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Clinical implication of plant protein based cookies for pulmonary TB patients

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Abstract

Tuberculosis (TB) is one of the 9th leading causes of death worldwide accompanied by under-nutrition results in compromised immune system. Many health organizations recommended plant based foods to improve human health. The study was designed to determine the composition, acceptability and effectiveness of plant protein based cookies in TB patients and was completed in nine months in two phases. In phase-I, cookies were prepared using mung bean, flaxseeds, peanuts, chickpea. Acceptability of cookies was determined by using nine-point hedonic scale. In Phase-II, efficacy trial was conducted at Gulab Devi Chest Hospital, Lahore, Pakistan. Patients in the experimental group were provided with 100g of plant-based high protein cookies and were assessed for BMI (Body Mass Index), MUAC (Mid Upper Arm Circumference) and serum total protein. Collected data were analyzed through paired sample t-test by using SPSS version 22. The results of sensory evaluation showed that sample S4 contained more protein content (11.2%) than sample S2 and S3, 9.97% and 8.83% respectively but the sample S2 showed higher overall acceptability. A total of 550.3 to 762.3kcal/g energy were provided by formulated cookies. After 6 weeks of intervention, cookie sample S2 showed significant difference in BMI and MUAC among experimental group $p=0.031$, $p=0.036$ as compared to control group. 7.1% increase in serum total protein was observed in intervention group ($p=0.001$). It was concluded that a balanced and healthy diet from the cheap plant sources added the value to the patient's nutritional status.

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Introduction

Chronic under-nutrition affects about eight million people globally out of which 98% belongs to tuberculosis endemic countries (low-middle income) (Sinha *et al.*, 2018). WHO reported one-third of world's population is infected with Mycobacterium tuberculosis, 8 million TB cases emerge annually resulting in 2 million mortalities/ year (Zumla *et al.*, 2015). To eradicate TB in Pakistan, National Tuberculosis Control Program (NTP) in collaboration with WHO initiated Directly Observed Treatment Short Course (DOTS) in 1995 (Naseer, Khawaja, Pethani, & Aleem, 2013). The root cause is insufficient intake of food both in quantity and quality. TB patients undergo in catabolic state that has negative effect on patient's immunity (vital component to control Mycobacterium tuberculosis infection), resulting in weight loss, micronutrient deficiencies (Organization, 2013) and Protein Energy Malnutrition (PEM) associated with severe illness that can be fatal to human health. The disease may leads to low body mass index (BMI) $<18.5\text{kg}/\text{m}^2$, mid-upper-arm circumference (MUAC), skinfold thickness, loss of appetite, vomiting and diarrhea leading to muscle wasting (Swaminathan *et al.*, 2008). Therefore, lack of adequate weight gain with treatment can be fatal to patient's life (Lombardo, 2012). Healthy food plays an important role in the proper functioning of the body and provides prospective health benefits. During the treatment, energy and protein requirements of the patient increases upto 35-40kcal/kg body weight (Nthiga, Mbithe, & Mugendi, 2017).

A good quality of protein in the diet of TB patient is essential for the proper functioning, repair and maintenance of body cells which helps in providing immunity to fight against fatal disease. 0.8 grams of protein per kilogram body weight is the recommended daily allowance in a diet of a normal person and it increases upto 1.2 - 1.5 grams per kilogram body weight in TB (Nthiga *et al.*, 2017). High protein diet should comprise around 15% of the total energy intake daily. Malnourished patients were provided with ready-to-use therapeutic food (RUTF) containing 16% proteins, 1.5% vitamins and found the product effective for treating severe acute malnutrition (Nisa, Baig, &

Sherwani, 2013). The diet of the patient must include high protein foods such as unrefined cereal grain (whole wheat, oats etc.), meat, fish, eggs, milk and dairy products including (cheese and yogurt), beans, legumes (lentils and chickpeas), nuts (almond, walnuts or peanuts etc), seeds, fruits and vegetables. The soy and maize based supplements were used in the Africa and showed successful rapid weight gain in malnourished patients (Warkentin *et al.*, 2013). WHO (World Health Organization) recommends liquid milk based therapeutic diet (F-100) that provide 100kcal/100ml energy for the treatment of severe malnutrition for rapid weight gain (Dargie, Tesfaye, & Worku, 2016). Various researches have been carried out to improve the nutritive value of food. As a result, many bakery products have been developed and incorporated with non-wheat flours along with conventional wheat products. The nutritive value of such food items can be boosted by addition of legume flours, nuts and seeds as they are rich in nutrients including proteins, carbohydrates, fibers, minerals and essential vitamins. Oats contain (16.89 protein content protein/100g), Chickpea (20.47 protein/100g), mung bean raw contains 23.86 proteins protein/100g, peanuts have 25.80 protein/100g and likewise flaxseed contain 18.29 proteins protein/100g (USDA) (Igbabul, Iorliam, & Umana, 2015).

Peanut (*Arachis hypogaea* L.) also known as groundnut, are the good source of proteins 22% to 30% and energy of 5.6 calories per gram (Nga *et al.*, 2013). Arginine (amino acid) present in peanuts is a precursor for Nitrogen Oxide (NO) which plays an important role against infections in human body (Ikuomola, Otutu, & Oluniran, 2017; Soh, Chee, Wang, Yuan, & Koh, 2017). A research was conducted in south Asia, where severe acute malnourished patients were provided with peanut-based RUTF had shown significant improvement in health status (Nga *et al.*, 2013). Chickpea (*Cicer arietinum* L.) is the valuable crop and serve 15% of the total pulses produced in the world. In Pakistan and other developing countries, it is one of the important components in the human diet as it is rich in proteins (Hussain, Aslam, Ghaffar, Irshad, & Din, 2015). Mung bean (*Vigna radiata*) also known as green gram, is

broadly grown in Asia and on the largest scale in Pakistan (Anwar & Saeed, 2014). It is an excellent source of proteins, carbohydrates, magnesium, phosphorus and essential vitamins (Thongram, Tanwar, Chauhan, & Kumar, 2016). Flaxseeds (*Linum usitatissimum* L.) also known as linseeds, are the beneficial source of nutrients and has tremendously served in pharmaceutical industry. It contains high amount of proteins and polyunsaturated fatty acids such as linolenic acid that prevents diseases such as cancers, cardiovascular diseases and other health problems (Clavier-Rogez, Rogez, Labrin, Branger, & Dabadie, 2015). Keeping in view the nutritional profile of the legumes, nuts and seed, the research was aimed to determine the nutritional profile, sensory characteristics and effectiveness of cookies incorporated with different flours. The results of this study may be helpful to promote and support the use of these ingredients as the cheap source of proteins to uplift the nutritional status of TB patients.

Materials & methods

Cookies Formulation

Wheat flour, Oat, chickpea, moong beans, peanuts, flaxseeds were procured from the local market of Lahore, Pakistan. All the ingredients were properly ground and weighed. in the prescribed quantity (Table 1). Straight dough method was used for cookies development (Fig. 1).

The cookie dough was dropped on to a greased tray using kitchen tool (cookie scoop) that measures 50g dough. Meanwhile, oven was preheated at 200°C for 15-20 minutes and cookies were baked at 150°C for 20 min or until light golden-brown texture was obtained (Ivan, Marie, & Barbora, 2017). Cookies were then taken out of the oven, cooled down and finally packaged in a tight polythene bag for further evaluation.

Physical attributes of cookies

The diameter was calculated by placing the cookies next to each other and total diameter was measured using ruler. The cookies were then rotated, and diameter was measured again to avoid any error. The width of cookies was measured using the Vernier

Calliper to the nearest 0.1mm. The readings were taken thrice and calculated the Mean \pm SD to avoid errors. Spread ratio was determined by dividing width by thickness (Shahzad, Anjum, Pasha, & Saeed, 2011).

Table 1. Formulation of cookies

SL	Ingredients	S1	S2	S3	S4
1	Chickpea Flour (g)	--	30	30	30
2	Moong Bean Flour (g)	--	110	110	110
3	Wheat Flour (g)	400	110	110	110
4	Peanut (chopped) (g)	--	55	55	55
5	Flaxseeds (g)	--	25	25	25
6	Butter (g)	50	100	100	100
7	Milk (ml)	100	50	100	0
8	Skimmed milk	--	50	0	100
9	Baking Powder (g)	3	6	6	6
10	Sugar (g)	150	150	150	150
11	Vanilla Essence (ml)	10	10	10	10
12	Egg	1	2	2	2
13	Salt (g)	2	4	4	4

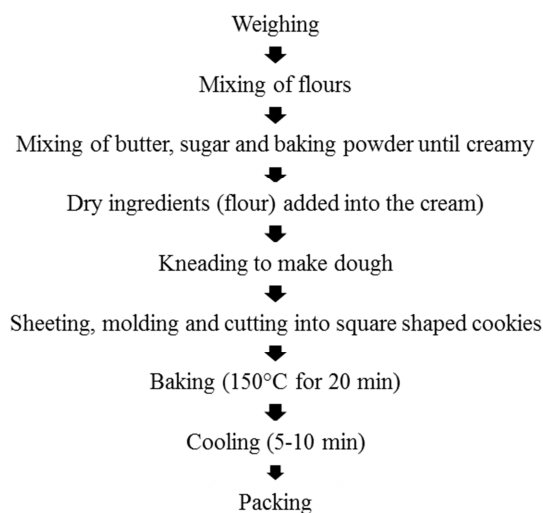


Fig. 1. Straight dough method for cookies preparation

Proximate analysis of Cookies

Moisture, ash, crude protein, crude fiber, crude fat and energy were analyzed by using methods of AACC (2000). The carbohydrate content was calculated by following formula (Adeyeye, Adebayo-Oyetoro, & Omoniyi, 2017).

$$\% \text{ Carbohydrates} = 100 - (\% \text{ Moisture} + \% \text{ Ash} + \% \text{ Protein} + \% \text{ Fiber} + \% \text{ Fat}).$$

Sensory Evaluation of Cookies

The cookies were evaluated by a panel of twenty judges for taste, colour, flavour, texture, and overall acceptability was determined using nine-point hedonic scale.

Each participant was instructed to rinse their mouth with water and was asked to have unsalted crackers to differentiate the taste between the recipes after tasting every sample (Akosua, Kwasi, Sedem, & Christopher, 2015).

Efficacy study for accepted formulation

One preferred formulation was selected on the basis of all analysis for efficacy study. The efficacy trial was approved from ethical board ref. no. IRB-UOL-FAHS/386/2018. Total 150 male tuberculosis (TB) patients were recruited from Gulab Devi Chest Hospital, Lahore, Pakistan from October 2018 to March 2019 (mention month, year).

The patients who fulfilled inclusion criteria (hospitalized adult male TB patients, BMI < 18.5 kg/m²) and signed the informed consent form were selected. Patients allergic to any ingredient in the product were excluded from the study. The selected participants (n=100) were divided into two groups, experimental (n=50) and control (n=50) by lottery method.

After completing baseline assessment including anthropometric measurements (height, weight, MUAC), serum total proteins and dietary assessment (total energy and macronutrients intake using 24-hours recall method), patients were provided with packets of 100g cookies/day for six weeks. They were prescribed to consume 1 packet/day providing approximately 22% daily value (DV) of supplemental protein by the formulation. Patients were reassessed after six weeks of intervention.

Table 3. Proximate composition of cookies

SL	Moisture Mean±S.D	Ash Mean±S.D	C. Protein Mean±S.D	C. Fats Mean±S.D	C. Fiber Mean±S.D	CHO Mean±S.D	Energy kcal/100g
S1	8.38 ± 0.25	3.36 ± 0.51	6.43 ± 0.27	21.65 ± 0.21	2.85 ± 0.2	72.11 ± 0.41	415.3
S2	5.77 ± 0.28	4.39 ± 0.1	9.97 ± 0.08	23.15 ± 0.11	1.62 ± 0.28	67.12 ± 1.97	550.3
S3	7.19 ± 0.27	3.98 ± 0.24	8.83 ± 0.36	25.2 ± 0.30	1.46 ± 0.29	68.50 ± 0.69	676.5
S4	5.47 ± 0.33	5.15 ± 0.19	11.2 ± 0.19	21.8 ± 0.37	1.64 ± 0.17	68.97 ± 1.02	762.3

Sensory attributes

Color is the important quality indicator, as it is the first thing that human eye perceive. Mean color score of formulated cookies was (7.65 ± 1.04) (7.25 ± 0.97) and (6.85 ± 0.93) S2, S3 and S4 respectively while

Results

Physical Attributes

The parameters including weight, diameter, thickness and spread ratio of the cookies were determined for all the samples. The diameter of S2 (0.584 ± 0.11mm) was lesser than other samples S3 & S4, 0.604 ± 0.07mm and 0.605 ± 0.06 mm, while 0.611 ± 0.07mm was recorded for control cookie S1. The thickness of sample S4 i.e (0.105 ± 0.09mm) was highest than S2 (0.086 ± 0.05mm) and S3 (0.095 ± 0.06mm) as shown in Table 2.

Proximate Composition

Control wheat cookies S1 had highest moisture content (8.38 ± 0.25%). Cookies sample S2 had lowest moisture content (5.77 ± 0.28%) as compared to others. The amount of proteins in wheat based cookie S1 was (6.43 ± 0.27%) and was highest in sample S4 (11.2 ± 0.19%). The amount of carbohydrates in the three samples of cookies was (67.12 ± 1.97%), (68.50 ± 0.69%), (68.97 ± 1.02%); respectively. Whereas, wheat based cookies contained 72.11 ± 0.41% carbohydrates. The amount of energy was highest in sample 4 i.e (762.3kcal/100g) but control cookie had (415.3kcal/100g) as clear in Table 3.

Table 2. Physical parameters of cookies

Parameters	S1	S2	S3	S4
Weight (g)	30.34 ± 0.47	32.54 ± 0.33	31.82 ± 0.82	32.73 ± 1.01
Diameter (mm)	0.611 ± 0.07	0.608 ± 0.03	0.604 ± 0.07	0.605 ± 0.06
Thickness (mm)	0.097 ± 0.01	0.086 ± 0.05	0.095 ± 0.06	0.105 ± 0.09
Spread Ratio	6.29 ± 0.73	6.84 ± 0.54	6.35 ± 0.34	5.81 ± 0.45

control (wheat-based cookies S1) showed highest mean score of (8.15 ± 0.75). In consumer response taste and mouth-feel are the most important attributes. Sample S2 showed highest mean score for taste and mouth-feel, (7.80 ± 0.89) and (7.75 ± 0.97)

respectively, as compared to sample S3 and S4. Whereas, for control cookie S1 was 7.00 ± 1.08 and 7.05 ± 0.89 . Among four samples of cookies, S2

achieved highest mean score (7.51 ± 0.44) for overall acceptability as shown in (Table 4) and was selected for efficacy trial.

Table 4. Sensory attributes of cookies

Sensory Attributes	S1	S2	S3	S4
	Mean \pm S.D	Mean \pm S.D	Mean \pm S.D	Mean \pm S.D
Color	8.15 ± 0.75	7.65 ± 1.04	7.25 ± 0.97	6.85 ± 0.93
Visual appearance	7.75 ± 0.85	7.15 ± 0.93	7.60 ± 1.23	6.55 ± 0.95
Texture	7.90 ± 0.72	7.20 ± 0.83	7.60 ± 0.94	6.60 ± 1.27
Taste	7.00 ± 1.08	7.80 ± 0.89	7.55 ± 0.99	6.85 ± 0.98
Flavour	7.00 ± 1.45	7.50 ± 0.95	7.25 ± 1.16	6.40 ± 1.27
Mouth feel	7.05 ± 0.89	7.75 ± 0.97	7.35 ± 0.98	6.50 ± 1.15
Overall acceptance	7.41 ± 0.53	7.51 ± 0.44	7.43 ± 0.82	6.59 ± 0.66

Effectiveness of cookies

A paired sample t-test was applied on collected efficacy trial data and there was strong association ($p = 0.018$) between the change in BMI and the high protein cookies intake in the experimental group Table 5. Patients in the experimental group had a mean BMI of 16.06kg/m^2 at baseline which increased to 16.36kg/m^2 . For mid upper arm circumference measurement (MUAC), patients in experimental group had a mean MUAC of 19.31cm which increased upto 19.49cm , whereas patients in control group had 20.84cm at baseline and 20.90

cm with 0.29% increase after trial completion. However, the control group showed the mean blood protein level of 7.30g/dl at initial stage which decreases upto 7.11g/dl at the end of study (Table 5). Patient's mean intake of protein, fats and CHO through their respective diets was also calculated. At baseline, patients in the control group consumed diet with mean of 67.87g proteins/day but an increased intake of mean protein was noticed at the end of trial while increase in mean protein intake of 65.60g to 75.17g with 14.6% increase was noted in the experimental group (Table 6).

Table 5. Mean difference between BMI, MUAC and Serum Total Protein among TB patients.

Parameters	Groups	Baseline Mean \pm S.D	After 6 weeks Mean \pm S.D	P-value
BMI	C. group	16.91 ± 1.53	17.07 ± 1.50	0.247
	E. group	16.06 ± 1.71	16.36 ± 1.72	0.031
MUAC	C. group	20.84 ± 1.47	20.90 ± 1.45	0.360
	E. group	19.31 ± 1.61	19.39 ± 1.63	0.036
Serum Total proteins	C. group	7.30 ± 0.54	7.11 ± 0.61	0.236
	E. group	6.34 ± 0.50	6.79 ± 0.49	0.000

Table 6. Mean difference between dietary intake and caloric consumption among TB patients.

24 Hrs. Food intake	Groups	Baseline Mean \pm S.D	After 6 weeks Mean \pm S.D	P-value
Protein intake	C. group	67.87 ± 10.8	76.20 ± 9.25	0.000
	E. group	65.60 ± 11.9	75.17 ± 16.5	0.009
Fats Intake	C. group	54.5 ± 12.1	58.7 ± 9.21	0.070
	E. group	57.2 ± 10.8	61.2 ± 8.32	0.055
CHO Intake	C. group	157.1 ± 35.4	158.1 ± 33.1	0.897
	E. group	170.8 ± 33.7	163.7 ± 31.3	0.224
Total Caloric Intake	C. group	1390.9 ± 201.3	1483.3 ± 184.3	0.012
	E. group	1493.5 ± 217.2	1656.5 ± 200.2	0.000

Discussion

For 4 cookies sample type, spread ratio was calculated as 6.84 ± 0.54 , 6.35 ± 0.34 , 5.81 ± 0.45 respectively

as compared to control i.e., 6.29 ± 0.73 . Increase in protein content also increases spread ratio of baked products.

This trend was also observed in study conducted by (Nanyen, Dooshima, Julius, & Benbella, 2016). Moisture content in food stuff affects the stability and freshness of food. High moisture contents promotes bacterial and yeast growth which shortens the shelf life (Khalesi, Jamaluddin, & Ismail, 2011). Cookies sample S2 had lowest moisture content ($5.77 \pm 0.28\%$) as compared to S3 ($7.19 \pm 0.27\%$) and S4 ($5.47 \pm 0.33\%$). Findings of present study i.e, flaxseed fortification decreases moisture content are in accordance with (Naseem *et al.*, 2013). Dietary proteins are essential for normal functioning of the body as they provide essential amino acids. The amount of protein was highest in sample S4 ($11.2 \pm 0.19\%$) than sample S2 and S3 ($9.97 \pm 0.08\%$) ($8.83 \pm 0.36\%$) respectively. The amount of proteins in wheat based cookie S1 was ($6.43 \pm 0.27\%$). Similar trend was observed by kaur in 2019 and they observed increase in protein content from 4.26% to 9.25% by addition of raw flaxseed flour (Kaur *et al.*, 2019).

The overall acceptability is the most quality parameter for final selection of product. Among four samples of cookies, S2 achieved highest mean score (7.51 ± 0.44) than mean scores for S3 and S4 (7.43 ± 0.82) and (6.59 ± 0.66), respectively. The result for the overall acceptability (6.59 ± 0.66 to 7.51 ± 0.44) were in accordance with results of cookies made with addition of legume flour (7.1 ± 0.80 to 7.9 ± 0.37) (Thongram *et al.*, 2016).

The tuberculosis patients in the experimental group had a mean BMI of $16.06\text{kg}/\text{m}^2$ at baseline which increased to $16.36\text{kg}/\text{m}^2$ (1.87% increase) while in control group 0.89% increase in BMI was observed at the completion of six weeks. 2-3kg Weight gain trend was observed after macronutrient supplementation for 6-8 weeks in study conducted by (Koethe & von Reyn, 2016).

MUAC of the experimental group provided with high protein cookies was improved from mean value of 19.31 ± 1.61 to 19.39 ± 1.63 cm with significant increase of 0.41% ($p=0.036$). These findings have been found to be contradictory to study conducted in South Africa which did not show any significant improvement after intervention in MUAC in children suffering from TB (Rudolph *et al.*, 2013). Formulated

cookies S2 consumption increased ($p=0.00$) serum total protein level from 6.34 g/dl to 7.1% after six weeks. In a study conducted among North Indian Patients increase in serum albumin levels was observed with supplementation of vitamin A and zinc (Ginawi, Ahmed, Ahmad, & Al-Hazimi, 2013).

Mean daily caloric intake of TB patients at baseline in the experimental group was calculated as 1493.5kcal with significant increase i.e, 1656.5kcal was observed at the end of trial ($p=0.00$). Increase in caloric intake 1390.9kcal to 1483.3kcal was calculated in the control group also ($p=0.01$) (Table 6). Similar trend of increase in total energy intake was observed in a study conducted in Singapore in nutritional supplement group ($2267 \pm 528\text{kcal}/\text{d}$) as compared to control group ($1628 \pm 370\text{kcal}/\text{d}$, $P = 0.001$) after 12 weeks of intervention (Paton, Chua, Earnest, & Chee, 2004).

Conclusions and recommendations

Under-nutrition and poor dietary habits affects about million people worldwide. The results intensely showed that incorporation of chickpea, mung bean, peanuts and flaxseeds flour in the cookies increases the nutritional value of the cookies and uplifted nutritional status of TB patients. Hence, plant proteins can serve as a cheap nutrient source to the consumers. Study was conducted for short period of time so for better results it is recommended to intervene patients for longer duration in future studies.

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Declaration

All authors contributed to reviewing, editing and approving the final version of paper for publication. The authors declare that there is no conflict of interest.

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