concentration tolerable for human health (Sunetra, 2009). High intake of sodium has been reported to cause hypertension (Apata and Ologbobo, 1994; Norman and Joseph, 2006).

**Table 2.** Mineral elements composition of varieties of *Mallotus subulatus* (mg/100g)

Mineral elements	White	Brown
Calcium	128.81a±1.02	126.78b±1.001
Zinc	20.57a±0.31	19.89a±0.41
Sodium	42.34a±0.05	40.57b±0.42
Iron	5.52a±0.02	9.05b±0.42
Magnesium	140.53a±1.02	137.69b±1.01
Phosphorus	240.24a±1.10	240.32a±1.02
Potassium	146.51a±0.81	128.77b±2.01

Values with different subscript in the same row are significantly different ( $p < 0.05$ )

**Table 3.** Some phytochemical components of *Mallotus subulatus* (mg/g)

	White	Brown
Phytic acid	71.31a±0.72	69.53a±0.43
Saponin	10.21a±0.43	12.19b±0.72
Trypsin inhibitor	34.37a±0.32	30.44b±0.13
Tannin	28.10a±0.22	38.87b±0.41

Values with different subscript in the same row are significantly different ( $p < 0.05$ )

The legumes contained non-haem iron. Although, the concentrations were low, there was significant difference in the quantity of iron present. The white variety had 20.57mg/100g of zinc while the brown variety had 19.89mg/100g. Zinc is a constituent of enzymes involved in the body metabolism. The two legumes contained appreciable quantity of magnesium. There was no significant difference in the concentration of phosphorus between the two legumes. The potassium content of the brown variety was lower -- 128.77mg/100g, than that of the white variety -- 146.51mg/100g. These values compare well with the values of 141.00mg/100g reported for cowpea but lower than 200mg/100g reported for yam bean (Edem *et al.*, 1990; Apata and Ologbo, 1994). Also, the quantity of potassium in lima bean- 143.04mg/100g as reported by Oyebode *et al.* (2005) was similar to that of the white *M. subulatus*-146.51mg/100g. Potassium is the principal intracellular cation which helps regulate osmotic pressure and pH equilibria.

### Phytochemical components of the legumes

The phytochemical components of each of the legumes are recorded in Table 3. The two varieties had tannin content of 28.10 mg/g for the white and 38.87mg/g for the brown. The tannin content of these lesser known

legumes were observed to be higher than those reported for the major legumes. For instance, soybean, groundnut and cowpea were reported to have tannin content of 1.80, 1.10 and 6.47mg/100g respectively. Also, the results agree with the observation of Nahad (2000) who reported higher tannin contents among coloured varieties of faba beans than those in the white seeds thereby suggesting a relationship between colour intensity and tannin content. Unlike tannin, trypsin inhibitor occurred more in the white variety. The inhibitor impairs protein hydrolysis thereby decreasing the nutritive value. As shown in Table 3, higher content of the saponin was observed for the brown variety containing 12.19mg/g compared to the white which contained 10.21mg/g. The saponin content of *Albizzia lebeck* another minor legume commonly grown in some northern parts of Nigeria was reported to be 18.00g/100g (Abdullahi *et al.*, 2007). The presence of saponin in foods induces astringent or bitter taste.

The phytic acid content of the legumes were 71.34mg/g for the white variety and 69.53mg/g for the brown variety. These values were high when compared with other values reported for some other legumes such as pigeon pea - 4.8g/100g and Lima bean -- 3.17g/100g (Fasoyiro *et al.*, 2006). There is a correlation between the presence of phytic acid and protein content and mineral availability (Muzquiz *et al.*, 1991; Sidthraju *et al.*, 2002).

**Table 4.** Cooking duration of *Mallotus subulatus*.

Sample	Volume of water used for boiling (cm <sup>3</sup> )	Duration of cooking (min)
White variety	4700a±141.42	328a±2.00
Brown variety	3000b±100.00	274b±2.00

Values with different subscript in the same column are significantly different ( $p < 0.05$ )

**Table 5.** *In vitro* multienzymes protein digestibility of white *Mallotus subulatus* before and after boiling.

Sample	10 min		15 min	
	pH	% digestibility	pH	% digestibility
RS1	9.91	31.06a±0.02	9.91	31.06a±0.06
CS1	6.73	88.63b±0.21 [185.35]	6.72	88.81b±0.00 [185.93]

Values with different subscript in the same column are significantly different ( $p < 0.05$ ). Values in parenthesis represent % increase in digestibility.

RS1= raw white *Mallotus subulatus* ; CS1 = boiled white *Mallotus subulatus*

**Table 6.** *In vitro* multienzyme protein digestibility of brown *Mallotus subulatus* before and after boiling.

Sample	10 min		15 min	
	pH	% digestibility	pH	% digestibility
RS	29.82	32.69a±0.13	9.82	32.69a±0.45
CS2	6.79	87.54b±0.01 [167.79]	6.77	87.91±0.06 [168.92]

Values with different subscript in the same column are significantly different ( $p < 0.05$ ). Values in parenthesis represent % increase in digestibility.

RS= raw brown *Mallotus subulatus* ; CS2 = boiled brown *Mallotus subulatus*

### Effect of boiling on cooking time

Table 4 shows the effect of boiling on cooking times and the corresponding volume of water required for the boiling process. It took more time of 328 minutes to effect cooking of the white variety compared with 274 minutes required for the brown variety. Thus the white variety is harder to cook than the brown variety. This might be due to the high amylase content which delays starch gelatinization and thus affects normal cooking properties (Fairweather- Tait and Hurrel, 1996). Prolong cooking time is a major limiting factor to the utilization of these legumes.

### *In vitro* multienzyme protein digestibility

The protein digestibility of the two varieties of legumes before and after boiling is presented in Tables 5 and 6.

Table 5 shows the protein digestibility for the white variety while Table 6 shows the values for the brown variety. Values in parenthesis represent percentage increase in digestibility after boiling. Earlier studies have shown that nutrient composition of food is not enough to determine nutrient bioavailability (Fagbemi, 2005; Ayo *et al.*, 2007). Hence, the need for *in vitro* digestibility studies. For each of the two legumes, boiling had significant effect ( $p < 0.05$ ) on the *in vitro* protein digestibility. Raw sample of white variety with digestibility of 31.06% increased to 88.81% digestibility representing 185.93% increase (Table 5) while percentage increase of 168.92 was observed for the brown variety after boiling. Low percentage digestibility reported for the raw samples were in agreement with the report of Adewusi and Osuntogun (1991) who reported low value of 5.4% for soybean and 36.6% for lima bean. Fagbemi *et al.* (2005) observed that the percentage digestibility of *T.*

*occidentalis* was 84.7% after boiling. The two varieties of *M. subulatus* are therefore highly digestible.

## CONCLUSION

The two varieties of *Mallotus subulatus* are good sources of highly digestible dietary protein. Provision of more information about the composition of these legumes, it is hoped, will enhance more utilization which will make critically important nutrients especially protein available to consumers in the developing countries at affordable prices. More utilization of these underutilized legumes will widen dietary pattern, solve the wider problems of hunger, poverty and malnutrition. It is expedient to state that our ongoing study to proffer solution to the challenge of prolong cooking using hydrothermal processing methods is yielding positive results.

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