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*Full Length Research Paper*

# Effects of fermentation and germination on the physicochemical properties of *Mucuna cochinchinensis* protein isolate

**Udensi, E. A.* and Okoronkwo, K. A**.

Department of Food Science and Technology, Abia State University, Uturu, Nigeria.

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**The proximate composition and functional properties of fermentated and germinated *Mucuna cochinchinensis* protein isolates were determined. *Mucuna* bean protein extracted at pH 8 and pH 10 had protein contents ranging from 89.6 to 90.1% for the fermented *Mucuna* bean flour while the germinated bean ranged from 88.1 to 89.8%. There were significant (P<0.05) differences in the results of some of the functional properties of the *Mucuna* bean isolates at different fermenting and germinating periods. However, oil and water absorption, emulsion capacity, bulk density, foaming capacity and gelation capacity compared favourably with soy and winged bean isolates. Thus *Mucuna* bean isolate with its high protein content and good functionality has a good potential for application in food systems**.

**Key words:** Fermentation, germination, physicochemical properties, *Mucuna*, protein isolate.

## INTRODUCTION

Velvet bean (*Mucuna cochinchinensis*) is one of the lesser known legumes and is yet to be utilized as a plant protein source in Nigeria. Udensi et al. (2001) and Ukachukwu and Obioha (1997) observed that the seeds are rich in protein, low in oil and contain a fair amount of crude fibre and mineral matter. However, the lack of knowledge of the nutritional qualities of lesser-known legumes grown in developing countries like Nigeria is responsible for the poor utilization of these traditional crops in different food formulations. Proteins not only provide nutritional components in foods but also perform other functions. Their functional properties determine their characteristics in food systems. In order to produce a high protein food, the protein in a legume may have to be concentrated and isolated from the legume.

Protein isolation and fractionation is aimed at separating the protein from other components in such a form that they remain (as much as possible) fully undenatured and thus retain their functionality (Zadow, 1993). Plant protein isolates are the most refined forms of proteins. When compared with other conventional legumes such as soybean, the functional properties of the *M. cochinchinensis* bean protein will largely determine its acceptability and application as an ingredient in food systems. Bean protein isolates are incorporated in food systems where heat treatment will be involved in order to eliminate or reduce significantly the antinutrients contained in them. Ukachukwu and Obioha (1999) has shown *M. cochinchinensis* to be a high protein source whose antinutrient factors can be brought to safe levels by traditional processing methods such as boiling and roasting.

The purpose of this study was to evaluate the effect of fermentation and germination on the physico-chemical properties of the protein isolate of *M. cochinchinensis.*

## MATERIALS AND METHODS

### Preparation of velvet bean seeds

Velvet bean seeds (*M. cochichinensis* ) were provided by the National Root Crop Research Institute (NRCRI) Umudike, Abia State, Nigeria. The mature, dry *Mucuna* beans were sorted to remove infested seeds. The whole seeds were cracked and winnowed to separate the seed coat. The beans were divided into

*Corresponding author. E-mail : emeobika@yahoo.com.

**Table 1.** Proximate composition of fermented and germinated *Mucuna* been protein isolate flour samples.

| Fermentation time (h) | Protein (% N x 6.25) | Crude fat | Crude fibre | Ash | Carbohydrate |
|---|---|---|---|---|---|
| 0 | 87.5$^{a}$ | 0.20$^{a}$ | 1.37$^{b}$ | 2.05$^{a}$ | 4.27$^{a}$ |
| 24 | 89.6$^{a}$ | 0.20$^{a}$ | 1.67$^{a}$ | 2.50$^{a}$ | 0.82$^{c}$ |
| 48 | 90.0$^{a}$ | 0.20$^{a}$ | 1.60$^{a}$ | 2.40$^{a}$ | 0.59$^{b}$ |
| 72 | 90.1$^{a}$ | 0.20$^{a}$ | 1.73$^{a}$ | 2.60$^{a}$ | 0.04$^{e}$ |
| **Germination time (h)** | | | | | |
| 0 | 87.5$^{a}$ | 0.20$^{a}$ | 1.37$^{b}$ | 2.05$^{c}$ | 4.27$^{a}$ |
| 24 | 88.1$^{a}$ | 0.30$^{a}$ | 1.53$^{a}$ | 2.30$^{a}$ | 3.07$^{b}$ |
| 48 | 89.2$^{a}$ | 0.20$^{a}$ | 1.43$^{a}$ | 2.15$^{b}$ | 0.00$^{c}$ |
| 72 | 89.9$^{a}$ | 0.20$^{a}$ | 0.73$^{a}$ | 1.10$^{d}$ | 0.00$^{c}$ |

Means with the same superscript in the same column indicates no significant difference (P<0.05).

three parts, with two portions (500 g each) subjected to fermentation and germination while the other was used as control.

**Fermentation of the velvet bean seeds**

The beans were soaked in water in a 1:6 (bean : water) ratio for 12 h, drained soaked grains were fermented naturally. This means that the beans were allowed to ferment without the addition of yeast. The fermentation periods were O, 24, 48 and 72 h.

**Germination**

The beans for germination were prepared to undergo germination separately for 0, 24, 48 and 72 h, by initially soaking the beans in water for 24 h and then spreading them on a damp cloth for the number of hours required. The *Mucuna* beans were germinated at room temperature (23°C)

**Preparation of flour samples**

Flours from both fermented and germinated beans were produced by drying the bean seeds in a hot air oven at 60°C to about 10 percent moisture content. The dried beans were milled using locally fabricated attrition mill to obtain sieve size of 0.5µm mesh size. The raw *Mucuna* beans (control) were equally milled to produce flour of the same sieve size (0.5µm)

**Protein isolation**

The method of Okaka (1997) was adopted. Exactly 2 g of the *Mucuna* flour sample was solubilized in 20 ml of distilled water in 100 ml beaker. It was then stirred intermittently to facilitate protein extraction. The extraction was carried out for 30 min followed by centrifugation at 10,000 x g for 10 min to allow for separation. The supernatant was then decanted and used. The supernatant was adjusted to pH 4, 6, 8 and 10 using dilute HCl (2 M) or NaOH (2 M) as appropriate. The HCl acidified the supernatant and consequently the protein precipitated out. The pH range (pH 8 and 10) at which there was maximum protein isolation was used for bulk extraction.

The resulting protein curd was separated by filteration with filter paper and washed three times using distilled water. Later the slurry was scroped out and placed in a low temperature dryer for 3 h. After drying, it was ground into fine powder using ceramic mortar and pistle.

**Proximate analysis**

Determination of the proximate composition of the samples was carried out by AOAC (1980) standard procedure.

**Functional properties**

The method of Abey and Ibeh (1988) was used for the determination of water and oil absorption capacity. Foaming capacity and gelation property were determined by the method described by Coffman and Garcia (1977), while emulsifying capacity was carried out by the method of Okerie and Bello (1988). The method of Okaka and Potter (1979) was used to determine the bulk density while wettability was obtained by the method described by Armstrong et al. (1979).

**Statistical analysis**

The data were statistically analysed using Analysis of variance (ANOVA) and Turkey's Test was used to separate the means.

## RESULTS AND DISCUSSIONS

### Proximate analysis

The proximate compositions of the fermented and germinated *M. cochinchinneses* protein isolate flours are shown in Table 1. Increase in both fermentation and germination time slightly increased the protein content of the *Mucuna* bean isolate flours. However, there was no significant difference (P>0.05) between the protein content of the different fermentation and germinated *Mucuna* beans isolate flours. The results recorded in

**Table 2.** Functional properties of fermented and germinated *Mucuna* bean isolate flour samples.

| Fermentation time (h) | WAC (g/g) | OAC (g/g) | Bulk density (g/cm$^3$) | Emulsion capacity (mg/g) | Wettability (s) |
|---|---|---|---|---|---|
| 0 | 6.00$^{b}$ | 2.20$^{a}$ | 0.50$^{a}$ | 12.50$^{b}$ | 10.00$^{c}$ |
| 24 | 6.40$^{a}$ | 1.76$^{b}$ | 0.50$^{a}$ | 12.20$^{b}$ | 18.80$^{b}$ |
| 48 | 5.20$^{c}$ | 1.76$^{b}$ | 0.50$^{a}$ | 11.90$^{b}$ | 19.00$^{b}$ |
| 72 | 4.90$^{cd}$ | 0.88$^{c}$ | 0.50$^{a}$ | 17.40$^{a}$ | 22.00$^{a}$ |
| **Germination (h)** | | | | | |
| 0 | 6.00$^{c}$ | 2.20$^{b}$ | 0.56$^{a}$ | 12.50$^{b}$ | 10.00$^{c}$ |
| 24 | 7.00$^{b}$ | 1.76$^{d}$ | 0.50$^{a}$ | 11.40$^{c}$ | 35.00$^{a}$ |
| 48 | 8.10$^{a}$ | 2.64$^{a}$ | 0.50$^{a}$ | 16.70$^{a}$ | 30.00$^{b}$ |
| 78 | 7.80$^{a}$ | 1.94$^{c}$ | 0.50$^{a}$ | 16.70$^{a}$ | 34.00$^{a}$ |

Means with the same superscripts in the same column indicated no significant difference (P<0.05).

(Table 1) are similar to the results of Okorie and Bellow (1988) which showed that winged bean isolate and soy isolate had a protein content of 91.10 and 96.50%, respectively. Protein isolate flours from both treatments (fermentation and germination) had low fat content ranging from 0.20 – 0.30%. Both isolates were almost fat free and therefore if introduced in a food system will not encourage rancidity.

The ash content of both fermented and germinated isolates ranged from 1.10 – 2.60%. These values are lower than the ash content of winged bean isolate (5.5%) and soy isolate (3.4%) (Okerie and Bello, 1988). The crude fibre content of the protein isolates are higher compared to values obtained for winged bean isolate (0.00%) and soy isolated (0.00%). The presence of the crude fibre in the protein isolates of *Mucuna* beans is very important nutritionally.

### Water absorption

Fermentation significantly (P>0.08) decreased the water absorption capacity (WAC) of *Mucuna* bean protein isolate while germination significantly (P<0.05) increased it. The WAC values (Table 2) of the protein isolates are higher than the values obtained for winged bean isolate and soy isolate (5.00 and 4.10 g/g, respectively) (Okerie and Bello (1988). Water binding capacity is a useful indication of whether flour or isolates can be incorporated into aqueous food formulations especially those involving dough handling (Okorie and Bello, 1988; Giami, 1993). The higher water absorption capacity results obtained suggest that *Mucuna* bean protein isolate flours could be useful in food systems such as bakery products which require hydration to improve handling characteristics.

### Oil absorption

As presented in Table 2, fermentation slightly decreased oil absorption capacity of the *Mucuna* bean protein isolates while oil absorption for the germinated *Mucuna* bean protein isolates remained fairly constant. The values obtained for winged bean isolate (9.65 g/g) and soy isolate (4.88 g/g) are much higher than those of *Mucuna* bean isolates. Oil binding capacity may determine whether the protein material will perform well as meat extenders or analogs.

### Bulk density

Data for bulk density are presented in Table 2. There were no significant differences (P<0.05) in the bulk density of *Mucuna* bean isolates from both fermented and germinated protein isolates. Okorie and Bello (1988) observed similar values of bulk density (0.48 g/cm$^3$) for soy isolate. Bulk density gives an indication of the relative volume of packaging material required. Generally, higher bulk density is desirable for the greater ease of dispersibility and reduction of paste thickness which is an important factor in convalescent child feeding (Padmashree et al., 1987).

### Foam capacity/ foam stability

The results of foam capacities are presented in Table 3. Both fermentation and germination significantly (P<0.05) increased the foaming capacity of *Mucuna* isolate. However, the results obtained for *Mucuna* protein isolates are lower than that obtained for winged bean isolate and soy isolate (Okorie and Bello, 1988). As shown in Table 3, foams from the *Mucuna* isolate are significantly stable after 2 h interval. Foam stability is important since the usefulness of whipping agents depend on their ability to maintain the whip as long as possible (Lin et al., 1974). Potential *Mucuna* based foods in which foams are important may include moi-moi and akara balls.

**Table 3.** Foam capacity and stability of fermented and germinated *Mucuna* bean isolate samples.

| Fermentation time (h) | Initial Vol after whipping | % volume increase | Volume of foam after deferent time 1hr, 1 ½hr, 2hr | | |
|---|---|---|---|---|---|
| | | | 1 hr | 1 ½ hr | 2 hr |
| 0 | $110^{d}$ | $10^{c}$ | 105 | 100 | 100 |
| 24 | $115^{c}$ | $15^{b}$ | 105 | 101 | 101 |
| 78 | $126^{b}$ | $26^{a}$ | 115 | 110 | 110 |
| 72 | $143^{a}$ | $43^{d}$ | 125 | 120 | 120 |
| **Germination time (h)** | | | | | |
| 0 | $110^{c}$ | $10^{c}$ | 105 | 100 | 100 |
| 24 | $148^{b}$ | $48^{a}$ | 133 | 125 | 125 |
| 48 | $153^{a}$ | $53^{b}$ | 143 | 125 | 125 |
| 72 | $155^{a}$ | $55^{b}$ | 140 | 130 | 130 |

Means with the same superscript in the same column indicates no significant difference (P<0.05).

**Table 4.** Least gelation concentration of fermented and germinated *Mucuna* bean protein isolate samples.

| Conc (%, w/v) | Fermentation time (h) | | | | Germination time (r) | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 24 | 48 | 72 | 0 | 24 | 48 | 72 |
| 2 | - | - | - | - | - | - | - | - |
| 4 | - | - | - | - | - | - | - | - |
| 6 | - | - | - | - | - | - | - | - |
| 8 | - | - | - | - | - | - | - | - |
| 10 | - | - | - | - | - | - | - | - |
| 12 | - | - | - | - | - | - | - | - |
| 14 | - | - | - | - | - | - | - | - |
| 16 | Gel | Gel | Gel | Gel | Gel | Gel | Gel | Gel |
| 18 | Gel | Gel | Gel | Gel | Gel | Gel | Gel | Gel |
| 20 | Gel | Gel | Gel | Gel | Gel | Gel | Gel | Gel |

## Emulsifying capacity

Table 2 shows the results of the emulsifying capacity for both fermented and germinated *Mucuna* protein isolates. Fermentation for 72 h significantly (P<0.05) increased the emulsified capacity of the *Mucuna* isolates while germinating for 48 and 72 h also significantly (P<0.05) increased the emulsifying capacity of the isolate. The value (12.5 mg/g) obtained for the *Mucuna* isolate is similar to that (12.90%) reported for winged bean isolate by Okerie and Bello (1988) but higher than that obtained for soy isolate (8.00%). The high emulsion capacity of *Mucuna* bean protein isolates makes them a potentially useful ingredient in preparing comminuted meat products and analogs.

## Wettability

Increase in fermentation and germination significantly (P<.05) decreased the wettability of the *Mucuna* bean protein. However, the period of time required to reach complete wetting was longer for germinated *Mucuna* protein isolate than fermented *Mucuna* isolate. Generally, the determination of wettability will provide a useful indication of the degree to which the dried *Mucuna* protein isolate flour is likely to posses instant characteristics.

## Gelation

Data for gelation capacity is presented in Table 4. The gelation capacities of both fermented and germinated

*Mucuna* protein isolates are not significantly (P<0.05) different irrespective of the fermentation and germination periods. The values (16- 20%) obtained for *Mucuna* protein isolates are similar to those reported for winged bean isolate and soy isolate (14-20%).

Gelation is an important property which influences the texture of various kinds of foods such as moi-moi, agidi and soup (Udensi et al., 2001).

## CONCLUSION

The high protein isolate coupled with good functional properties makes it a possible good quality protein source in food applications. It is hoped that the results of this investigation will help to increase the awareness on the uses of *Mucuna* in food systems. Such increased use of *Mucuna* could lead to a significant alleviation of the nutritional problems associated with developing countries where it is cultivated.

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