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DEVELOPMENT, EFFICACY AND COMPERATIVE ANALYSIS OF NOVEL CHICKPEA BASED READY-TO-USE THERAPEUTIC FOOD

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ABSTRACT

The development of ready-to-use therapeutic foods (RUTF) using indigenous sources in under developing countries is highlgy required. In the present study, chickpea based RUTF was prepared, analyzed and evaluated for true digestibility (TD), net protein utilization (NPU), biological value (BV), protein efficiency ratio (PER) and feed efficiency ratio (FER) in Sprague Dawley rats in comparison to peanut based *Plumpy'nut* and casein as standard. The proximate analysis of novel RUTF showed that it contains 97.9% dry matter, whereas crude fat, crude protein, crude fiber and nitrogen free extract were 31.33%, 12.4%, 2.17% and 50.6%, respectively and was statistically similar to *Plumpy'nut.* The protein quality experiments showed that the TD of RUTF, *Plumpy'nut* and casein was 83.78, 86.98 and 93.16, NPU was 73.54, 77.42 and 86.58, BV was 87.77, 89.01 and 92.98, respectively, and these parameters of RUTF and *Plumpy'nut* were statistically similar to each other but lower than casein. The PER was statistically different for all treatments and was 1.92, 2.13 and 2.82 for RUTF, *Plumpy'nut* and casein, respectively. The FER of RUTF (0.228) was statistically higher than *Plumpy'nut* (0.193) but lower than casein (0.275). It can be concluded that novel indigenous source chickpea based RUTF is similar to *Plumpy'nut* with respect to nutritional value and protein quality.

Key words: Chickpea, Malnutrition, *Plumpy'nut*, RUTF, Therapeutic Food.

INTRODUCTION

Therapeutic foods are specially designed, energy and nutrient dense foods, used in emergencies as medicine or supplements to treat severe and moderately acute malnutrition in infants and children (Michael *et al.* 2011). Most of the ready-to-use thrapeutic foods (RUTF) are oil based which make them free from microbial growth and suitable for use in hospital setup as well as in the house, safely without refrigeration and even in unhygienic conditions. The World Health Organization (WHO), four decades back, considered the importance of socioeconomic factors in providing good quality, low-cost foods for children in developing countries and recommended the use of traditional local foods especially of plant origin like cereals and pulses with suitable addition of skim-milk powder, vitamins, minerals and flavorings to produce a nutritious and readily-acceptable food (Kim *et al.* 2009). However, with the exception of the standards, modifications of the ingredients, process approach, packaging and storage conditions might be significant contributors to variability in the quality and safety of the finished products. Considerable variability has also been reported relating to form (liquid vs. powder), inherent levels of nutrients in product ingredients, source of protein & other micronutrients, nutrient stability and component nutrient interactions (Hoppe *et al.* 2008; Kunyanga *et al.* 2012). Therefore, safety, efficacy and sufficiency of the newly developed product, with respect to its specific use, needs to be scientifically proven/documented in specific population groups.

Lack of quality infant supplementary foods at affordable prices is one of the main causes of malnutrition in Pakistan. Despite the country's economic development, most of the people are not financially sound enough to buy high priced imported formulas like *Plumpy'nut* for malnourished infants. In fact, there is no such formula which is prepared in the country from indigenous resources. For the reason, consumption of cheaper but good quality plant proteins and protein isolates to replace more expensive animal proteins in food products like infant and young children's formulas, is the need of time in countries like Pakistan. Legumes represent an important protein source, especially for low income families. Being a low cost coupled with better protein quality, legume proteins should be included to prepare cost effective infant foods (Isanaka *et al.* 2009).

In the indian subcontinent, chickpea (*Cicer arietinum* L.) is mostly consumed as whole seed in several differnet forms but is also used to produce "*dhal*" (cotyledons) and to make flour (*besan*) after grinding. With respect to nutritional point of view, chickpea provides carbohydrates – from mono-to polysaccharides especially resistant starch, protein – quality is better than other pulses, low amounts of lipids – especially unsaturated fatty acids like linoleic and oleic acids, vitamins – such as thiamin, riboflavin, niacin, folate and vitamin A (β-carotene), minerals – Ca, Mg, P and K and important bioactive compounds like β-sitosterol, campesterol and stigmasterol (Bampidis and Christodoulou 2011; Jukanti *et al.* 2012). Pakistan is an agricultural country and is among one of the main producers of chickpea in world after India and Australia (FAO 2014). In Pakistan, chickea is locally called as "channa" and both distinct types i.e. Desi and Kabuli are produced. Due to the increasing rate of natural and human-instigated disasters, sever conditions of malnutrition and hidden hunger in Pakistan as shown in National Nutritional Survery (Anonymous. 2011), several non-governmental organizations (NGO's) and government based projects are importing therapeutic foods and infant formulae with the expense of lot of money. In the current scenario it is highly required to explore indigenous plant based sources to produce value-added products and to evaluate their potential to reduce malnutrition problems. Same kind of effort has been done in the current work and a chickpea based therapeutic food was prepared and its efficacy was assessed in Sprague Dawley rats.

MATERIALS AND METHODS

Procurement of material: RUTF was prepared in the Laboratory of Department of Food Science and Human Nutrition, University of Veterinary and Animal Sciences, Lahore using method given by Manary (Manary 2006) with little modifications. *Plumpy'nut* was the curtesty of Mayo Hospital, Lahore and other raw materials were purchased from the local market of Lahore and were of the highest purity and quality.

Preparation of RUTF: For the preparation of RUTF, chickpea (*Kabuli* type) were roasted in sand having temperature of 250 ±20ºC for 10 min with continues mixing and husks were removed through rubbing, sieving and winnowing. The stone mill was used to prepare chickpea flour after proper dry cleaning. After that, all other ingredients like sugar, corn oil, skim milk powder, and emulsifier as per amount given in Table 1 were mixed in chickpea powder and cooked at 70ºC for 25 to 30 minutes. The material was cooled and packed in airtight packets and kept at room temperature (25±2 ºC). Before preparation of new RUTF, lot of efforts were done to establish recipe similar to *Plumpy'nut* for major nutrients. The standard food composition tables were used for detailed composition of RUTF for vitamins and minerals given in Table 1. Finally the the recipe of RUTF was adjusted in the way that 100 g of RUTF provide 12.4 g of protein, from which almost 50% was taken from the Chickpea and remaining 50% was provided by skimmed milk powder, sugar provided 16 g carbohydrate, and vegetable fat provided dense energy.

Proximate composition of RUTF: The *Plumpy'nut* and newly produced RUTF were analyzed for total dry matter, moisture, crude fat, crude protein, ash content, crude fiber and nitrogen free extract (NFE) according to their standard methods described by American Association of Cereal Chemists. Approved Methods Committee (2000).

Calculations of vitamins and minerals: The quantity of vitamins and minerals in RUTF was calculated using the recipe/composition of novel product and Food Composition Tables /Data (Ene-Obong *et al.* 2013). The following tables were used; (1) Food Composition Table for Pakistan, 2001 (2) Food composition table/ the McGraw-Hill Companies, NY, USA (3) FAIS Food Composition Table, World Food Program (WFP).

Protein quality of RUTF on Sprague Dawley rats: The novel chickpea based RUTF was evaluated for protein quality and efficacy in comparison to *Plumpy'nut* (Developed by Nutriset and the Institute for Research and Development, France) and casein as standard diet in various groups of Sprague Dawley rats. The protein quality was evaluated on the basis of true digestibility (TD), net protein utilization (NPU), biological value (BV), protein efficiency ratio (PER) and feed efficiency ratio (FER).

Housing of rats: Twenty four (24) weanling male Sprague Dawley rats were used for the study and housed at Animal Room, Department of Microbiology, UVAS, Lahore. Relative humidity (~55%), temperature (23±2 °C) and light conditions (12 hour light-dark cycles) were maintained throughout the study period. The rats were fed on basal diet having casein as source of protein, prepared according to Chonan and Watanuki (1995) containing 5% mineral-vitamin mixture, for a period of one week and randomly divided into 4 groups (A, B, C and D) having 06 rats in each group. The four groups were fed on RUTF, *Plumpy'nut*, standard casein diet and no protein diet, respectively. The groups were fed separately on these iso-nitrogenous diets (having 10% protein), along with no protein diet for a period of 10 days (Eggum 1973).

Sample collection and analysis: At the end of study overnight fasted rats were decapitated by following standard procedures and their bodies were dried. The spilled diet, fecal matter and dried rats' bodies were subjected to nitrogen analysis to determine TD, NPU, BV, NPR and PER following the processes described by Proll (1982).

Statistical analysis: The proximate analysis of RUTF and *Plumpy'nut* were conducted in triplicate and the data were analyzed using t-test, whereas one way analysis of variance under completely randomized design was used for protein quality analysis in Cohort Costat-2003 software (Steel *et al.* 1996). The Duncan Multiple Range test was used to compare the means for significance of difference (Duncan 1955). The confidence interval defined as P≤0. 05.

RESULTS

Proximate composition of RUTF and *Plumpy'nut*: The proximate compositions, i.e. total dry matter, moisture, crude protein, crude fat, crude fiber and NFE of novel chickpea based RUTF prepared from indigenous sources and *Plumpy'nut* supplied by UNICEF are given in Figure 1. Proximate analysis of *Plumpy'nut* and RUTF revealed that *Plumpy'nut* comprise 98.01% dry matter, 2.02% moisture, 3.63% Ash, 32.33% crude fat, 2.15% crude fiber, 12.56% crude protein and 49.33% NFE while RUTF comprise 97.90% dry matter, 2.07% moisture, 3.51% ash, 31.33% crude fat, 2.17% crude fiber, 12.42% crude protein and 50.63% NFE. The statistical analysis (t-test) revealed that there is non-significant difference in proximate composition of RUTF and *Plumpy'nut* (p=0. 5286). 100 g of RUTF provided approximately 530 kcal which is sufficient for infants and child's energy requirement for the whole day.

The proximate composition of both products showed that these are very hard and dry with very little moisture and mainly constituted on energy giving components, i.e. carbohydrates (~50%), lipids (~32%) and proteins (~12.5%) and micronutrients comprise on ~3.5%. This composition makes these products more energy dense.

Protein quality

True Digestibility (TD): The results of TD for various treatments are presented in Table 2 showing significant difference (P=0.0456) among treatments. Casein contains highest TD i.e. 93.16±5.26% on the other hand novel RUTF has lowest 83.78±1.4%. It can be observed that the TD values between standard casein and RUTF are statistically significant from each other while casein & *Plumpy' nut* as well as RUTF & *Plumpy' nut* are statistically same. The results showed that RUTF and *Plumpy' nut* have same digestibility.

Net Protein Utilization (NPU): The mean values of NPU for various products are presented in Table 2 and show that there is a significant difference (P≤0.05) among various treatments. The standard casein has highest NPU (86.58±3.76%) whereas novel chickpea based RUTF has lowest (73.54±2.16%). It can be observed that NPU for casein is statistically higher than *Plumpy'nut* and RUTF (p=.0049). But NPU for *Plumpy'nut* and newly prepared supplementary food are statistically non-significant from each other. It is clear from the data that NPU for casein is higher than other two because milk protein are more digestible and have good BV as compared to chickpea (RUTF) and peanuts (*Plumpy'nut*).

Biological value (BV): The mean values with standard deviations of BV for various treatments are presented in Table 2 and show significant difference between various treatments (P≤0. 05). It is clear that standard casein has the highest value for BV i.e. 92.98±1.65%, which is significantly higher than *Plumpy'nut* (89.01±0.65%) and novel RUTF (87.77±1.32%). It can also be observed that *Plumpy'nut* and RUTF are statistically not different to each other, although they have different protein sources i.e. peanuts and chickpea, respectively.

Protein Efficiency Ratio (PER): The results for PER of different foods/treatments are presented in Table 2. It is clear that casein (2.82±0.16) has highest efficiency ratio as compared to *Plumpy'nut* (2.13±0.04) and chickpea based RUTF (1.92±0.04). It can be observed that all FER values among casein, *Plumpy'nut* and RUTF are statistically different to each other (P≤0.05). The novel RUTF has a lowest PER because it contain ~50% protein from chickpea and rest from skim milk powder, whereas casein is a purely milk protein and *Plumpy'nut* have skim milk and protein from peanut.

Feed Efficiency Ratio (FER): The results for FER of casein, *Plumpy'nut* and chickpea based RUTF are presented in Table 2. The casein shows highest highest FER value (0.275±0.013) followed by RUTF (0.228±0.017) and the lowest was secured by *Plumpy'nut* (0.193± 0.011). It can be observed that all FER values among casein, *Plumpy'nut* and RUTF are statistically different to each other (P≤0.05). Although the chemical composition of *Plumpy'nut* and newly prepared chickpea based RUTF is not different, but still the sources of macronutrients have changed the FER of all products.

Table 1. Recipe and Nutritional composition of ingredients used in novel chickpea based ready-to-use-threapeutic food (RUTF) (Per Kg).

Ingredients	Chickpea powder	Corn oil	Skim milk powder	Sugar (Sucrose)	Emulsifier	Vitamin & Mineral Mixture	RUTF Composition
Weight (g)	315	300	200	160	5	20	**1000**
Energy (Kcal)	1243.54	2700	722	640	--	--	**5305**
Protein (g)	63.32	--	65	--	--	--	**128.32**
Carbohydrate (g)	203.27	--	111	160	--	--	**474.27**

Fats (g)	19.70	300	2	--	--	--	**321.7**
Monounsaturated fats (g)	1.91	81.43	--	--	--	--	**83.33**
Polyunsaturated fats (g)	3.61	158.58	--	--	--	--	**162.19**
Fiber (g)	20.8	--	--	--	--	--	**20.8**
Ash	8.7	--	17	--	--	--	**45.7**

Final composition of microminerals in novel chickpea based RUTF (Per 1000g)

Vitamins		**Minerals**	
Vitamin A (µg) (RAE)	84.17	Calcium (mg)	3000
Thiamin (mg)	5	Iron (mg)	100
Riboflavin (mg)	16	Magnesium (mg)	800
Niacin (mg NE)	50	Phosphorus (mg)	3000
Vitamin B6 (mg)	6	Potassium (mg)	11000
Vitamin B12 (µg)	10	Sodium (mg)	2900
Folic acid (µg)	2000	Zinc (mg)	110
Vitamin C (mg)	500	Copper (mg)	140
Vitamin D (µg)	150	Selenium (µg)	200
Vitamin E (mg)	200	Iodine (µg)	700
Pantothenic acid (mg)	30		
Biotin (µg)	600		

Vitamins and Minerals

- The vitamins and minerals were calculated using various Food Composition Data.
- The main sources for microminerals were vitamin and mineral mixture, chickpea powder and skim milk powder

Table 2. Protein quality evaluation of novel chickpea based ready-to-use-threapeutic food (RUTF) and market available peanut based *Plumpy'nut* in comparision to Casein as control in Sprague Dawley rats.

Treatments Parameters	Casein	*Plumpy'nut*	RUTF	P≤0.05
True Digestibility (%)	93.16±5.26[a]	86.98±2.88[ab]	83.78±1.4[b]	0.0456
Net Protein Utilization (%)	86.58±3.76[a]	77.42±2.94[b]	73.54±2.16[b]	0.0049
Biological Value (%)	92.98±1.65[a]	89.01±0.65[b]	87.77±1.32[b]	0.0059
Protein Efficiency Ratio	2.82±0.16[a]	2.13±0.04[b]	1.92±0.04[c]	0.0001
Feed Efficiency Ratio	0.28±0.01[a]	0.19±0.01[c]	0.23±0.017[b]	0.0013

Means sharing the same letter in a row are not significantly different to each other @ P≤0.05. Casein; groups of rats fed on basel diet prepared using casein as protein source, *Plumpy'nut*; the rats group fed on *Plumpy'nut*, RUTF; group of rats fed on novel chickpea based therapeutic food. All the readings ± SD in the table is the mean value of at least 06 rats.

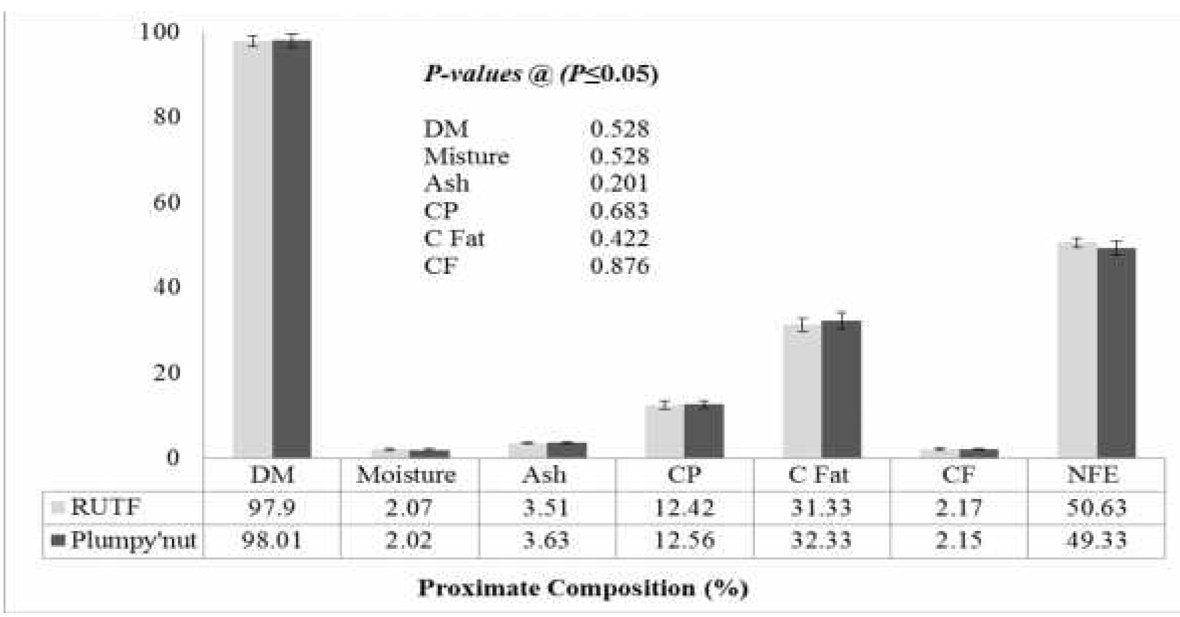

Figure 1. Comparision of proximate composition of novel chickpea based ready-to-use threapeutic food and market available *Plumpy'nut* (t-test). All the values are the average of at least 3 readings. DM, Dry Matter; CP, Crude Protein; C Fat, Crude Fat; CF, Crude Fiber; NFE, Nitrogen Free Extract

DISCUSSION

The development of RUTF is the requirement for underdeveloped and developing countries because most of them have high rate of moderate and/or acute malnutrition and unfortunately these countries are also more vulnerable to natural and man-made disasters and uncertainties. The other reasons for RUTF development for the management of community-based uncomplicated severe acute malnutrition is; they provide all nutrients for recovery, have good shelf-life and they are mostly oil based so less risk of bacterial growth and can be used without refrigeration even in unhygienic conditions (Manary 2006). The utilization of indigenous sources for RUTF is highly recommended by UNICEF and WHO with some basic recipe and standard protocols.

It can be observed from proximate analysis of newly prepared RUTF that it's mainly comprised of protein, lipid and carbohydrates and very small quantity of moisture and micronutrients and this chemical composition is statistically similar to *Plumpy'nut*, a peanut based RUTF prepared by Nutriset, France (Table 1). This chemical composition makes the product resistant to spoilage even at room temperature and can be utilized even in unhygenic conditions for example in areas of flood and disaster where common food preparation is not possible. Several medical formulations and supplementary foods have been prepared using plant based proteins with more or less same composition. Some of them have discussed below; Ulloa and Valencia (1993) prepared a medical formula using protein concentrate from chickpea and with the addition of sucrose, milk flavor, mixtures of corn and coconut oil and vitamins and minerals with final proximate composition as protein 16.0%, fat 25.8%, moisture 4%, ash 3.2% and carbohydrates 51.0%. On the basis of the results of the net protein ratio, nitrogen utilization and their relative values to casein, the authors reported that chickpea protein concentrate can be a potential ingredient for RUTF preparation. In another study in India, totally indigenous based therapeutic food was prepared "RUTF Agra" by the equal combination of jaggery, peanut, puffed rice and Bengal gram and its proximate composition was 9.5% protein, 26.25% fat and 62% carbohydrate (Sandeep and Mona 2014). The authors had recommended this local product for nutritional rehabilitation of malnourished children admitted in hospital. Similarly, (Thakur *et al.* 2013) prepared a local ready to use therapeutic food (LRUTF) from groundnut (25%), sugar (30%), milk powder (30%) and vegetable oil (15%) by weight, giving 544 kcal/100g. The new LRUTF was found to be better than F100 for weight-gain in severe acute malnutrition patients during the rehabilitation phase. Most recently, (Malunga *et al.* 2014) prepared a fully chickpea based infant follow-on formula through germination of seeds for 72 hours in distilled water followed by boiling to reduce anti-nutritional factors and concluded that these kind of products fulfill the requirement of WHO/FAO with minimal addition of minerals and vitamins as we have incorporated milk powder to enhance nutritional importance of the product. In our study similar kind of effort has been done and is suggested to be continued with main purpose to produce more economical and affordable products for developing countries without compromising the nutritional quality. In conclusion, chemical composition of these kind of products showed that they are mainly composed of carbohydrates, proteins and lipids whereas having less contents of non-digestible fibers, minerals and vitamins which make them energy dense and suitable to treat chronic and acute malnutrition.

As far as the efficacy of chickpea based RUTF is concerned, Sprague Dawley rats were used to determine various parameters of protein quality evaluation. The results show that TD was 83.78±1.4, NPU was 73.54±2.16, BV was 87.77±1.32, PER was 1.92±0.04 and FER was 0.22±0.017. The results showed that TD, NPU and BV were significantly higher for standard casein whereas *Plumpy'nut* and chickpea based RUTF were statistically similar to each other. The reason behind is the casein which is the main milk protein and have the highest biological value, excellent digestibility and complete range of amino acids to fulfill the requirement of "*all-or-none*" principle of protein synthesis, whereas other two products have protein of less quality as compared to casein i.e. *Plumpy'nut* contain peanuts and RUTF have chickpea (~50%). Several findings have received results which are in line with our study. In a study, three different formulations were prepared on the basis of chickpea, rice, soybean meal, banana, sugar and different amino acids. In all recipes the major part was chickpea. In the final composition, moisture was ranging from 3.92 to 5.43%, protein from 14.51 to 17.98%, fat from 1.61 to 4.05%, carbohydrates from 69.39 to 76.74% and ash from 1.98 to 2.42%. When these formulations were evaluated for PER and NPU in rats in comparison with cerelac as standard, all got almost similar results, but lower than standard (Valencia *et al.* 1988). Similar higher values for PER, NPR and NPU were obtained for products based on chickpea supplemented with methionine (Sotelo *et al.* 1987). In another study, (Livingstone *et al.* 1993) prepared a roller dried weaning food by mixing of mildly toasted and debranned wheat and dehusked chickpea flours. The final formulation was composed of 60% wheat, 30% chickpea, 5% skim milk powder and 5% sucrose and having about 16% protein and 1.0 kcal/g energy density. The formulation showed 88.3 BV, 87.5 TD and 2.91 PER. In a similar study, a variety of malted weaning foods were prepared using different techniques but the final recipe contained 60% processed barley, 30% chickpea, 5% skim milk powder and 5% cane sugar. The protein content of the formulations were ranged from 14.8% to 15.6% and energy content were 1.1 kcal/g. All formulations indicated good growth-promoting quality of the proteins in 21-days-old male Wister albino rats with 85-87% TD, 93-97% BV, 79-84% NPU and 2.6-3.1 PER (Wondimu and Malleshi 1996). So final of the discussion is that the protein quality parameters of novel chickpea based RUTF are in a range of other similar products and it can be used successfully at larger scale.

Like other legumes and plant based proteins, chickpea also have several anti-nutritional factors (ANF) including phytic acid, tannins, saponins, polyphenols, oxalates, and trypsin inhibitors which reduce the biological value of these products. Several treatments have been used to reduce these factors like soaking, germination, pressure cooking, autoclaving, and roasting, etc. The roasting of chickpea in sand at 120°C for 15 min can be used for significant reduction of ANF (Rajni *et al.* 2012). In our study, chickpea were roasted in sand having temperature of 250 ±20ºC for 10 min with internal chickpea temperature of ~140±10°C. It was assumed that at these conditions most of the ANF were removed which result in good BV, TD, NPU, and PER compareable to casein and *Plumpy'nut.*

It can be concluded from the present study that indigenous plant sources like chickpea, can successfully replace milk protein upto ~50% in supplementary foods as these have better protein quality comparable to commercially available therapeutic formulae. This is highly needed for agriculture based developing and underdeveloped countries like Pakistan to utilize indigenous plant based proteins in therapeutic foods. With regard to future perspective, it is extremely recommended that other plant proteins, e.g. sesame, flaxseed, sunflower, etc. should be used in functional foods and their efficacy should be conducted at the community level.

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