***Original Research Article***

# ACCEPTABILITY OF YOGHURT PREPARED FROM MILK SUBSTITUTED WITH BENTH SEED (*Adenopus breviflorus*) PROTEIN ISOLATE

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## Abstract

Protein has been isolated from *Adenopus breviflorus* seed flour and used as a supplement to the production of yoghurt. The chemical composition of the seed flour and protein isolate was determined using standard methods. The yoghurt so produced was analysed for its pH, total solids, titratable acidity and the sensory qualities evaluated using the nine point Hedonic scale from dislike extremely (1) and like extremely (9). The results obtained from the chemical composition of the seed flour and protein isolates showed that they are good sources of protein (30.44% and 94.14% respectively). There was s significant reduction in the mineral content and antinutrients of the seed flour after protein isolation. The result obtained from the sensory evaluation showed that milk can be substituted for *Adenopus breviflorus* protein isolate from the production of yoghurt up to 25% level of substitution without affecting the sensory qualities.

Keywords: *Adenopus breviflorus*, protein isolate, yoghurt

## 1.0 Introduction

*Adenopus breviflorus* Benth is a tree plant, commonly known as *"Lagenaria breviflora* Roberty," belongs to the family Cucurbitaceae (Yasuyuki, 2005). Different parts of the plant (stem, seeds and fruits and leaves) have been used in folklore medicine in West Africa as herbal treatment for various diseases in man and livestock (Ajayi *et al.*, 2002; Sonaiya, 1999; Tomori *et al.*, 2007). In addition to its medicinal application, so much has been reported on the taxonomy (Morimoto *et al*, 2005) and chemical constituents of the plant. *Adenopus breviflorus* bend seed is an oil seed commonly found in the savannah and semi - savannah forest region in southern Nigeria. The seeds are used as soup ingredient. They can be ground with hulls or without hulls for preparation of soup. It is also known that within some ethnic groups, the seeds are roasted and eaten whole. Oshodi (1992) reported the proximate chemical composition, nutritionally valuable minerals and functional properties of *Adenopus breviflorus* bend seed flour which could be used to assess its value in the food industries other than direct consumption by farmers.

Protein–Energy–Malnutrition (PEM) is a serious problem facing most developing nations as a result of inadequate intake of good quality protein from sources such as meat, fish and poultry products, which are out of reach to much populace due to poor economy, increase from population pressure and other natural calamities such as drought and flood (Nordeide *et al.*, 1996). In order to arrest this situation, much attention has been focused on the exploitation and utilization of plants. Ordinarily, plants provided nearly two thirds of the world supply of food protein for human and animals in which 10 – 15% comes from legumes (Pirman *et al.*, 2001; Abegunde, 2018). Protein isolates are the most refined form of protein products containing the greatest concentration of protein but unlike flour and concentrates contains no dietary fibre (Jay and Michael, 2004). They are very digestible and easily incorporated into different food products. Protein isolates are nowadays believed to have played a major role in the development of new class of formulated foods. It is high concentration of protein with the advantage of colour, flavour and functional properties making it an ideal raw ingredient for use in beverages, infant foods and children milk food, texture protein products and certain types of specialty foods (Olaofe, 1998).

Yoghurt is a fermented milk product obtained from milk or milk products by lactic acid fermentation through the action of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (FAO/WHO, 1977). When a sufficient quantity of lactic acid is produced then the milk coagulates and this coagulated milk is called yoghurt. Lactic acid fermentation of legume based milks has been used as one of the approaches to prolong the shelf life of the products, create variety, improve the nutritional value and as well enhance the acceptability of the product. The probiotic yoghurt, having probiotic effect is a fermented milk product of adjuvant microorganisms. Yoghurts varies from appearance, flavor and ingredients. There is a symbiotic relationship between the two species of bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*; that is why there is more rapid acid development than in the single strain culture (Tamime and Deeth, 1980). Various combinations of starter cultures are selected during manufacturing of yoghurt to achieve desirable characteristics of product and also to provide the consumers with a wide choice of therapeutic benefits. Depending on its activity, manufacturer usually adds 2-4 % yoghurt starter culture. Now a days, there has been increasing trends to fortify the dairy product with fruits (natural fruit juice, pulp, dry fruits) (Ghadge *et al.*, 2008). Yogurt-like products have been prepared by some workers from soybean, cowpeas, coconut and mug beans (Terna and Musa, 1998).

This study seeks the possibility of using *Adenopus breviflorus* seed protein isolates from substituting or fortifying with milk in yoghurt production.

## 2.0 Materials and Methods

### Collection and Preparation of Sample

*Adenopus breviflorus* seeds used were obtained in the dried form, from a local market in Edo State, Nigeria. The seeds were screened to remove stones, bad ones and dirt after which they

were de-hulled, dried and milled into powder. The powdered samples were stored in screw-capped air-tight container.

**Preparation of Defatted Flour Sample**

Defatted sample of the seed flour was prepared by continuous extraction method using n-hexane for nine hours in a soxhlet apparatus. The defatted flour was then recovered and residual solvent was removed by air-drying.

**Preparation of Protein Isolate**

In preparation of the protein isolate, defatted seed flour, was dispersed in distilled water at a meal ratio of 1:20 w/v (flour /water). The mixture was stirred with a stirrer for 30 minutes after which the pH of the slurry was adjusted to the pH at which the protein in the flour is most soluble (pre-determined) using 0.1M HCl drop wisely. The solution was further stirred for 2 hours at $30\pm2^0$C using a stirrer to enhance high degree of protein solubility. The slurry was centrifuged at 4,000 rpm for 30 minutes at $4^0$C. The residue obtained after decanting the supernatant was re-extracted with half the volume of the same solvent under similar conditions. The pH of the combined supernatants was adjusted to the pH at which the protein in the flour is least soluble (the isoelectric point which has been predetermined) with 0.1M HCl to precipitate the protein. The isolate was recovered by centrifugation for 30 minutes at 4°C after which it was dispersed in distilled water and dialyzed against distilled water for 18 hours. The dialysate was freeze dried and then stored in air-tight container in the deep freezer for further analysis.

Proximate composition of the raw seed and protein isolate was determined according to the standard method of AOAC (2005). Ca, Mg, Mn, Ni, Cd, Cu, Fe, Pb, Co, Zn and Cr were determined using Atomic Absoption spectrophotometric method (Buck 210VGP model). Na and K were determined by flame photometric method (Jenway PFP7 model) while phosphorus was determined spectrophotometrically by the use of phosphovanadomolybdate solution (AOAC, 1990).

Various standard methods were used in determination of antinutritional composition of the raw seed and protein isolate. The method of Young and Greaves (1940) as described by Reddy and Kove (1999) was used to determine phytin. Tannin content was determined by modifying the procedure of Makkar (1994). Oxalate was determined by the method described by Day and Underwood (1986). Standard method of AOAC (2005) was used for the determination of cyanide. Saponin was determined using the method of Obadoni and Ochuko (2001). Boham and Kocipal-Abyazan (1994) method was used for the determination of flavonoid. Alkaloid was determined according the method of Harborne (1993).

**Production of Yoghurt**

The milk and the protein isolates were weighed and mixed at different concentrations (25%, 50%, and 75%), while 100% milk was used as control sample. The mixtures were heated at 82 to $92^0$C for 30 minutes to pasteurize it and then cool about $45^0$C. The mixed starter culture was added and stirred very well for complete mixing. The whole mixtures were allowed to stand in an incubator for about 5 hours of $45^0$C. The mixtures were transferred into the refrigerator and allowed to cool about thereby stopping further fermentation.

**Determination of pH of Yoghurt**

pH was measured using a pH meter (WTW-pH 330, Weilheim Germany).

**Determination of Titratable Acidity**

Acidity was determined by titrating the samples against 0.1M NaOH using phenolphthalein as indicator.

**Determination of Total Solid of Yoghurt**

The weight of the residue obtained from moisture content analysis was expressed as percentage total solid using the formula:

$$Total\ solid\ (\%) = \frac{weight\ of\ dry\ yoghurt}{weight\ of\ sample} \times 100$$

**Sensory Evaluation of Yoghurt**

Sensory evaluation was carried out on the samples of yoghurt by panel of 10 judges selected from their consistency in scoring and the samples were evaluated for colour, taste, flavor consistency and overall acceptability using the nine point Hedonic scale from dislike extremely (1) and like extremely (9).

**Statistical Analysis of Data**

The data generated from all the results obtained were subjected to statistical analysis of variance (ANOVA) using SPSS17 computer package and the mean values separated by Duncan′s multiple range test. Values reported are mean of triplicate determinations.

## 3.0 Results

**Table 1:** Proximate composition of raw seed flour and protein isolate (%)

| Parameters | Moisture content | Fat | Crude protein | Ash | Crude fibre | Carbohydrate |
|---|---|---|---|---|---|---|
| Raw seed | 4.05 ± 0.09 | 52.63 ± 0.33 | 30.44 ± 0.19 | 3.33 ± 0.11 | 3.15 ± 0.03 | 6.40 ± 0.31 |
| Protein isolate | 2.58 ± 0.04 | 1.21 ± 0.01 | 94.10 ± 0.02 | 1.08 ± 0.11 | 1.03 ± 0.06 | ND |

ND = not detected

**Table 2:** Mineral composition of raw seed flour and protein isolate (mg/100g)

| Minerals | Raw seed | Protein isolate |
|---|---|---|
| Iron | 12.32 ± 0.81 | 1.46 ± 0.04 |
| Chromium | BDL | BDL |
| Nickel | BDL | BDL |
| Copper | 0.39 ± 0.54 | 0.28 ± 0.02 |
| Cobalt | BDL | BDL |
| Manganese | 0.40 ± 0.30 | 0.07 ± 0.02 |
| Zinc | 21.32 ± 0.30 | 3.63 ± 0.01 |
| Lead | BDL | BDL |
| Calcium | 120.53 ± 0.62 | 25.16 ± 0.08 |
| Magnesium | 125.06 ± 0.11 | 18.51 ± 0.02 |
| Potassium | 129.62 ± 0.77 | 116.60 ± 0.09 |
| Sodium | 151.26 ± 0.31 | 86.43 ± 0.04 |
| Phosphorus | 164.94 ± 0.05 | 95.13 ± 0.03 |
| Cadmium | BDL | BDL |

BDL = below detection limit

**Table 3:** Antinutritional composition of raw seed and protein isolate

| Parameters | Raw seed | Protein isolate |
|---|---|---|
| Phytate (mg/g) | 11.74 ± 0.02 | 4.67 ± 0.02 |
| Tannin (mg/100g) | 0.14 ± 0.02 | 0.13 ± 0.01 |
| Saponin (%) | 1.32 ± 0.02 | 0.37 ± 0.03 |
| Oxalate (mg/g) | 1.33 ± 0.01 | 1.24 ± 0.02 |
| Cyanide (mg/kg) | 2.34 ± 0.02 | 0.66 ± 0.02 |
| Alkaloid (%) | 0.46 ± 0.02 | 0.14 ± 0.02 |
| Flavonoid (%) | 1.31 ± 0.01 | 0.26 ± 0.01 |

**Table 4:** General Notation of Yoghurt Samples Formulation

| Samples | Dano milk (0% fat) | Protein isolate |
|---|---|---|
| Sample 1 | 25% | 75% |
| Sample 2 | 50% | 50% |
| Sample 3 | 75% | 25% |
| Sample 4 (control) | 100% | --- |

**Table 5:** The Result of Chemical Properties of Yoghurt Samples

| Samples | pH | Titratable Acidity (%) | Total Solid (TS) (%) |
|---|---|---|---|
| Sample 1 | 5.49 | 0.91 | 13.30 |
| Sample 2 | 5.36 | 0.97 | 16.60 |
| Sample 3 | 5.29 | 1.00 | 16.60 |
| Sample 4 | 6.19 | 0.89 | 7.60 |

**Table 6:** Sensory Evaluation of Yoghurt

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Colour | $3.80^{b} \pm 2.20$ | $2.80^{b} \pm 1.14$ | $7.00^{a} \pm 1.15$ | $7.70^{a} \pm 1.06$ |
| Taste | $3.80^{b} \pm 2.39$ | $2.70^{b} \pm 1.83$ | $6.50^{a} \pm 1.43$ | $7.40^{a} \pm 1.35$ |
| Flavour | $3.80^{b} \pm 2.80$ | $2.50^{b} \pm 1.84$ | $6.70^{a} \pm 0.95$ | $6.60^{a} \pm 1.96$ |
| Consistency | $2.60^{b} \pm 1.78$ | $2.40^{b} \pm 1.71$ | $6.80^{a} \pm 1.23$ | $7.80^{a} \pm 1.03$ |
| Acceptability | $2.50^{b} \pm 1.78$ | $2.80^{b} \pm 1.75$ | $7.30_{a} \pm 0.82$ | $8.20^{a} \pm 0.92$ |

Values with different superscript on the same row are significantly different ($p \leq 0.05$)

## 4.0 Discussion

The moisture content of the seed flour and protein isolates ranged from 2.58% to 4.05%. The moisture content of the seed flour is lower than that for similar legumes like Cassia floribida, 6.0% (Vandevel and Janardhanam, 2001), Larthyrus martimus, 9.7% (Chavan *et al*, 1999), Lupin

Species, 6.6% (Ruin-lopez *et al*, 2009). The moisture content obtained for the sample and isolate are lower than the 10% recommended for storage stability of flours and this might be advantageous in terms of prolonging the shelf-life and retaining their qualities. The moisture content decreased significantly on isolation.

The high crude fat obtained (52.63%) suggests that it is an oil seed. The value, however, compared very well with 47.9- 51.1% in Citrullus vulgaris, 47.02% in flute pumpkin and with the range of 42.9- 57.3g/100g reported for some species of cucurbitaceae (Oshodi 1992; Ige *et al;* 1984; Fagbemi and Oshodi 1991; and Fokou *et al*; 2004 respectively). There was reduction in the value obtained for the protein isolate.

30.44% was recorded for the crude protein in the seed flour. This value is lesser than 49.8% protein in soya beans reported by Osundahunsi and Aworh (2003). Higher protein content was recorded in the protein isolate, 94.10 %. The value was higher than those reported for similar legumes; mung beans isolate, 87.9% (Rahman *et al*, 2000), Chickpea isolate 88.1% (Sanchez *et al*, 1999), Canarvalia einsformis, 73.3%, (Chel-guerrero *et al*, 2002). The variations in protein contents of the different legume protein isolate are attributed to genetic make-up of legumes along with some environmental factors (Kaur, 2007). The high protein content obtained in the isolate suggests that it may be a better protein supplement than the seed flour and also contribute significantly to alleviating the problem of protein malnutrition in the third world and developing countries.

The total ash content of the seed flour, 3.33%, is comparable with some reported works (Oshodi 1992; Fokou *et al*, 2004; Aremu *et al*, 2006). The value is in excellent agreement with an acceptable total range values for legumes which are between 2.4 -5.0% (FAO, 1989). There was significant reduction in the ash content of the isolates.

The crude fibre content of the raw seed and protein isolate are generally low. The values obtained are much higher than that of green peas (0.5- 0.93%). The results are in agreement with those reported for mung peas and field pea (Naezk *et al*, 1986; Summer *et al*, 1981).

The mineral composition of the raw seed flours and protein isolate are presented in table 2. The levels of phosphorus, sodium, potassium, magnesium and calcium were relatively high in the raw seed flour than the isolate. The values of iron, zinc and copper were low while chromium, nickel, lead and cadmium were not detected.

Though isolation decreased the values of these mineral elements, the potassium and phosphorus is still high. The high calcium content has been reported to reduce blood pressure (Ranhotra *et al;* 1998). Calcium is important to bone and teeth formation, blood clothing and in muscle contraction. Magnesium in the blood as an activator of many enzyme systems and maintains the electrical potential for nerves. Iron is important to blood formation and it is especially needed by pregnant and lactating mothers. Manganese is an antioxidant nutrient that is important in the breakdown of amino acids and the production of energy. The more important minerals involved

in the building of rigid structures to support the body i.e. Ca, P and Mg were more abundant in the raw seed flour than the isolates. There may be need for fortifications of the isolates before use.

The antinutritional composition of the raw seed and protein isolates are shown in tables 3. The antinutrients were higher in the raw seed flour but on isolation they were significantly reduced. This may be due to the procedures involved in isolation. Phytic acid might have been lost during the process of isolation from the protein since it is soluble in water. It has been reported that processing methods reduces the phytic acid of seeds to the minimum levels (Enujiugha and Agbede, 2000). The concentration of tannin in this work is within the range obtained for chickpea seeds, 0.07 % – 0.22 % (Sharma *et al*., 2013). Polyphenols and tannin are known to inhibit digestive enzymes, and also reduce absorption of vitamins like B12 (Liener, 1989). They also form complexes about Ca, Zn, Mg and Fe thereby reducing protein and mineral bioavailability. Though tannin-protein complexes are insoluble in water and thus decreases protein digestibility (Carnovale *et al*, 1987), the low concentration of tannin observed in this work will have no nutritional significance.

The values of alkaloid, flavonoid, saponin and oxalate obtained are very low, therefore no nutritional discomfort are expected. The low levels of the antinutrients reported on the isolates are desirable from the functional and nutritional viewpoint and in the preparation of high quality food products.

The percentage protein content of the protein isolates was found to be 94.10%. This showed that the protein extracted is an isolate and not a concentrate. The results of the chemical properties of four yoghurt samples produced are presented in table 5. The pH of the samples range from 5.29 to 6.19, addition of isolate reduced the pH of the yoghurt. This result is relatively high compared to the result of Rodrigues *et al* (2010) who concluded that pH of yoghurt is 4.30 to 5.08. The increase in pH of yoghurt may lead to short time of preservative of the yoghurt. The titratable acidity of the four yoghurt samples ranges from 0.89 to 1.0. The addition of the isolate increased the titratable acidity of the yoghurt. This result is in agreement with Younus *et al*, (2002) who analysed the Quality evaluation of market yoghurt/ dahi and recorded 0.89 and 1.13 titratable acidity. The percentage of the total solid ranges from 7.6 to 16.6 and the addition of the isolate increases the total solid of the yoghurt. This content agrees with the findings of Muhammed *et al*, (2005) who reported a higher total solid of 17.11%. However, Weaver (1993) reported that low percentage of total solids in yoghurt can lead to malfunction of the starter culture.

The panelist evaluation indicated that there was no significant difference between the yoghurt sample of up to 25% level of substitution and the yoghurt produced for 100% milk (0% fat) in all parameters evaluated. This shows that at higher level of substitution (above 25%) the yoghurt produced gets more undesirable to the panelist.

## 5.0 CONCLUSION

The result of chemical composition of the seed flours and protein isolates revealed that they are good sources of proteins and carbohydrate. It also revealed the possibility of substituting the conventional milk with protein isolates from A. breviflorus in the production of yoghurt up to 20% level of substitution without affecting the sensory qualities.

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