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Protein Isolation and Proximate Evaluation of Sweet and Bitter Cassava Leaves (Manihot esculenta crantz)

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Abstract
The need for an alternative source of protein has become pertinent due to the economic situation of developing countries. It has however been revealed by various studies that plant leaves are a good source of protein. Having realized that cassava is a plant that is readily available in tropical Africa, therefore, this project is centered on cassava leaves with the aim of preparing protein isolates and ultimately evaluate the proximate composition of the cassava leaf meal and its protein isolates. Both sweet and bitter cassava leaves were identified and obtained IITA Ibadan, Nigeria, the leaf meal was prepared and the protein isolate was subsequently obtained. Proximate composition analysis was carried out on both the leaf meals and protein isolates according to standard AOAC (2000). Protein digestibility was also carried out on the protein isolates. Sweet Cassava Leaf Protein Isolates possess 87.59% protein digestibility compared to Bitter Cassava Leaf Protein Isolates with protein digestibility of 88.73%. The moisture content was higher in Sweet Cassava Leaf Meal, 78.22% than that of Bitter Cassava Leaf Meal, 77.58%. Crude fibre content in Sweet Cassava Leaf Meal is 10.28% against 9.86% expressed by Bitter Cassava Leaf Meal. The Ash content found in Sweet Cassava Leaf Meal is 7.88% compared to 7.61% present in Bitter Cassava Leaf Meal. Furthermore, Sweet Cassava Leaf Meal contains 3.01% fat compared to 3.00% fat obtained in Bitter Cassava Leaf Meal obtained in Bitter Cassava Leaf Meal. The carbohydrate content is higher in Sweet Cassava Leaf Meal with 46.25% than Bitter Cassava Leaf Meal which contains 44.24%. Sweet Cassava Leaf Meal contains 39.59% protein while Bitter Cassava Leaf Meal contains 39.33% protein. Analysis carried out on the protein isolate revealed that Sweet Cassava Leaf Protein Isolate is higher (57.35%) than that of Bitter Cassava Leaf Protein Isolate (56.49%). The result obtained from this study revealed that cassava leaf is a good source of protein and their chemical supports the probability that they could find their use in food formulation and food supplementation.
Keywords: Sweet and bitter cassava, protein isolates, proximate composition

1. Introduction

In developing countries, the supply of animal proteins for use as food and food ingredients is inadequate, which has necessitated the quest for cheap and abundant sources of protein with desirable functional and nutritional properties (Gbadamosi *et al*, 2000). In recent years, many plants have attracted a great deal of interest as sources of low-cost proteins to human diets. Among the various sources of vegetable proteins considered as food ingredients are peanuts and soya beans. Several authors have demonstrated the potential application of oil seed proteins such as peanut protein concentrate (Wu *et al*, 2009), mustard proteins (Alireza & Bhagya, 2009) and lesser known leguminous seed fractions (Ogunwolu *et al*, 2009) for product formulation and food fortification, particularly for developing countries (Wu *et al*, 2009).

The green leafy vegetable has long been recognized (Byers, 2003; Oke, 2003) as the cheapest and most abundant potential source of protein because of its ability to synthesize amino acids from a wide range of virtually unlimited and readily available primary materials such as sunlight, water, carbon dioxide and atmospheric nitrogen. For example, cassava leaves, a byproduct of cassava root harvest are (depending on the varieties) rich in protein (14-40 % dry matter), minerals, vitamins B_1, B_2 and carotene (Adewusi and Bradbury, 2007; Aletor and Adeogun, 2004).

Studies have showed that the process of photosynthesis is the only non depletable protein source and can supply some essential amino acids as well as provide adequate nitrogen in the diet for synthesis of nonessential amino acids (Kinsella, 2000; Staman, 2002). Available literature clearly indicate that apart from lower methionine content, the amino acid profile from most leaf species compare favorably with those of soya bean, meat, fish and egg and generally, surpass FAO essential amino acid pattern (Eggum, 1999).

Leaf vegetable protein is about half the vegetable protein content in the human diet and probably contributes more to the world protein, although less attention is given to it. Two factors limit the nutritional value of plant leaves to monogastric animals: The high fiber content and the indigestibility of cellulose (Kinsella, 2000). Normally animals assimilate the plant protein and they are in turn consumed by human and through this food chain man avoid the cellulose. However, this food chain system is very inefficient in some developing countries since most people do not eat enough animal protein for economic reasons. Moreover, only 8-20% of the plant protein consumed by animals is recoverable as protein for human nutrition (Parrish *et al*, 1997). Thus, more efficient ways of utilizing plant protein must be found.

Manihot esculenta (cassava) called yucca or manioc in the South-East and South-Western parts of Nigeria, respectively. The leaf belongs to the family Euphorbiaceae (Ugochukwu and Babady, 2002) cassava leaves are used by the people of Southern Nigeria for treatment of some ailments and as food. Some communities such as the Efik and Ibibio have for several generations used this plant for medicinal and nutritional purposes, and the leaves of this plant are used for the treatment of hypertension, headache and other form of pain. (Atangwho *et al*., 2009).

Manihot esculenta crantz is one of the most popular green leafy vegetable in Nigeria and is gaining equal popularity as a delicious food leaf in other African countries such as Cameroon, Gabon, Congo and Angola (Eyo & Abel, 2003).. The mineral element content, amino acid content and proximate composition of the leaves has been reported by (Eyo & Abel, 2003).

Thus to increase their utilization due to the increased utilization of conventional sources of proteins coupled with rapid population growth which has prompted research efforts into finding alternative sources of proteins that are cheap and abundantly available, there is need to process the whole leaves into a high protein product such as protein isolate and then examine the suitability of this isolate as functional ingredients in food formulations and as food supplements. However, the ultimate success of utilizing any plant protein as food ingredients depends largely on its physico-chemical and functional properties. The present study therefore is aimed at isolating and evaluating the proximate composition of the protein isolates in *Manihot esculenta crantz*

2. METHODOLOGY

Collection and Preparation of Raw Material

The two leafy vegetables were harvested fresh from a farm site located in Ede and Ife, towns in Osun state, the South western part of Nigeria. Each vegetable was spread out on a table and inspected for presence of variegated, dried and extraneous materials such as dirt and insect larva. After removing the unwanted ones, the leaves were shredded with a chopping knife and oven dried at 50°C. The dried leaves were comminuted using a grinder to obtain vegetable meal. The leaf meal was sieved through 60-80 mesh screens to obtain uniform particle sizes.

Preparation of protein isolate from leaf meal

A modified method of Gbadamosi *et al,* (2000) was adopted for the preparation of protein isolates from *Manihot esculenta* leaves(sweet and bitter) .

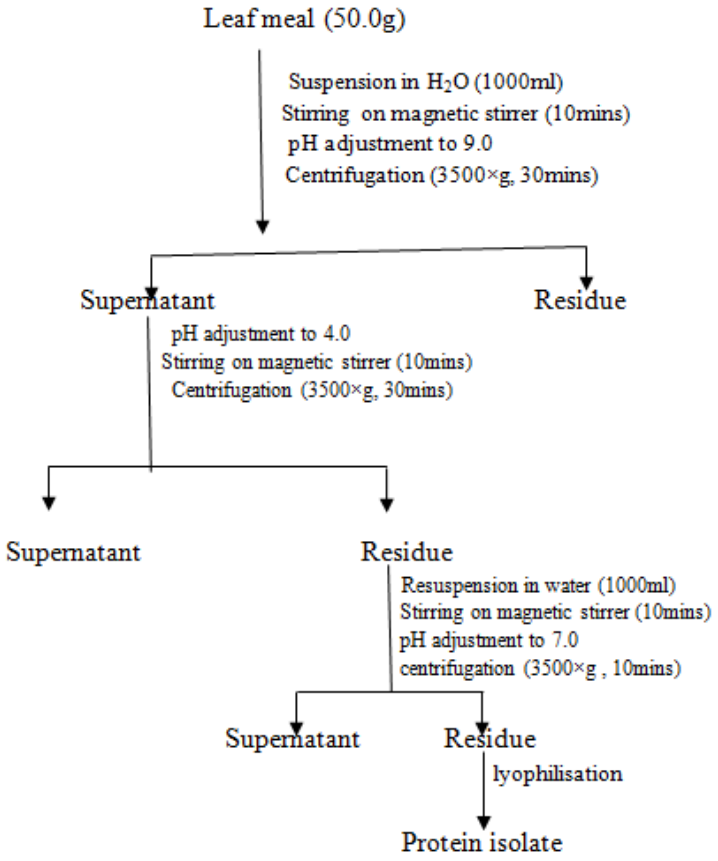

Fig 1 : Scheme for the production of leaf protein isolate. (Gbadamosi, 2000)

Proximate composition

Proximate composition of the leaf meal and the protein isolate was determined according to standard recommended by AOAC (2000).

3. Results and Discussion

Isolation and proximate evaluation of *Manihot esculenta (sweet and bitter)* Protein isolates.

***In-vitro* protein digestibility**

The results obtained for *in-vitro* protein digestibility was 87.59% for sweet cassava leaf protein isolate and 88.73% for bitter cassava leaf protein isolate. The *in-vitro* protein digestibility compares favorably with the range of values (86.3-93.9%) for lupin protein isolates (Lqari *et* al., 2002). The results showed that the proteins of both leaves are susceptible to decomposition by the enzymes of the digestive systems.

It has been reported that the presence of trypsin and chymotrypsin inhibitors and the global structure of protein lower digestible enzymes activity. Thus, the removal of protease inhibitors during the extraction process increases the invitro protein digestibility observed in the protein isolates (Richardson, 1991). In addition leaf proteins are denatured during isolation, rendering the protein isolates more accessible to digestive enzymes and improve the hydrolysis (Lynch *et* al., 1977).

Proximate composition

Table 1 depicts the proximate composition of both sweet and bitter cassava leaf. The moisture content was higher in sweet cassava leaf (78.22% ±0.02) than bitter cassava leaf (77.58% ±0.29). Protein was high with values of 39.59 ± 1.1 g/100 g (sweet cassava leaf) and 38.88 ± 1.2 g/100 g (bitter cassava leaf). The two leaves are good sources of protein. Their protein content is quite high and compares favorably values reported for chickpea (24.0%), cowpea (24.7%), lentil (26.1%), green pea (24.9%), bitter leaf (35.1%) and fluted pumpkin leaves (22.4%) (Glew *et al*., 1997; Akwaowo *et al*., 2000.

Other proximate parameters were (g/100 g) and; crude fat, 3.01 ± 0.28 and 3 ± 0.27; ash, 7.88 ±0.18 and 7.61 ± 0.17; crude fibre, 10.28 ± 0.15, 9.86 ± 0.08; carbohydrate, 46.25 ± 0.4 and 43.76 ± 2.4 in in both sweet and bitter cassava leaf respectively which compared with the results obtained by Aletor (1999) for crude fat, 5.6 ± 0.31 and 11.9 ± 0.16; ash, 22.3 ± 0.06 and 11.4 ± 0.43; crude fiber, 1.1 ± 0.35 and 1.8 ± 0.24; carbohydrate, 31.4 and 38.3 in *Amaranthus hybridus* and *Telferia occidentalis* respectively.

The proximate compositions of both protein isolates suggest that they may find use in food/ feed formulation/ supplementation operations. This would be particularly so where protein content is of prime importance.

4. Conclusion

Isolation and proximate evaluation of *Manihot esculenta crantz* (sweet and bitter) protein isolates have been the study in this work. This study reveals that cassava leaf is a good source of protein. The chemical composition of both leaves suggest that they may find their use in food/ feed formulation/ supplementation operations. This would particularly be so where protein content is of prime importance. Most functional properties of the protein isolates obtained from both leaves were comparable with that of Bambara nut, lentil seed, soybean and winged bean protein isolates which were already being used as the functional ingredients in many food products.

The results of this study therefore revealed that protein isolates of suitable proximate composition could be produced from the leaves of *Manihot esculenta crantz* (sweet and bitter).

Further studies could still be conducted on the fortification of cereal based foods, infants' formula with *Manihot esculenta crantz* protein isolate as a protein supplement and food formulation as animal protein replacer or extender which could be possibly incorporated in beverages or soup. Also further research is needed to relate potential functionality of *Manihot esculenta crantz* protein isolate to performance in specific food systems including sensory evaluation to determine acceptability of the new product.

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Table 1: Proximate Composition (%) of *Manihot esculenta crantz* (sweet and bitter)

Food component	SCLM	BCLM	SCLPI	BCLPI
Moisture Content	78.22± 0.02	77.58 ±0.29	---	---
Crude Fibre	10.28 ±0.15	9.86 ± 0.08	---	---
Ash	7.88± 0.18	7.61± 0.17	---	---
Fat	3.01 ± 0.28	3.00 ± 0.27	---	---
Protein	39.59	39.33	57.35	56.49
Carbohydrate	46.25	44.24	---	---

SCLM: sweet cassava leaf, **BCLM**: bitter cassava leaf meal,
SCLPI: sweet cassava leave protein isolate, **BCLPI**: bitter cassava leaf protein isolate.

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