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Studies on preparation of leaf protein concentrate from leaves of *Amaranthus hybridus, Moringa oleifera* and *Leucaena leucocephala* and its utilization in weaning food

R.N. MEDA*, A.R. SAWATE, B.M. PATIL AND R.B. KSHIRSAGAR
Department of Food Engineering, College of Food Technology, Vasantrao Naik Marathwada Krishi Vidyapeeth, PARBHANI (M.S.) INDIA
Email: medarohit4@gmail.com

*Author for Correspondence

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SUMMARY :
Protein deficiency and protein calorie malnutrition (PCM) is one of the major nutritional problems in the developing world. As a novel source to combat malnutrition, leaf protein concentrate was prepared and further utilized in weaning food. Three different leaves: *Amaranthus hybridus, Moringa oleifera* and *Leucaena leucocephala* (Subabul) were utilized for leaf protein concentrate (LPC) preparation. AMS (Amaranth, Moringa and Subabul) mix consisting of LPC from each leaf at the ratio of 1:1:1 was further studied. Proximate analysis of AMS mix showed protein content as 36.48 per cent and moisture content as 8 per cent. Weaning food was prepared using wheat, mung bean and rice to which LPC mix was incorporated. On the basis of sensory evaluation, sample with 2 per cent LPC mix was selected. Nutritional analysis of the weaning food showed protein content as 19.26 per cent and ash content as 2.6 per cent. Thus, formulation of weaning food with LPC makes the food more nutritious thereby alleviating the problem of malnutrition.

KEY WORDS : Malnutrition, Leaf protein concentrate (LPC), Weaning food, Protein, AMS mix

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The world is coming to recognize the grim truth that ultimately the population growth will outstrip food suppliers with apocalyptic results. About 36 million people die every year due to hunger or as a result of hunger (Gasperini and Maguire, 2001). There are 16 million people undernourished in developed countries (FAO, 2012).

Protein deficiency is at the root of problems of growth and immunity. Iron deficiency, even more widespread, since according to United Nations International Children's Emergency Funds (UNICEF), 500 million children suffer chronic anemia with its accompanying immunity problems and hindrance to physical and mental growth. For decades, WHO has had a programme for fighting these three deficiencies but while the need is great the cost is high.The widening gap between the supply and demand can be bridged by increasing the production of food and by supplementing the existing resources with novel food stuffs. The development of novel protein sources such as fish protein concentrate (FPC) (Pariser *et al.*, 1978 and Sikka *et al.*, 1979), single cell protein (SCP) (Tannenhaum and Wang, 1975 and Ferrianti and Fiechter, 1983), soybean protein (SBP) (Mendez *et al*., 2002 and Bhatia and Greer, 2008) and insect protein (Ghaly and Alkoaik, 2010) have made significant contributions toward the alleviation of the world protein deficiency (Kuijer and Wielenga, 1999).

However, there is still an estimated one billion people suffering from protein deficiency and malnutrition (FAO, 2008 and WHO, 2002b). Therefore, new methods of feeding the underfed world population, especially in the less developed countries, have to be developed. Out of all the unconventional source of protein, leaf protein appears to have better exploitation in the light of excessive of photosynthesis and availability of abundant green vegetation (Srivastava and Mohan, 1981). Leaf concentrate can be a powerful tool in the effort to defeat malnutrition. Pirie (1966) suggested incorporation of leaf protein concentrate (LPC) into human food.

EXPERIMENTAL 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, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani during year 2016-17. Leaves of Amaranth, Moringa and Subabul were obtained from the local village area of the Parbhani region.

Proximate analysis of leaves :

Proximate analysis of leaves were estimated according to the standard A.O.A.C. method (1990).

Preparation of leaf protein concentrate :

The fresh leaves were plucked, weighed and washed prior to pulping as described by Fellows (1987). In brief, the leaves were pulped using a village scale mill and the juice which contained the proteins was squeezed out. The separated leaf juice was heated in batches at 80-90^{0}C for 10 min. This procedure coagulated the leaf proteins from the whey. The coagulated proteins were, thereafter, separated from the whey by filtering through muslin cloth and passed with screw press to dryness. The leaf protein concentrate was then washed with water, repressed and sun-dried.

Formulation of weaning food :

Weaning product prepared from cereals with different formulations which is ready to eat and rich in different nutrients. Dried wheat, mungbean seeds and

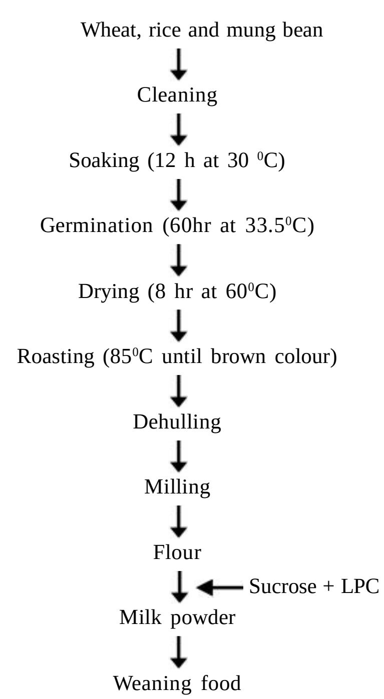

Fig. A: Flow diagram of weaning food formulation from rice flour, germinated wheat and mungbean flour

Table A: Standardized recipe for preparation of LPC incorporated weaning food

Sample	Rice flour (g)	Malted flour (g)	Milk powder (g)	Sucrose (g)	AMS mix (LPC)
Control	45	45	5	5	____
W_1	40	50	4	5	1
W_2	40	50	3	5	2
W_3	40	50	2	5	3

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, mungbean flour mixture, rice flour, skim milk powder and sucrose were blended. The standardized recipe for weaning food preparation is shown in Table A. The complete processing steps of weaning food formulation are shown in Fig. A.

EXPERIMENTAL FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Proximate composition of the leaves :

For the preparation of leaf protein concentrate three different leaves were used: *Amaranthus hybridus, Moringa oleifera* and *Leucaena leucocephala* (Subabul). All proximate and mineral analysis was according to the standard A.O.A.C. method (1990). The proximate composition of the leaves is depicted in Table 1.

It is recorded from Table 1 that amaranth and subabul leaves had the highest moisture content than moringa leaves. The protein content of subabul was highest amongst all three thus, indicating the suitability for preparation of leaf protein concentrate. The high crude protein suggestsits richness in essential amino acids. These aminoacids serve as alternative source of energy when carbohydrate metabolism is impaired viagluconeogenesis. While the ash content of the leaves as 0.4, 2, 0.38 per cent in amaranth, moringa and subabul leaves. This confirms that, thereare, more minerals in leaves. Fibre content in the leaves of Amaranth was more among all *i.e.* 17.3 per cent.

High amount of ash content indicates good composition of minerals. There was significant quantity of calcium, Magnesium and iron in leaf samples. The calcium content in the leaves of Amaranth, Moringa and Subabul (mg/g) was 26.40, 18.97 and 0.26, respectively. Similarly the magnesium content of the leaves was 12.48,4.73 and 0.010, respectively, finally the iron content of the samples were 0.81, 0.49 and 6.42, respectively (Table 2).

Proximate composition of the leaf protein concentrate :

All proximate and mineral analysis was according to the standard A.O.A.C. method (1990).

Table 1 : Proximate composition of the leaves

Leaves	Parameters (%)					
	Moisture	Ash	Protein	Fat	Carbohydrate	Crude fibre
Amaranthus hybridus	7.21	0.4	18.04	9.4	47.65	17.3
Moringa oliefera	4.37	2	18.5	12.28	46.65	16.2
Leucaena leucocephala	7.22	0.38	23.06	19.25	32.99	17.1

*Each value is average of three determinations

Table 2 : Mineral composition of the leaves

Minerals	Amaranth	Moringa	Subabul
Calcium	26.40	18.97	0.26
Magnesium	12.48	4.73	0.010
Iron	0.81	0.49	6.42

*Each value is average of three determinations

Table 3 : Proximate composition of the leaf protein concentrate (AMS mix)

Parameters (%)	AMS Mix (LPC)
Ash	0.14
Moisture	8
Fat	1.6
Protein	36.48
Crude fibre	2.0
Carbohydrate	51.78

*Each value is average of three determinations

The results of proximate analysis of leaf protein concentrate (AMS) mix have been revealed in Table 3. The moisture content of the sample is 8 per cent. Moisture in food indicate the keeping quality of food and determines largely rate of food absorption and digestion.The study shows the protein content of the AMS mix to be as 36.48. The ash, fat, crude fibre and carbohydrate are 0.14, 1.6, 2.0 and 51.78 per cent, respectively.

Table 4 shows the mineral composition of the AMS mix that comprises the leaf protein concentrate from the leaves of Amaranth, Moringa and Subabul. The calcium, magnesium and iron content (mg/100g) in AMS mix was 430.4, 853.3 and 0.8, respectively. Magnesium plays a vital role in calcium metabolism in bone. It is mineral element in connection with circulatory diseases such as ischemic heart diseases.

Table 4 : Mineral content of AMS mix (LPC) (mg/g)

Minerals	AMS mix (LPC)
Calcium	430.4
Magnesium	853.3
Iron	0.8

*Each value is average of three determinations

Organoleptic evaluation of weaning food sample:

The panel of judges consisting of 10 members was given the weaning food samples for evaluation of organoleptic characteristics *viz.*, flavour, body and texture, colour and appearance and taste. It was served to judges on the day of preparation. By summing up the scores obtained for all these characteristics the overall acceptability scores was obtained. The results of the organoleptic evaluation of weaning food samples are depicted in Table 5.

From Table 5 it was revealed that the overall acceptability of control sample was 8.9. The overall acceptability of samples W_1, W_2 and W_3 were 8.3, 8.5 and 7.7. The colour of the control sample was scored as 8.5. The colour of sample W_3 was rated as 8.0 as addition of more amount of LPC gave dark undesirable green colour. While W_1 was rated as 8.2 as it gave a dull colour.

Table 5 : Organoleptic evaluation of weaning food samples

Samples	Sensory attributes					
	Colour	Flavour	Taste	Texture	Consistency	Overall acceptability
Control	8.5	8.5	8.6	8.0	8.6	8.9
W_1	8.2	8.3	8.5	7.8	8.2	8.3
W_2	8.5	8.5	8.6	8.0	8.5	8.5
W_3	8.0	8.0	8.2	7.5	8.0	7.7
S.E ±	0.044	0.045	0.036	0.045	0.053	0.097
C.D. (P=0.05)	0.137	0.136	0.109	0.136	0.158	0.292

*Each value is average of three determinations Control - without addition of LPC W_1- With addition of 1 per cent LPC
W_2- With addition of 2 per cent LPC W_3- With addition of 3 per cent LPC

Table 6 : Nutritional composition of the weaning food

Parameters (%)	Control	W_2 Sample
Ash	2.2	2.6
Minerals	**Control sample**	**AMS mix (LPC)**
Moisture	8	6.6
Protein	17.10	19.26
Fat	3.4	3.9
Crude fibre	1.08	2.7
Carbohydrate	68.22	64.94

*Each value is average of three determinations W_2- With addition of 2 per cent LPC

Table 7 : Mineral composition of the weaning food samples mg/100g

Minerals	Control sample	AMS Mix (LPC)
Iron	2.7	3.5
Calcium	195.2	76.0
Magnesium	137.3	236.1

*Each value is average of three determinations W_2 - With addition of 2 per cent LPC

Sample W_2 was acceptable with desirable colour. Taste attribute for control sample was rated as 8.6. Addition of high amount of LPC gave unacceptable bitter flavour. Thus, sample W_2 had an acceptable taste.

The consistency of the control sample was rated as 8.6. The consistency of Sample W_2 was acceptable. Finally it could be concluded that sample with 2 per cent of leaf protein concentrate *i.e.* W_2 had the highest scores and thus, it was selected for further studies.

Nutritional composition of the weaning food:

The results of the nutritional composition of control and W_2 samples were presented in Table 6. The moisture as (8 and 6.6), protein (17.10 and 19.26), ash (2.2 and 2.6) crude fibre (1.08 and 2.7) and carbohydrate (68.22 and 64.94) were found in control and W_2 sample, respectively. The moisture content is used as a quality factor for prepared cereals which should have 2-8 per cent moisture content. The low moisture content of formulation was required for convenient packaging and transport of products. The fat content was 3.4 and 3.9 for control and W_2 sample, respectively. It is low as compared to the specifications. Fat content in formulations is less because the fat content of a food sample canaffect its shelf stability. The prepared weaning food formulations were close to the specification for weaning foods (Imtiaz *et al.*, 2011).

Mineral composition of the weaning food samples :

The results of mineral analysis of weaning food is shown in Table 7. The results showed that W_2 sample contained higher amounts of iron and magnesium. Because this combination had higher amount of mungbean as compared to other samples and mungbean had higher amount of minerals. The results were in accordance with earlier research (Khalil, 2001).

Conclusion:

It can be finally concluded that extracted leaf protein concentrate had a significant amount of minerals like iron, calcium and magnesium. Owing to its high mineral concentration and protein content it can help in combating malnutrition among affected population, especially children. The prepared LPC that had protein of about 36.48 per cent can be used in any food as supplementation for protein. The weaning was food prepared by incorporating LPC mix, that had protein content of about 17.1 per cent. The fortification of LPC helps in increasing the protein content and mineral composition.

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