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Studies on amino acid and functional properties of leaf protein concentrate based weaning food

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Abstract

Leaf protein concentrate (LPC) was extracted from the leaves of Amaranths hybridus, Moringa oleifera and Leucaena leucocephala and was mixed at the proportion of 1:1:1. Then LPC mix was mixed with weaning food prepared. 2 per cent LPC was added to the prepared weaning food sample. The prepared sample was assessed for amino acid and functional properties. The prepared sample had 16-17 percent protein. Protein is a food component that has functional properties that affect the properties of food products. Thus, this research was aimed to study amino acid profile and functional properties of the prepared weaning food sample to determine the quality of the weaning food.

Keywords: LPC, Weaning food, Amino acid, functional properties and Rheological properties.

1. Introduction

The weaning period is a crucial period in an infant's life. At the age of 5–6 months, most infants begin to eat supplementary semisolid foods. At this stage homogenized infant foods play a major role in their nutrition (Martinez *et al*. 2004) [13]. Weaning foods (WFs) for a child in a developing country where WFs are relatively expensive is out of reach of a majority of the people and may result in malnutrition and pose a risk to the life of a child, particularly if the parents are low-income earners. Most WFs commonly sold in Nigeria are composed mainly of cereal grains which contribute about 42% of the total daily calories and 49% of the total daily protein (Keshinro *et al*. 1993) [10]. Emerging evidence (Baker 1994) [3] indicates that diseases such as hypertension, cardiovascular diseases, respiratory diseases, and diabetes are related to poor health and nutrition of the infant; thus, the need to provide a low-cost, nutritious weaning supplement for infants cannot be overemphasized. Baker (1994) [3] argues that malnutrition during infancy permanently changes the body's structure, physiology, and metabolism, leading to coronary heart disease and stroke later in life. There is therefore the need to explore the nutritional potential of affordable, alternative carbohydrate- and protein-based food crops. Leaf concentrate can be a powerful tool in the effort to defeat malnutrition. Pirie (1966) [15] suggested incorporation of leaf protein concentrate (LPC) into human food. Shah (1976) extracted proteins from grasses which contained 35%-60% protein, 6%-10% crude lipids and 1.01-1.47 mg/g of carotene. Research interest has been focused on different leaf meals as protein sources in animal feeds (Abowei and Ekubo, 2011). Leaf proteins have been found to have great nutritive value than most of the pulses, resembling skim milk in the diet of infants recovering from Kwashiorkor. How infants are fed appears to influence their long-term development and health (Baker 1994) [3], thus heightening the importance of improving infant food.

This article therefore investigates the use of Leaf protein concentrate with common grains (Wheat, Rice and green gram) in the formulation of Weaning Food and study of the properties to determine the quality of the sample.

Materials and Methods

The present investigation was carried out in Department of Food Engineering with collaboration of Department of Food Science and Technology and Department of Food Chemistry and Nutrition, College of Food Technology, VNMKV, Parbhani during year 2016-17. Leaves of Amaranth, Moringa and Subabul were obtained from the local village area of the Parbhani region.

Formulation of weaning food

Weaning product prepared from cereals which is ready to eat and rich in different nutrients. Dried wheat, green gram seeds and Rice were purchased from the local market of Parbhani and incorporated with prepared LPC.

Weaning food was prepared as per the method given by Yaseen *et al*., (2014).
Germinated wheat flour, green gram bean flour mixture, rice flour, skim milk powder and sucrose were blended. The complete processing steps of weaning food formulation are shown in Figure 1.

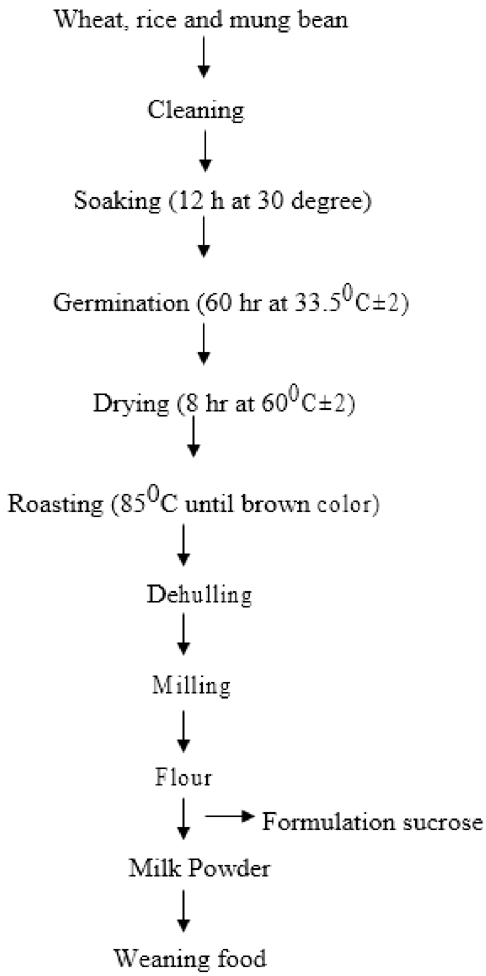

Fig 1: Flow diagram of weaning food formulation from Rice flour, germinated Wheat and Green gram flour.

Amino Acid Profile

Sample preparation

About 100 g of sample with 10 ml of 6N HCI Was digested at 110 ^{0}C in sealed tubes for 24 hr, The solution from the tubes were filtered and flash evaporated thring using distilled water to remove HCI and taken in buffer (Sodium citrate tribasic, perchloric acid, n-caprylic acid, pH 2.2).

HPLC analysis

The HPLC analysis was carried out according to the method of Ishida *et al.,* (1981) [8]. The sample thus prepared was filtered using a membrane filter of 0.45J.Im and 20J.It was injected into Shimadzu HPLC-LC 10AS,fited with a packed column (ISC-07IS1504-NA).The column material was a strong acidic cation exchange resin i.e. Styrene divinyle benzene copolymer with sulfunic acid. The elution buffers used were as described in the HPLC manual. Oven temperature was kept at 60 ^{0}C.The amino acid identification was done by non-switching flow method and flurorescence detection after the post column derivitisation of amino acid with O-phthaladehyde.

Amino acid score was determined with the use of following formula

Amino acid scores = [g/100g of amino acid in sample ÷ (g/100g of amino acid in reference protein)] x 100.

Functional properties:

Water and fat absorption capacity

The water absorption capacity (WAC) and fat absorption capacity (FAC) were determined using a reported method by Narsing Rao *et al*., (2010). Dispersing 1 g of Sample in about 10 ml of distilled water or sunflower oil. The contents were vortexes for 2 min and centrifuged at 5500 revolutions for 15 min at RT. The supernatant water or oil was decanted and the residue weight was noted. The weight difference was noted before and after water or oil process. Calculated WAC and OAC and express d as g/g sample.

Rehydration ratio

An initial amount of 1 g of dehydrated samples were used. The sample was rehydrated by immersion in 250 ml beaker filled with water. Temperature was maintained by placing beaker in a thermostatically controlled water bath. Beaker were withdrawn from the water bath after 15 minutes. After specified soaking time, the hydrated samples were blotted free of excess surface moisture with an absorbent cloth and weighed. The increase in the weight was taken as the amount of water absorbed. Rehydration continued until the difference between two consecutive weighings was significant. Rehydration ratio of the samples were determined using the method described by Ranganna (1986) [17].

$$\text{Rehydration ratio} = \frac{\text{Weight of the rehydrated material}}{\text{Weight of the dehydrated material}}$$

Emulsification capacity (EC)

Emulsification capacity was determined following a reported method in literature (Gagne and Adambounou, 1994) [7]. 1 g of sample was taken, homogenate and slowly adding of vegetable oil while stirring until the separation of oil globules was observed. The oil volume was noted after separation and emulsifying capacity was expressed as mL of oil/g sample.

Foam measurements

Foam capacity (FC) and foam stability (FS) of SLP were measured by following the methods described by Lawhon *et al*. 1972 One gram of SLP dispersed in100 ml distilled water and whipping for 10 min. The increase in volume by foam was measured, calculated and expressed as foam per cent. Foam volume was measured during a time intervals such as 15, 30, 45 and 60 min, calculated the foam volume and expressed foam stability.

Results and Discussion

Amino acid composition of the leaf protein concentrate mix:

Carefully made dry leaf protein concentrate is said to contain 36% true protein and 0.1-0.2% beta-carotene. The apparent differences in the amino acid composition might be due to the use of leaves of differing age and antecedents, or different processing techniques, as from real species differences (Pirie, 1979) [16].

Table 1: Amino acid composition of AMS mix

Amino acid	Results (g/100g)
Glutamic acid	5.50
Methionine	1.15
Proline	0.75
Isoleucine	2.15
Aspartic acid	3.90
Tyrosine	0.98
Valine	2.16
Alanine	1.46
Leucine	3.20
Histidine	1.01
Phenylananine	2.10
Tryptophan	1.47
Lysine	3.19
Threonine	2.65
Cysteine	0.88
Arginine	0.95
Serine	1.99
Glycin	0.90

*Each value is the average of 3 determinations

The amino acid profile of the leaf protein concentrate was analysed for quantitative proportions. The quantity of amino acids in leaf protein concentrate from the respective leaves showed the results as 0.95, 3.19, 3.9 and 5.50 g/100g for Arginine, Lysine, Aspartic acid and Glutamic acid. The values for Aspargine, Histidine, Serine, Threonine and Tryptophan are 0.95, 1.01, 1.99, 2.65, 0.98 and 1.47 respectively. The values for Methionine and cysteine were 1.15 and 0.38, while the values for valine, proline and glysine are 2.16, 0.75 and 0.90 repectively. Finally the value for alanine, isoleucine, leucine and phenylalanine are 1.46, 2.15, 3.20 and 2.20 respectively.

A 50:50 mixture of LPC and milk gave nearly as good nitrogen retention by malnourished infants as the same amount of protein given wholly as milk. Children 6-11 years old grew more on a diet supplemented with lysine or sesame (Pirie, 1979) [16].

Mothers gave 10g of LPC daily to a group of 2-to-6 year old Nigerian children with kwashiorkor. Besides curing the children, this improved their appetites and mental alertness (Pirie 1979) [16].

Amino acid profile of the weaning food

Table 2 shows the amino acid profile of the weaning food. All diets acquired lower essential amino acids profile compared to that of the reference diet. However, with increasing the amount of leaf protein concentrate in the formula the content of each amino acid significantly increases. The proportional rise in the amino acids values with increasing the LPC substitution is logic because leaf protein is known for its complete amino acids profile compared to that of cereal protein that is deficient in some essential amino acids such as lysine and methionine. Interestingly, the content of the most limiting amino acid lysine of the diets were significantly improved and was 0.95% in Sample A. These values were lower than the range 3.02-4.68 (Asma *et al.*, 2006) [2] reported for some locally made weaning foods.

Lysine gave the lowest value of chemical score for all of the diets. For all the formulated diets, the lysine score improved concomitantly with addition of LPC in the diet. The chemical score of all formulated diets was lower than that of the reference diet. The diets will be prepared by the addition of milk or other nutritious solutions which will increase the chemical score. Sulphur containing amino acids gave lower chemical score when compared with the reference diet.

Table 2: Amino acid profile of the weaning food

Amino acid	Results (g/100g)
Glutamic acid	1.54
Methionine	0.78
Proline	0.75
Isoleucine	1.08
Aspartic acid	2.15
Tyrosine	0.39
Valine	0.57
Alanine	0.96
Leucine	1.32
Histidine	0.72
Phenylananine	0.96
Tryptophan	0.52
Lysine	0.95
Threonine	1.16
Cysteine	0.63
Arginine	0.87
Serine	1.06
Glycin	0.66
Total 17.07	

* Each value is average of three determinations.

Analysis of the weaning food for functional properties

Functional properties denote those physicochemical properties of food proteins that determine their behavior in food during processing, storage, and consumption. The results on the functional properties of the diets are presented in table 3

Table 3: Functional properties of weaning food

Parameters	Control	Sample
Water absorption capacity (g/g)	3.2	3.5
Oil absorption capacity (g/g)	2.6	2.3
Emulsifying capacity (ml/g)	8.7	8.53
Foam capacity	78.22	76.14
Rehydration ratio	1.6	1.8

*Each value is the average of three determinations

Data pertaining to water absorption capacity, Fat absorption capacity, Emulsifying capacity and rehydration capacity were presented in table no 3. The leaf protein concentrate exhibited higher water absorption (3g/g) than oil (1.69). The phenomena can be explained based on the capacity of hydrophilic peptides binding to water molecules and high hydrogen bonding. (Cubertson et al., 2006) [5]. The leaf protein concentrate was found to be good emulsifying agents. Their ability to emulsify water oil dispersion had good industrial applications. Leaf protein concentrate exhibited marginal emulsification capacity of 8.80 ml/g. protein, carbohydrates and fat play an important role in water absorption capacity, fat absorption capacity and emulsifying capacity. The higher value of water absorption capacity of leaf protein concentrate (3g/g) indicates the presence of more hydrophobic sites in leaf protein concentrate, fat absorption plays an important role in food preparation, because fat plays an important role in food preparations, because fat improve the flavor and mouthfeel of products (Kinsella 1982) [82]. High water absorption capacity and fat absorption capacity of leaf protein concentrate may find useful application in the preparation of different products.

The foam capacity of leaf protein concentrate was found to be 70.30 (ml/g), leaf protein concentrate exhibited a foam stability of 20.67 ml over a period of 60 min. Foam stability depends on protein content with hydrophilic nature of peptides and their content with hydrophilic nature of peptides and their characteristics are desirable in preparation of beverages. The lower protein solubility and quality of denatured proteins might be the causes for lower foam capacity (Narsing Rao *et al*, 2011) [14]

The weaning food samples exhibited higher water absorption capacity (70-75 percent) than oil (45-55 per cent). The phenomenon can be explained based on the capacity of hydrophilic peptides binding to water molecules and high hydrogen binding. Sample a (74 per cent) had more water holding capacity as compared to control samples (72.44 per cent).

The water holding capacity was concurrently increased with increase in the LPC. Hydration or rehydration is the first step (Damodaran 1993) [6] and may be most critical step in impacting desirable functional properties to proteins in a food system. Intrinsic factors are affecting water binding properties of food flours relatively affecting high protein content include amino acid composition, protein conformation and surface polarity/hydrophobicity (Barbut 1999) [4].

The difference in protein structure and the presence of different hydrophilic carbohydrates might be responsible for variation in the water holding capacity of the sample. Flour with high water holding capacity has more hydrophilic constituents such as polysaccharides. (Mao and Hua 2012) [12]

Conclusion

It can be finally concluded that with increasing the amount of leaf protein concentrate in the formula the content of each amino acid significantly increased. The water holding capacity of the weaning food was concurrently increased with increase in the LPC. The foam capacity of leaf protein concentrate was found to be 70.30 (ml/g), leaf protein concentrate exhibited a foam stability of 20.67 ml over a period of 60 min. The weaning food samples exhibited higher water absorption capacity (70-75 percent) than oil (45-55 per cent). Thus indicating the suitability of the product for the consumption of the infants.

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