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Evaluation of nutritional value of a nonconventional seed protein

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Abstract

A world wide supply of nutritionally balanced food is of fundamental importance. More than 800 million people in the world are under ñourished today and protein is of primary concern in this context. The more the essential amino acids the better is the quality of food. The proportion of èssential amino acid to the total amino acids and in vitro digestibility of protein isolate using sequential pepsin and trypsin digestion model have been recognised às nutritional parameters for the evaluation of proteins which were studied on the isolated proteinss from a nonconventional seed of native land, india. Different processing conditions were applied for getting protein powders and it was observed that methionine and alanine were absent at low temperature where as leucine was fòund to be diminishing at elevated temperature and the essential amino acid to the tòtal varies within 42%-50%. The invitro digestion study on sds page revealed that this proteins become soluble and get slowly digested with in 120 minute and showed a trace fraction of protein band of molecular weight of 32 kd-40 kd. Thus the present study focuses the untapped area of the said seed proteins for its further use to develop newer food ptoducts to alliviate protein mal nutrition.

Keywords: *Jackfruit seed, flour protein, nonconventional protein, nutritional evaluation, amino acids, sds page electrophoresis*

1. Introduction

In developing countries, like India, the major nutritional problem occurs due to inadequate supply of protein through diet as its deficiency is a serious case of ill-health causing kwashiorkor. It is estimated that more than 800 million people in the world today are under nourished. Proteins in particular are of primary concern in this context [1]. The nutritional value of food or feed depends on several factors; protein content, amino acid composition, crude fibre content,

contents of vitamins and minerals and possible presence of toxic substances [2]. Having all the different nutrients in appropriate amounts through foods and a world wide supply of nutritionally balanced food is of fundamental importance. The more the essential amino acids the better is the quality of food. The proportion of essential amino acid, EAA (E) to total amino acids (T) that is E/ T%, determination of nitrogen release during digestion [3] and the in vitro digestibility of protein isolate using sequential pepsin and trypsin digestion model [4,5] have also been recognized as a nutritional parameters for the evaluation of proteins. In order to get the full nutritional benefit, the functional properties of the protein should also be considered

The high cost of animal proteins has made it highly imperative to search for cheap and abundant sources of proteins with desirable functional and nutritional properties. In recent years, research attention has been focused mainly on inexpensive vegetable proteins and underutilized seeds [6] as sources of low- cost proteins to supplement human diets for product formulation and food fortification [7].

In spite of its high nutrient content, seeds of jackfruits have found limited applications in the food processing industry due to lack of research information. Research efforts have been focused mainly on proximate composition, physicochemical characteristics of flours and jackfruit seed starch and their limited use in food fortification [8-11].

Jackfruit (*Artocarpus heterophyllus Lam.*) is a popular fruit crop which is abundant in India, Bangladesh, Thailand and other parts of the Tropics [12,13]. The edible bulbs of ripe jackfruit are either consumed fresh or processed into canned products in India. Jackfruit is gaining popularity even in the United States due to emerging ethnic and mainstream marketing opportunities [14]. Seeds make up around 10 -15% of the total fruit weight and have high carbohydrate and protein contents [15,16]. As the seeds are normally discarded and are usually considered as underutilized agro waste, utilization of its flour and protein would be worth exploring.

Detailed characterization of jackfruit seed proteins when subjected to heat treatment needs to be focused more. Hence the effective study of nutritional behaviours of heat treated jackfruit seed proteins are aimed at for their utilization in food formulations. The complete study of functionality, amino acid score and in vitro digestibility of isolated protein was targeted to correlate the effect of drying on these attributes with a view to inclusion of proteins as functional ingredients and food supplements.

Wheat flour and bakery products serve as staple food and is probably the largest snack food among all age groups for rural as well as urban sectors of India [17] and also all over the world [18] because of their low manufacturing cost, more handling convenience and long shelf life. As the seeds can not be stored for a longer period because of its high moisture level, they are subjected to drying operation to achieve a shelf stable useable form for utilization.

For efficient utilization of jackfruit seed flour or proteins blended with wheat or rice flours for preparing bakery items, idli or extruded food, it is necessary to study its functional and nutritional properties.

2. Materials and methods

2.1 Seeds

Indian Jackfruit seeds (58.43 % average moisture content) with white seed coverings were collected from local markets of Kolkata, West Bengal, India and were used for this study.

2.2 Preparation of jack fruit seed flours

Four varieties of flours were prepared with varying heat treatment on lye peeled jackfruit seed chips by keeping at 50^0C, 60^0C, 80^0C and 105^0C following the method of Roy Chowdhury et al. (2012) [19]. The other variant of flours were prepared without removing the outer brown seed coat followed by drying the chips as per the method described above. The colour of this flour was brown and was stored for subsequent studies.

2.3 Amino acid analysis

The amino acid composition of the flour samples was determined by an automatic amino acid analyzer (Waters M510, USA), using PICO.TAG column. The determination was carried out at 38 ^{0}C and the detection wavelength 254 nm and flow rate 1.0 micro L per minute. The samples were hydrolyzed with 6 N HCl for 24 h at 110 ^{0}C in a sealed tube. The amino acid composition was reported as g/100 g of flour sample.

2.4 Evaluation of nutritional parameters

The amino acid composition of the jackfruit seed flours was used for calculating of the nutritional value of jackfruit seed proteins following the method of Chavan, McKenzie, & Shahidi (2001)[20].The proportion of essential amino acids (E) to total amino acids (T) of the protein is summarized below.

$$E/T\ \% = \frac{Ile + Leu + Lys + Met + Cys + Phe + Tyr + Thr + Val + His}{Ala + Asp + Arg + Gly + Glu + Ile + Leu + Lys + Met + Cys + Phe + Tyr + Pro + Ser + Thr + Val + His} \times 100$$

2.5 Preparation of Jackfruit seed protein isolates

The protein isolates were prepared from 60 ^{0}C flour, maintaining the ratio of defatted flour : deionised water as 1: 20 . The mixture was adjusted to pH 10 with 1.0 N NaOH. After continuously stirring for 1 h, the suspensions were centrifuged at 5500 rpm for 30 minutes. The pH of the supernatants was adjusted to pH 4.5 to 4.8 with 1 N HCl and the precipitates were collected by centrifugation . The obtained precipitates were washed with pre-cooled deionized water, and dispersed in the deionized water. The dispersions were adjusted to pH 7.0 with 1 N HCl, and then concentrated. The sample was subjected to tray drying at 50 ^{0}C overnight.

2.6. Proximate composition was carried out using AOAC, 1984 methods and Functional properties of protein isolates were determined.

2.7 Sequential in vitro protein digestion

The in vitro digestibility of protein isolates was evaluated using sequential pepsin and trypsin digestion model according to the method of Chavan et al. (2001) and Nunes et al. (2004)[21]. For SDS-PAGE analysis of digestion process, aliquots (200 microL) of the protein and enzyme mixtures were taken after specific periods of incubation time (0–120 min),

2.8 Electrophoresis of the protein digest

The one dimensional SDS- PAGE was carried out according to the methods of Laemmli (1970) [22].

3. Results

3.1 Proximate analysis of protein isolate

The protein, moisture, ash and fat contents of flour (treated at 60 ^{0}C) protein isolates prepared under tray drying, are shown in **Table 1.**

Table 1 : Proximate compositions and functional behaviour of flour protein isolates (Tray dried)

Proximate composition of tray dried protein isolate		**Functional properties of flour protein isolate**	
Constituents	wet basis % , gm / 100gm of isolate	Properties	Tray dried protein
Moisture	9.02	Water absorption capacity	4.84 ml/gm
Protein	73.07	Oil absorption capacity	42.4% +/- 2.1
Ash	2.38	Bulk Density	38 gm/100ml +/-2.8
Fat	0.36	Foam capacity & stability	400 ml/gm, 400ml/gm (15 min) 300ml/gm (30 min) 250ml/gm (1hr)
Carbohydrates	15.17	Gelation capacity	No gelation observed within 2% - 20% concentration
		Protein solubility	38.7%

3.2 Amino acid composition and evaluation

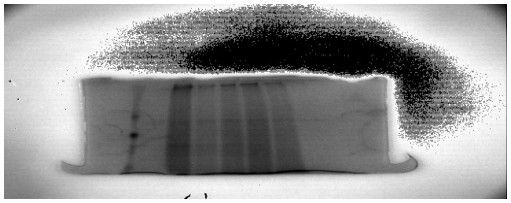
Table 2: Amino acid compositions of JFS Flour Proteins under various heat treatments

Amino acid	JFS flour 1 50 ^{0}C	JFS flour 2 60 ^{0}C	JFS flour 3 80 ^{0}C	JFS flour 4 JFS flour 60^{0}C with seed coat	FAO/WHO suggested requirements (2–5 year old) for essential amino acids
S(serine)	5.5620	2.6528	1.4625	2.6659	-
G(glycine)	10.2905	4.7528	2.5356	4.7133	-
H(histidine)	1.9291	0.9663	0.5597	0.9980	1.9
R (arginine)	24.8356	3.1449	1.2616	2.5036	-
T (threonine)	0.9462	2.3047	1.2216	2.4526	3.4
A (alanine)	absent	1.4831	0.8511	1.4668	-
P (proline)	0.0452	2.1202	1.3325	2.1619	-
Y (tyrosine)	3.9016	1.1406	0.6048	1.2479	-
V (valine)	5.4368	2.5676	1.5595	2.6386	3.5
M (methionine)	absent	0.3502	0.3433	0.3514	-
C (cysteine)	0.7490	0.8678	0.3660	0.7205	-
I/L (isoleucine)	2.3334	1.4406	0.8394	1.5364	2.8
L(leucine)	0.1326	0.1120	absent	absent	6.6
F (phenylalanine)	6.0050	1.2155	1.7178	1.6144	-
K (lysine)	0.9748	2.3495	1.0687	2.5369	5.8

Table 3 : Nutritional evaluation of heat treated jack fruit seed proteins under different conditions in drying

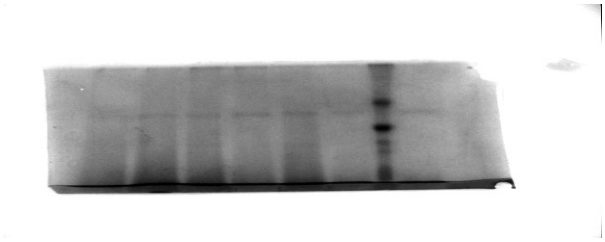
Amino acid	**JFS flour 1** **50 ^{0}C**	**JFS flour 2** **60 ^{0}C** **without seed coat**	**JFS flour 3** **80 ^{0}C**	**JFS flour 60^{0}C with seed coat**	**FAO/WHO suggested requirements (2–5 year old) for essential amino acids**
Total sulfur containing amino acids (Met & Cys)	0.7490	1.218	0.7093	1.0719	2.5
Total aromatic amino acids (Phy & Tyr)	9.9066	2.3561	2.3226	2.8623	6.3
E, Total essential amino acids	18.5069	12.1742	7.676	12.8488	32.8
Total non-essential amino acids	24.8808	14.1544	7.4433	13.5115	-
E/T (%)	42.6547	46.2395	50.7695	48.7429	-
T, Total amino acids	43.3877	26.3286	15.1193	26.3606	-

3.3 In vitro digestibility



B P0 P5 P10 P30 P60

Figure **4 P :** Picture of SDS PAGE of JFS seed protein with pepsin



P60 P30 P10 P5 P0 P120 M

Figure **5P :** Picture of SDS PAGE of Jack fruit Protein with pepsin and trypsin

4. Discussions

The amino acid compositions (gm /100 g of protein/ flour) of Jackfruit seed flours upon various heat treatment during drying operation are shown in **Table 2**. The FAO/WHO suggested requirements of the essential amino acids for 2–5 year old are also included (FAO/WHO, 1990) [23]. Jackfruit seed flour (JFS flour) proteins are rich in arginine, leucine , serine and valine which are to some extent consistent with the data of the amino acids found in soya protein isolate [24, 25, 26]. Although the sulfur-containing amino acids (Metheonine and Cystine) might be to some extent destroyed by the HCl-hydrolysis method used for this study, their contents in JFS flour were remarkably low (Metheonine 0.3502 gm/100 gm of flour at 60 ^{0}C). In comparison, the essential amino acids Valine , Phenylalanine and Tyrosine of JFS flour treated at 50^{0}C were retained to greater extent than that of treated at higher temperature. The Arginine content in less heat treated flour was found more. The proportion of essential amino acids to the total amino acids (E/T) for JFS flour , suggests that the JFS flour could be used as a functional adjunct to have more nutritional amino acids. The E/T % of JFS flour protein showed an increasing pattern on heat treatment starting from 50 ^{0}C flour to 80 ^{0}C one though the retention of essential amino acids was found to be present in excess at lower temperature treated flours. According to the FAO/WHO suggested requirements for 2–5 year old infants, only Leucine was found to be limiting at elevated temperature on drying. Except this amino acid, other essential amino acids are sufficient for the FAO/WHO suggested requirements for 2–5 year old infants. Interestingly, the sulfur-containing amino acids were found to be in excess in case of JFS flours with and without coat, treated at 60 ^{0}C. There is the evidence of the presence of all types of essential amino acids for heat treated flours but more retention was observed for flour treated at low temperature, 50^{0}C . The retention of phenylalanine, arginine and hystidine was more in 50^{0}C flour, and hence this flour can be utilized in developing food for children. A comparison was also drawn for two varieties of flours; treated at 60 ^{0}C with or without seed coat, on the basis of E/T% and the result suggests that flours with seed coat having E/T % 48.74 which is 2.5% higher than that for flours without seed coat. This may be inferred that a few essential amino acids were conserved in seed coat.

The 60^0C heat treated flours with or without seed coat provide better lysine for supplementation. As the wheat flour is limiting in lysine content, blending with these varieties flours and proteins can provide better nutrition in food preparation. The presence of total essential amino acids in the flours treated at lesser temperatures, 50^0C and 60^0C, suggests that the supplementation of these flours with conventional flours could be a tool for developing foods with higher nutritional status.

The in vitro digestibility of tray dried jackfruit seed protein was evaluated using the sequential pepsin and trypsin digestion model, by SDS-PAGE as shown in **Figures 4P and 5P**. During the pepsin digestion, the protein constituents of jackfruit seeds were rapidly digested by pepsin within about 5 minute, to release oligo-peptides with molecular weight (MW) less than 40.0 KDa in SDS-PAGE profile **(Fig 4P , Lane 2 and 3).** The protein band which will have similar mass with the marker protein as 195 or within the value of 195-127 KDa was found to be released immediately after 5minute of pepsin digestion and became gradually fade as time proceeded which indicates that the proteins with high molecular mass was slowly being digested by the action of the enzyme pepsin.

After the pepsin-digested hydrolysates were adjusted to pH 8.0, the addition of trypsin led to further decline in the MW distribution of the oligo-peptides **(Fig. 5P, Lanes 1-6)** after 60 – 120 minute. The evidence of protein with molecular mass of 195 KDa or similar was not found in **Fig 5P** for pepsin –trypsin digest. Only protein band observed even after trypsin digestion was of molecular weight within 32 KDa - 40 KDa value. The Gel picture revealed that jackfruit seed protein having molecular weight within 32 KD-40 KD value were much less prone to pepsin-trypsin digestion (**Fig.5P, Lanes 1-6).** This may be attributed to the difference in protein stability of these subunits in acid medium (at about pH 2.0). The difference in the pepsin digestion pattern among two bands of molecular weight 195 KDa and 32 KDa - 47 KDa and may be attributed to the difference in the availability of catalytic sites of protein substrates to pepsin. The poor solubility or the presence of insoluble aggregates of JFS protein might result in direct decline in the availability of these sites, during the initial pepsin digestion. The closer observation was reported in case of hemp protein isolate (Tang et al., 2006) .The pepsin-released peptides with high hydrophobicity are usually unstable at pH 8.0, and might interact to form some kinds of aggregates. Thus, the difference of trypsin digestion pattern for JFS protein may be attributed to the differences in the form and size of aggregates formed from the pepsin digested hydrolysates. It is obvious that the formation of the aggregates largely comes from the contribution of the digestion of the subunits.

5. Conclusion:

As the current study focuses on a nonconventional seed, jackfruit seed, its flours and proteins , information on the flour protein with their amino acid building blocks for different heat treated flours were obtained through analysis. The flours prepared at varied heat treatment from the raw seeds under different conditions of processing showed that methionine and alanine are found to be absent at low temperature whereas at elevated temperature treatment leucine was found to be diminished. The heat treated flours contained essential amino acids.

Information of food value of 100 gm of edible portion of dried seeds is scanty. Presence of antinutritional factors such as tannin and trypsin inhibitor has been reported, resulting in digestive ailment when eaten raw [27]. Also wet and dry heat treatments have been reported to be effective in detoxifying protease inhibitors and heat is the most efficient Pepsin and trypsin digest of the extracted Jackfruit seed flour proteins showed that the protein with KDa value of 195 along with 32 KDa - 40 KDa was more in zero hour and slowly gets digested indicating that no inhibition of enzyme takes place.But even after 120 minute fraction of the hydrolysate, showed a trace fraction of protein band having molecular weight of 32 KDa - 40 KDa. This might be the protein which is not acted upon by the enzyme system whereas other proteins present initially are not been retained in the digest with the extended run time.

It has been reported that severe heat treatment denatures protein better than inactivation of inhibitors [28]. Hence the extracted protein from heat treated flour and the subsequent invitro digestibility study of it would address suitably the nutritional aspects. However 60^0C treated flour is the choice for better sensory characteristics though nutritionally 50^0C is also good. The gross amino acid content of Jackfruit seed protein treated at 60 ^{0}C will be treated as the higher quality flour with a view to nutrition [29].

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