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Formulation, development and standardization of ready to eat food supplement for tuberculosis patients

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Abstract
Malnutrition is common among patients with tuberculosis. A high protein high calorie diet with high vitamins and minerals is often recommended for tuberculosis patients. The present work has been undertaken to formulate a ready to eat food supplement for persons with tuberculosis from commonly consumed ingredients like Ragi (Eleusine coracana), Soyabeans (Glycine max), Peanuts (Arachis hypogea) and Blackgram (Phaseolus mungo). The processed ingredients were mixed to prepare four different blends. Ragi and blackgram were taken 50 grams and 5 grams in each of the four blends but the proportions of soyabeans and peanuts were adjusted and mixed in different proportions. A traditional product laddu was prepared from all the four blends separately with the addition of jaggery and ghee to find the acceptability. The blend with 30 grams of soybeans and 15 grams of pea nuts was found to be acceptable through organoleptic evaluation. The accepted blend can be taken directly as laddu or porridge or can be incorporated into commonly consumed Indian breakfast items like Plain dosa, Masala vada, Mysore bonda, Chapati at 50 percent level. Thus the formulated ready to eat food mixture was accepted well by the experts and the shelf life was also found to be good.

Keywords: ragi, soyabeans, peanuts, blackgram, nutritional composition, food mixture, tuberculosis

Introduction
India is the country with the highest burden of tuberculosis (TB). There was an estimated 10.4 million new TB cases Worldwide according to The World Health Organization TB statistics 2017. Seven countries accounted for 64% of the total burden with India bearing a brunt, followed by Indonesia, China, Phillippines, Pakistan, Nigeria and South Africa. TB was considered as a nutritional disorder prior to the discovery of tubercle bacillus by Robert Koch 1882. Under nutrition can be considered as one of the risk factors in the development of TB. Since under nutrition is known to adversely affect the immune system (Onwubalili, 1988; Kennedy *et al.*, 1996; Karyadi, 2000) [14, 10, 9] still their remains a question was to whether malnutrition predisposes to tuberculosis, or whether it is a consequence of the disease. Irrespective, a vicious cycle is known to exist between TB and malnutrition such that one is promoting the others.
Further acute infectious illnesses, such as TB are accompanied by a complex variety of nutritional and metabolic responses within the body. The response to infection is associated with an increase in the energy expenditure of the patient and various degrees of tissue breakdown. Additionally, in the body's attempt to fight the infection energy expenditure is increased there by increasing energy needs in the TB patients. Complex changes occur in the metabolism of all the macronutrients i.e. proteins, carbohydrates and fat. An increase in protein breakdown for example leads to muscle wasting in these patients. The breakdown of protein and other reserves due to fever may also worsen under nutrition and further impair resistance against the infection.
The response to infection also includes a profound impact on the micronutrient status of the patient. Vitamins and minerals are compounds that are essential for normal growth and maintenance of body functions, playing key roles in many different metabolic processes in both health and disease. The increased energy expenditure and tissue breakdown associated with infection are thought to increase the requirements of micronutrients such as vitamin A, E, B_6, C, D and folate. It is also known that a decrease in blood levels of trace elements such as iron, zinc and selenium occur during the infection (Karyadi, 2000; Usha, 2002) [9, 16]. Hence there is a need for high calorie and high protein diet with high vitamins and minerals during infection.
Ragi is rich in calcium which is necessary for the healing of the tuberculin lesion and it is also a highly accepted cereal. Soyabean is a pulse rich in protein and micronutrients such as B-carotene, calcium and iron. Soy protein contains naturally higher levels of arginine and glutamine, these two amino acids are conditionally essential and they enhance the immune system. Peanut is rich in protein and a concentrated source of energy. Peanuts have arginine, a precursor of nitric oxide (No). Nitric oxide is thought to play a key role in mobilizing the body's defenses against infection. Black gram is a source of protein and a commonly consumed pulse. A very limited published data exist on the formulation of food supplements to TB patients. Therefore the study was planned to formulate a food supplement for TB patients and to find the acceptability.

Materials and Methods
Raw materials such as Ragi, Soyabeans, Peanuts and Black gram were procured from local market, cleaned and stored at 8 $\pm$ 2 ºC till use.

Processing of Ingredients
Ragi, Soyabeans, Peanuts and Black gram were soaked overnight in luke-warm water. The water was removed and the grains were kept for germination in damp wet cloth. After germination the grains were pressure cooked, dried and pulverized in a plate mill to obtain fine flour. The above said processing methods were used to eliminate antinutritional factors and unpleasant beany flavor of pulses and also for easy digestibility.

Composition of Food mixtures
The processed ragi, soyabeans, peanuts and black gram were mixed to prepare four different blends. Ragi and black gram were taken 50 grams and 5 grams in each of the 4 blends, but the proportion of soyabeans and peanuts were adjusted and mixed in different proportions inorder to find the acceptability.

Table 1: Composition of Formulated Food mixtures

Ingredients	FM- I	FM- II	FM- III	FM- IV
Ragi (g)	50	50	50	50
Soyabeans (g)	25	30	35	40
Groundnuts (g)	20	15	10	5
Black gram (g)	5	5	5	5

Nutrient Composition of Formulated Food mixtures
The Nutrient compositions of four formulated food mixtures were computed on the basis of "Nutritive Value of Indian Foods" (Gopalan *et al.*, 2014) [5].

Product Development
All the four food mixtures were developed into traditional product "laddu" with the addition of jaggery and ghee. Flow chart for the preparation of laddu is given in fig.1.

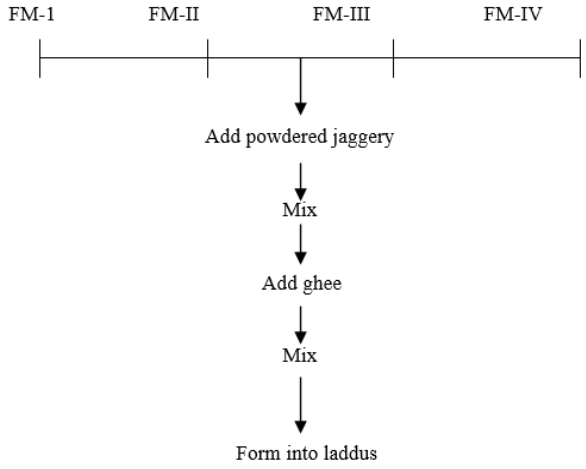

Fig 1: Flow chart for the preparation of laddu

Organoleptic Evaluation
The food mixtures that were prepared into laddus were used for evaluating the sensory properties. A panel of 10 judges evaluated the samples. Various characteristics like taste, flavor, texture, appearance and general acceptability were assessed using a nine - point Hedonic scale of excellent = 9, very good = 8, good = 7, below good and above fair = 6, fair = 6, below fair and above poor = 4, poor = 3, very poor = 2, and extremely poor = 1 (IS : Indian Standard 6273 -1971) [6]. The food mixture which is having the significantly best score was selected among the other food mixtures for further experimentation.

Evaluation of Proximate Composition
The proximate composition of the best scored food mixture viz, moisture, fat, ash and protein were analysed by (AACC, 1995) [1].

Preparation of some commonly consumed recipes by incorporating the best scored food mixture at 50 percent level.
The ready to eat food mixture can be consumed directly as laddu, porridge etc. or can also be incorporated into various dishes to avoid monotony.

Method of preparation
Commonly consumed Indian breakfast items like Plaindosa, Pesarattu, Masala vada, Mysore bonda and Chapati were selected. Provisons as well as vegetables required for the preparation of recipes were purchased from the local market

Plaindosa
It forms an essential component of majority of South Indian dishes. It is a product prepared from fermented batter of rice and blackgram dhal in the proportion ranging from 3:1 to 4:1.

Pesarattu
The main ingredient in this dish is greengram, which is soaked and ground into batter. The taste is improved by adding onions, green chillies and curry leaves. The batter is made into dosas and consumed along with chutney.

Masala vada
It is prepared from the thick batter of bengal gram. The nutritive value of the dish is enriched by the addition of curry leaves and coriander leaves.

Mysore bonda
It is also a snack item, famous in Southern India, which has black gram dhal as the main ingredient. Addition of cumin seeds, ginger and coriander leaves improve the aroma and flavor.

Chapati
It is prepared from whole wheat flour.
All the recipes were prepared in the traditional way. In all the recipes, the best scored food mixture was incorporated at 50 percent level. Traditional recipe with no substitution of food mixture served as control.

Sensory Evaluation
The recipes prepared by adding food mixture at 50 percent level were used for evaluating sensory properties. A panel of 10 judges evaluated the recepies. A Multiple Sample Difference Test was used to assess how the flavor and taste of the modified recepies differed from the basic recepies (Maynard, 1965) [11]. Modified recepies are the food mixture

incorporated recepies and basic recepies are the traditional recepies. A hedonic rating scale was also used to measure the degree of pleasurable and unpleasurable experience of tasting the recipes on a scale of nine points ranging from "like extremely" to "dislike extremely" with scores, like extremely=9, like very much=8, like moderately=7, like slightly=6, neither likes nor dislikes=5, dislike slightly=4, dislike moderately=3, dislike very much=2, dislike extremely=1 (Maynard, 1965) [12].

Shelf Life Studies

The best scored food mixture was packed in air tight containers and evaluated after 24 hrs., 30, 45 and 60 days of storage for total bacterial count, yeast and mould counts, moisture content and free fatty acids employing the standard methods. The insect infestation and change in color were observed visually. The observations were recorded in the beginning, at 30, 45 and 60 days considering the fact that such a shelf life should be adequate for a snack product.

Statistical Analysis

The data obtained through sensory evaluation were statistically analysed using Means, Standard deviations and ANOVA.

Results and Discussion

Proximate Composition of Food mixtures

The proximate composition of four food mixtures that were calculated with the help of "Nutritive Value of Indian Foods" is shown in Table No.2.

Table 2: Proximate Composition of Four Formulated Food mixtures

Food Mixture	Moisture (%)	Protein (g)	Fat (g)	Carbohydrate (g)	Energy (K cal)
I	9.72	20.71	13.61	49.42	402.75
II	9.97	21.60	12.58	49.16	396
III	10.23	22.5	11.55	48.9	359.25
IV	10.48	23.3	10.52	48.64	382.5

The data indicates that all the four formulated food mixtures for the present study are rich sources of calories and proteins. The protein content ranges from 21-23 g, whereas calorie content ranges from 383-403 kilo calories. The food mixture I which contains soybeans upto 25 percent had high calorie content and less protein content compared to food mixture IV which is having soy upto 40 percent had high protein and low calorie content. This shows that as the percentage of soy increased, the calorie content decreased and protein content increased.

Organoleptic Evaluation of the Product made from Food mixtures

The mean score values of the four food mixtures for different attributes such as Appearance, Texture, Taste, Flavor and General Acceptability were studied and presented in Table No.3. The sensory scores of four food mixtures were subjected to ANOVA for each sensory attribute and are also presented in Table No.3.

Table 3: Organoleptic Evaluation of Four Food mixtures

Sensory Attribute	Food Mixture	Mean Scores	f-value	p-value	Remark
Appearance	FM I	5.00	8.533	0.000	Sig@1%
	FM II	5.89			
	FM III	4.33			
	FM IV	4.78			
Texture	FM I	6.56	2.171	0.111	Not Sig
	FM II	7.00			
	FM III	6.11			
	FM IV	6.33			
Taste	FM I	8.11	31.673	0.000	Sig@1%
	FM II	8.89			
	FM III	7.00			
	FM IV	6.33			
Flavor	FM I	7.89	46.798	0.000	Sig@1%
	FM II	8.00			
	FM III	5.22			
	FM IV	4.78			
Overall Acceptancy	FM I	8.44	29.037	0.000	Sig@1%
	FM II	8.67			
	FM III	7.22			
	FM IV	6.56			

The results in Table No.3 show that food mixture II got highest score for overall acceptability. Regarding appearance, food mixture II was found to be highly significant ($p<0.05$), followed by I, IV and III. There was no significant difference between the food mixtures for texture. Regarding taste and flavour food mixture II was found to be highly significant ($p<0.05$) followed by I, III and IV. The results thus indicate that the food mixture II was well accepted by the panelists. Soyabeans generally possess unpleasant beany flavour and bitter taste. As soya content increased, the bitterness of the product III and IV might have increased, thus obtaining lower scores. Hence, the food mixture II having soyaflour upto 30 percent was well accepted because it was devoid of off flavour and possessed acceptable characteristics. The results were in accordance with Sahay and Kachru, (1988); Deshpande (1990); Deshpande *et al.*, (2001); Deshpande Sumedha *et al.*, (2004) [15, 4, 3, 2] who obtained similar results while evaluating different products prepared as soy-blended snacks at domestic level

Proximate Composition of the Developed Product

Based on the Organoleptic Evaluation and Nutritive value calculation, Food mixture II was subjected to food analysis. Moisture, ash, fat and protein were analysed and the results are presented in Table No.4.

Table 4: Proximate Composition of the Product

Proximate Composition	g/100g
Moisture	6.0
Ash	2.96
Fat	8.37
Protein	19.86

The product had moisture content of 6.0 g and ash content of 2.96 g. The fat and protein contents of product were 8.37 g

and 19.86 g respectively. The protein content of the product was due to soya beans, peanuts and black gram. Comparing with the calculation using "Nutritive Value of Indian Foods", the protein and fat content were low. This may be due to binding of anti nutritional factors present in soya beans with proteins in other ingredients. Proteins (19.86 g) present in the food mixture met 1/3 rd of the daily protein requirement which helpes in overcoming wasting of body tissues and helpes in increasing cell mediated immunity.

Kanchana, (1990) [8] formulated high protein snack food using soyabeans had a protein content of 41g. Deshpande Sumedha *et al.*, (2004) [2] developed barley based sattu by soy fortification containing 17.2 – 24.8 g protein.

Photograph - 1

Fig 2: Laddus prepared from Food mixture – II

Sensory Evaluation of the recepies prepared by incorporating the best scored Food mixture at 50 percent level.

The data on the Degree of difference for taste and flavor for recepies prepared by incorporating the best scored food mixture at 50 percent level is given in Table No.5.

Table 5: Mean scores of Degree of difference for taste and flavor

Attribute	Reciepe	Mean ± S.D	f-value	p-value	Remarks
Taste	Plaindosa	1.4 ± 0.4	4.120	0.014	Sig@5% level
	Pesarattu	1.8 ± 0.74			
	Mysore bonda	2.4 ± 0.48			
	Vada	0.8 ± 0.7			
	Chapati	1.0 ± 0.63			
Flavour	Plaindosa	0.6 ± 0.48	1.529	0.232	Not Sig
	Pesarattu	1.4 ± 1.2			
	Mysore bonda	0.6 ± 0.8			
	Vada	0.4 ± 0.48			
	Chapati	0.2 ± 0.4			

As far as the Degree of difference for taste between the modified and basic recepies is concerned, a significant difference ($p<0.05$) existed. This difference was more for Mysore bonda, thus indicating that Vada and Chapati were more acceptable followed by Plain dosa and Pesarattu. Mysore bonda was less accepted compared to other recepies.

Analysis of Degree of difference in flavor showed that no significant difference existed in flavor between the modified and basic recepies. All the recepies were well accepted except Pesarattu.

The data on Direction of difference for taste and flavor for recepies prepared by incorporating the best scored food mixture at 50 percent level is given in Table No.6.

Table 6: Mean scores of Direction of difference for taste and flavor

Attribute	Reciepe	Mean ± S.D	f-value	p-value	Remarks
Taste	Plaindosa	2.0 ± 0.63	2.136	0.114	Not Sig
	Pesarattu	1.6 ± 0.48			
	Mysore bonda	2.4 ± 0.48			
	Vada	1.4 ± 0.48			
	Chapati	1.4 ± 0.8			
Flavor	Plaindosa	2.2 ± 0	0.187	0.942	Not Sig
	Pesarattu	1.8 ± 0.74			
	Mysore bonda	2.0 ± 0.63			
	Vada	1.8 ± 0.4			
	Chapati	1.8 ± 0.4			

Direction of difference for taste showed that no significant difference existed between the modified and basic recepies. All the recepies were equal to that of basic recipe except for Mysore bonda. The Direction of difference for flavor showed that all the modified recepies were similar to that of basic recepies and no significant difference existed.

Table 7: Mean scores of the Degree of liking

Name of the reciepe	Samples	Mean ± S.D	t-value	p-value	Remarks
Plaindosa	B	8.2 ± 0.44	4.714	0.002	Sig@1%
	M	6.2 ± 0.83			
Pesarattu	B	8.4 ± 0.54	2.683	0.028	Sig@5%
	M	7.2 ± 0.83			
Mysorebonda	B	7.6 ± 0.54	5.367	0.001	Sig@1%
	M	5.2 ± 0.83			
Vada	B	8.6 ± 0.54	2.121	0.067	Not Sig
	M	7.4 ±.14			
Chapati	B	8.4 ±.54	0.447	0.667	Not Sig
	M	8.2 ±.83			

B- Basic Recepie, M-Modified Recepie

The mean scores for Degree of liking were presented in Table No.7 and fig.2. Here the scores are given based on the degree of pleasurable and unpleasurable experience of tasting the recepies.

Analysis of the mean scores for the Degree of liking of modified and basic recepies showed that no significant difference was observed for Vada and Chapati. It clearly indicates that Vada and Chapati were well accepted. The scores obtained were almost closer to the scores of the basic recipes. For Plaindosa, Pesarattu and Mysore bonda a significant difference ($p<0.05$) existed, which clearly reflects lesser acceptability of recepies. Since Mysore bonda and Plain dosa are fermented products, soyabeans present in the food mixture might have altered the taste.

From the above observations it is noted that the food mixture incorporated recepies were well accepted by the panelists. The food mixture can be consumed directly as porridge or can be incorporated into various dishes to avoid monotony.

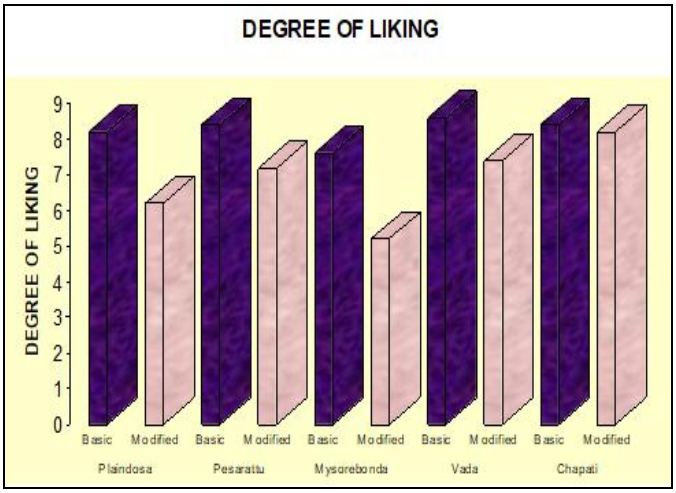

Fig 2: Comparison of mean scores for Degree of Liking

Shelf Life Studies

The shelf life of any type of food mixture depends upon its chemical composition, packaging material selected and environmental factors such as temperature, relative humidity and light. In the present study the parameters tested are as follows.

Moisture Content

Table 8: Changes in moisture content of the food mixtures at Different storage periods

Storage period (Days)	Moisture (%)
0	6.0
15	6.04
30	6.09
45	6.17
60	6.18

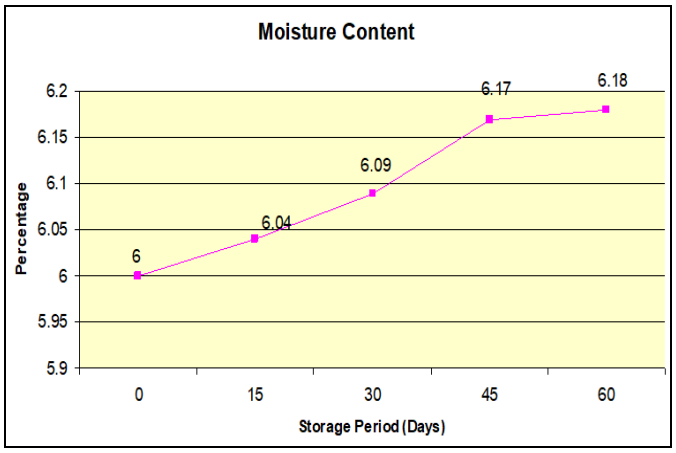

Fig 3: Changes in Moisture Content of Food mixture at different storage periods

change in the moisture content of the food mixture is given in Table No.8 and fig.3. The change in the moisture content was negligible being in the range of 6.0 to 6.1 percent from 0 to 60 days. The values are well within the range specified by the Bureau of Indian standards of nine percent of moisture content (IS: 7837, 1975) [7].

Free Fatty Acid

The changes in free fatty acid content at different storage periods are shown in Table No.9 and fig.4. The free fatty acid content increased at the time of storage. The values however increased from 0.39 to 0.64 percent. This was due to high fat content of the sample.

Table 9: Changes in Free fatty acid content

Storage period (Days)	FFA, Percent of Oleic acid (%)
0	0.39
15	0.45
30	0.53
45	0.59
60	0.64

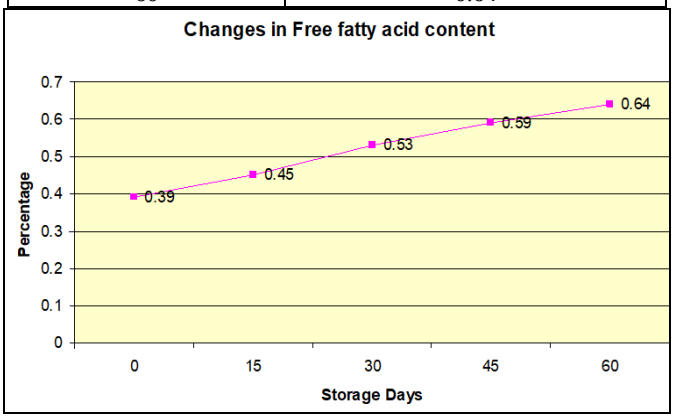

Fig 4: Changes in Free Fatty Acid content of Food mixture during storage

A value of 0.99 percent free fatty acid (% oleic acid) was used as the cut-off value for acceptability of the sample during storage in accordance with the recommendations of Mustaka and Griffin (1964) [13] for soy-based products. Here the increased value after two months was only 0.64. Free fatty acids alter the flavor of the product as well as they are carcinogenic in nature. Since free fatty acids are under normal limit, the mixture can be well accepted.

Changes in Microbial Load during storage of Food mixture

The changes in bacterial load during storage period were presented in Table No.10. The increase in bacterial load was well within the acceptable limits of total bacterial counts of $5.0x10^4$ per gram. There were no coliforms, enterobacteria, yeasts and moulds during and at the end of storage. As the moisture content is within the normal limits the growth of the bacteria is also under normal limits.

Table 10: Changes in Bacterial load during storage

Storage period	Standard plate count
15	$3x10^4$
30	$3x10^4$
45	$3.2x10^4$
60	$4.1x10^4$

As the moisture and free fatty acid contents are within the normal limits, the food mixture can be prepared in bulk amounts and can be stored upto two months.

Conclusion

The ready to eat food mixture made with 50grams ragi, 30grams soyabeans, 15 grams peanuts and 5grams black gram was found to be highly acceptable. The food mixture can be made in bulk amounts and can be stored as it had a good shelf life period. It provides additional calories and proteins necessary for Tuberculosis patients. Hence it can be supplemented to patients with Tuberculosis along with their regular diet.

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