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Comparative Evaluation of the Composition, Digestibility and Functionality of Chemically Modified Protein Isolates from Soya bean and some Under-Utilised Legumes

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Abstract
Native proteins, particularly those from indigenous under-utilised legumes have limited application especially in industrial food systems largely because of information dearth on their quality and functionality. Because of the rising cost of soya bean, there is renewed interest in evaluating the potentials of alternatives. Consequently, two (2) under-utilised legumes, namely: Pigeon pea (PP) (*Cajanus cajan*) and African yam bean (AYB) (*Sphenosstylis sternocarpa*) were processed into their protein isolates using alkaline (NaOH) solubilisation and acid (HCl) precipitation at their various isoelectric pH. The protein isolates were modified using acetic anhydride. The protein isolates and their chemically modified forms were thereafter analysed with respect to their proximate composition, metabolisable energy (ME),invitro multi-enzyme protein digestibility(IVPD) and functional properties. The findings were compared with the more conventional soya bean protein isolate (SI). On the average, the PP and AYB had 92.54 and 90.13g/100, SI had 85.8g/100g crude protein while the modified under-utilised had 92.4 and 90.5g/100g, respectively. Ash was higher in protein isolate of PP (3.6g/100g), AYB (3.3g/100g) and low in SI (1.0g/100g) while the modified form had 1.9 and 1.8g/100g respectively. However, ME was highest in the Soya isolate (512.4Kcal/100g) than those of PP and AYB which ranged between 369.01 and 380.07 Kcal/100g. The protein isolates of PP and AYB were more digestible and ranged between 91.0 and 98.8% when compared to 85.9% in Soya bean. The Soya bean isolate had better water holding capacity (WHC), oil holding capacity (OHC) and foaming stability than the PP and AYB Isolates. The foaming capacity, emulsion capacity and emulsion stability of the modified PP and AYB protein isolates were generally higher than those of soya bean isolates. Modification generally improved protein functionality when compared with the unmodified isolates. Given the higher *in vitro* protein digestibility and other functional attributes of pigeon pea and African Yam Bean than Soya isolates, it was concluded that these under-utilised legumes seeds could serve as useful alternatives for the much more expensive soya bean.

Keywords: Chemical modification, protein isolates, soya bean, underutilised legume

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Introduction
Legumes are inexpensive important sources of protein with high nutritional profile after cereal. They are also good sources of complex carbohydrates, dietary fibre and contain significant amount of vitamins and minerals .However, native protein have limited functionality and this has necessitated the need for the development of processes to improve plant protein functionality. Recently, protein isolates are being used to fortify all types of pasta products such as spaghetti and other carbohydrate based meals in order to improve nutritional value and functional properties. Modification of proteins improve the palatability, storage and functionality of the available protein resources . While some convectional legumes such as soya bean and cowpea have well detailed information as regards their protein isolation and modification (Lawal and Adebowale,2004), indigenous legumes such as the pigeon pea (*Cajamus*

cajan) and African yam bean (*Sphenosstylis sternocarpa*) had little information. Therefore the present study investigates the protein isolation and chemical modification of the isolates of two unconventional legumes and characterised them with respect to proximate composition, metabolizable energy, *invitro* multienzyme protein digestibility and functional properties.

Material and Methods

Proximate analysis and functional properties were determined using the standard method of (AOAC,2006).IVPD was done using the method of (Hsu et al., 1977), extraction was by the method of (Lawal and Adebowale,2004) and acylation of protein was carried out as described by (Lawal and Adebowale,2004).

Defatted legume + water (1:20)
↓
pH adjument (9.5)
↓
Centrifugation
↓
Super natant
↓
pH adjustment
↓
Centrifugation
↓
Precipitate (protein isolate)
↓
dialyzation
↓
Freeze dry
↓
Storage

Fig 1: Flow chart for protein isolate extraction

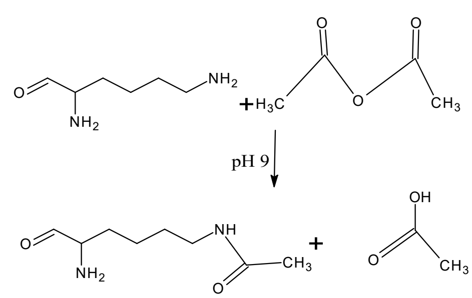

Fig. 2: acetylation of amino acid L-lysine

Results and Discussion

The results on proximate composition of protein isolate and modified isolate Tables 1and 2 show that the moisture content of the isolates SI, PP& AYB were lower 4.0, 2.69 and 4.2g/100g respectively than the modified (5.0 and 5.7g/100g) respectively, which indicate better shelf life in the isolate compared to modified. The crude fat of the isolated SI, PP and AYB were 0.6, 1.0 and 0.7g/100g, while the modified PP and AYB were 0.5 and 0.6g/100g respectively. The fat was low in the isolate and further reduced in the modified. The protein contents of SI, PP and AYB isolated were 85.8 92.54 and 93.13g/100g while modified PP and AYB had 92.4 and 90.5g/100g respectively. Only slight increase was observed in the modified

Table 1: Proximate Composition (g/100g),Energy and *invitro* multienzyme protein digestibility (IVPD) of some protein isolates

	(SI)	(PP)	(AYB)
MC	**4.0±2.0**	**2.7 ± 0.06**	**4.2 ± 0.05**
Fat	**0.6±0.4**	**1.0 ± 0.01**	**0.7 ± 0.02**
CP	**85.8 ±0.3**	**92.5 ± 0.01**	**90.1 ± 0.45**
Ash	**1.0 ± 0.11**	**3.6 ± 0.06**	**3.3 ± 0.08**
CF	**0.2 ±0.4**	**0.2 ± 0.01**	**1.7 ± 0.38**
E(KCal/100g)	**512.4**	**380.07**	**369.09**
IVPD(%)	**79.9±0.13**	**94.3 ± 0.01**	**98.8 ± 0.02**

Table 2: Proximate Composition (g/100g), Energy and *invitro* multienzyme protein digestibility (IVPD) of modified protein isolate

	(PP)	**(AYB)**
Moisture	5.0 ± 0.05	5.7 ± 0.35
Fat	0.5 ± 0.02	0.6 ± 0.01
Crude protein	92.4 +0.04	90.5 ± 0.25
Ash	1.9 ± 0.05	1.8 ± 0.20
Crude fibre	02 ± 0.01	1.3 ± 0.01
E(KCal/100g)	375.28	369.83
IVPD(%	91.0 ± 0.02	91.0 ± 0.20

The crude fibre CF of the isolates SI, PP & AYB (1.0,0.21 and 1.67g/100g) and modified PP &AYB (0.2 and 1.3g/100g) respectively were generally low. The metabolizable energy of the isolates and the modified isolates PP &AYB ranged 369.09-380.07KCal/100g indicating they are good sources of energy, though lower than 512.4Kcal/100g recorded for SI. The IVPD of the isolates PP & AYB (94.3&98.8%) respectively, showed higher digestibility when compared to SI (79.9%) and 91% in modified PP & AYB. The ability of the protein to imbibe water and retain it against a gravitational force with a protein matrix is the water holding capacity (WHC) Tables 3& 4. The WHC for PP and AYB isolates were 3.1&2.6% respectively and improved slightly by the modification 3.2 &3.0% respectively, however, SI showed better interaction with solvents with very high WHC (100%) The slight increase in WHC in the modified may be due to charge effect of the acyl groups and the unfolding of the protein might have exposed to more hydrophilic groups thereby increasing the binding sites (Ishaya and Aletor, 2019). An important functionality that influences taste of the product/food is the ability of protein to absorb oils (Aletor, 2010). Oil holding capacity (OHC) for isolates PP &AYB (2.9 &2.0%) and modified 3.8 & 2.2%) respectively. The increase observed in the modified may be attributed to denaturation of the protein due to chemical modification however, the same trend of WHC for SI was recorded for OHC(100%).The results were in consistent with those reported by (Aletor,2010) for soy bean isolate and mung bean by (Dua et al .,1996). Foaming capacity & stability of the isolates SI (3.3;120%), PP(16.4;34.0%) & AYB(32.2;56.4%)respectively, while the modified showed an increase in capacity (24.0 &38.0%) but decrease in stability (16 & 20%) respectively. This implies an increase in the surface activity probably due to increase in the number of polypeptide chains that arose from acylation which increases the viscosity and facilitates the formation of multilayer cohesive protein film at the interface thereby allowing more air to be incorporated (Aletor, 2012)

Table 3: Functional properties (%) of the protein isolate

	(SI)	(PP)	(AYB)
WHC	100±0.6	3.1 ± 0.12	2.60 ± 0.03
OHC	100±0.0	2.9 ± 0.06	2.0 ± 0.02
FC	3.3±1.5	16.4 ± 0.08	32.2 ± 0.13
FS	120±1.3	34.0 ± 0.15	56.4 ± 0.02
EC	46.3±1.1	59.0 ± 0.01	52.41 ± 0.12
ES	_	40.0 ± 0.03	45.1 ± 0.16

Table 4: Functional properties (%) of the modified protein isolate

	(PP)	(AYB)
Water holding capacity	3.2 ± 0.04	3.0 ± 0.02
Oil holding capacity	3.8 ± 0.01	2.2 ± 0.02
Foaming capacity	24.0 ± 0.04	38.0 ± 0.01
Foaming stability	16.0 ± 0.10	20.0 ± 0.25
Emulsion capacity	64.4 ± 0.12	68.5 ± 0.15
Emulsion stability	64.4 ± 0.15	52.5 ± 0.04

However, stability reduced after modification due to the negative charge imparted during chemical modification causing breakage in the intermolecular cohesiveness and elasticity (Aletor,2019). Emulsion capacity and stability of the SI, PP& AYB were 46.3 & 0; 59.0 & 52.4%; 40.0&45.1% respectively. Modified PP and AYB showed an appreciable increase 64.4 & 68.5%; 64.4& 52.5% respectively. The ability of proteins to form stable emulsions is important owing to the interactions between proteins and lipids in food system. An increase in the number of peptide molecules and exposed hydrophobic amino residue due to acylation would contribute to an improvement in the formation of emulsion. The highest protein solubilities for the isolates PP and AYB was at pH 11 while the modified were 6 and 9 respectively.

Conclusions and Outlook

The study revealed practical relevance of these underutilized legumes as a good source of desirable quality protein source in food industry. The chemically modified isolates having superior functionality over isolates.

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