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Studies on development of high protein cookies

Nihir Soni, Anant S Kulkarni and Luv Patel

Abstract
Cookies are good carrier of nutrients like carbohydrate and fat which can be enriched with protein by partially replacing refined wheat flour with protein rich flour up to an acceptable level. The research study was conducted to identify most suitable blend of pulses flour for partial replacement of wheat flour. Protein energy malnutrition can be combated with such high protein cookies. Soybean, Moth bean and Chickpea were recognized as potential source of protein and sufficient biological value according to the available literature. In this study these flours were replaced with the base flour i.e. refined wheat flour at different replacement level (10, 15, 20 & 25 per cent) and cookies of the above blend were developed. These cookies were studied with respect to physical, chemical, textural and sensory analysis and Cookies with 20% Chickpea and 80% refined wheat flour was finalized and protein content increased from 2.9% to 6.8%.

Keywords: cookies, chickpea, moth bean, soybean, protein, wheat flour, protein

Introduction
Cookies refer to a baked product generally containing three major ingredients flour, sugar and fat; these are mixed with other minor ingredients to form dough. Due to its appreciated rheological characteristics, wheat is principally used in bakery products. Wheat flour is the basic structural component of most batter and dough products. It is able to perform these textural functions because of gluten content, which allows expansion of air cells and provide rigidity after baking**.** However, wheat protein is deficient in some indispensable essential amino acids and it has lower protein in comparison with oilseeds and pulses (Kulkarni *et al*. 2013 and Murugkar 2014) [8, 10]. Composite flour technology for wheat supplementation with protein rich materials like oilseeds and pulses can become an approach to overcome the malnutrition. The annual growth of bakery industry is about 10% and the demand of bakery products are increasing among all sections of people. Cookies are having wider consumption base, relatively long shelf-life, more convenience and good palatability which make it attractive for protein fortification and other nutritional improvements. Cookies are predominantly based on refined wheat flour (RWF) and the blending of RWF with oilseeds and pulses such as soybean, moth bean and chickpea can upgrade the nutritional quality. Soybean is one of the most important oil and protein crops of the world. Soybeans contain 30 to 45% protein with a good source of all indispensable amino acids. Defatted soy flour (DSF) is a cheaper, convenient, conventional and richest source of protein for the fast expanding population worldwide. Soy proteins are unique among plant proteins by virtue of their relatively high biological value and presence of essential amino acid lysine which is a limiting amino acid in most of the cereals. (Kulthe *et al*. 2011; Sreeram *et al.* 2010) [9, 19].
Mothbean has been identified as one of the potential protein food source. It is rich in protein, calcium and it can make an excellent supplement to cereal diet as illustrated by Ismail *et al*. 2003 [5].
Chickpea is also one of the affordable sources of protein, carbohydrates, minerals and vitamins, dietary fiber, folate. Moreover, it has good antioxidant activity. Scientific studies provide some evidence to support the potential beneficial effects of Chickpea components in lowering the risk of various chronic diseases such as cardiovascular diseases, obesity, cancer and diabetes. Chickpea is a good source of isoflavones, which are high in concentration in chickpeas embryonic axe fraction, particularly genistein and daidzein which have been associated with reduction of breast cancer, prostate cancer, and cardiovascular diseases. (Sreerama *et al.* 2010) [19].

In developing countries including India people subsist mainly on starchy diets which lead to malnutrition and children are the worst sufferers. Protein Energy Malnutrition (PEM) is most prevalent which leads to diseases like Kwashiorkor and Marasmus in children. The bakery products are occupying an important place in food industry due to its consistent quality and therefore, has potential to serve as a vehicle for nutrition improvement of population at low cost and without much convincing. Indian biscuit industry is the largest among all food industry and India is the 2nd largest manufacturer of biscuit and cookies in the world followed by USA. Protein enrichment of such food items will certainly improve the nutritive value of the food to help combat protein deficiency.

Indian Population, being mainly vegetarian, by economies rather than by choice, the unbalanced diets results in deficiency hazards. To help combat this problem, inclusion of low cost protein rich materials in diet is necessary. Considering the 55% consumption in rural areas and 37% consumption by lower income groups, biscuits and cookies can serve as vehicle for providing additional nutrition at affordable cost.

Looking towards the health benefits of protein we have tried to replace wheat flour with soybean, moth bean & chickpea which are naturally good source of protein.

Methodology

Procurement of raw materials

The raw material required for the research includes Refined wheat flour, Soybean, Moth bean, Chickpea, Sugar, Shortening, Leavening agent and Flavoring agent (Custard powder) which were procured from D-Mart super store.

Preparation of raw materials

Sprouting

Pulses such as soybean, mothbean and chickpea were sprouted before converting into flours. In this process seeds were cleaned thoroughly to remove dust, dirt, stubbles and foreign matter. Seeds were soaked in distilled water for 4 h at room temperature. Then, the excess water was drained, sample were further rinsed with distilled water, and soaked again in water for 4 hours after that the seeds and cotyledons were utilized for the further processes and the hulls were discarded (Murugkar, 2014) [10].

Defatting of soy flour

In order to increase the protein concentration of soy flour, defatting was done to extract its oil. Hexane based solvent extraction method was followed as hexane has ability to extract almost all of the oil present in soybeans (Russin *et al*. 2011) [17].

Development of recipe for the preparation of protein enriched cookies

The recipe for protein enriched cookies was developed using different ingredients such as refined wheat flour, shortening, sugar, leavening agent, flavouring agent and different pulses such as soybean, mothbean and chick pea flour.

The cookies were prepared using the following method shown in Fig.1 (Khetrapaul *et al*. 2012) [7] with standard control recipe (Kamaliya and Kamaliya, 2001) [6] and formulated trials (Table 1, 2 and 3).

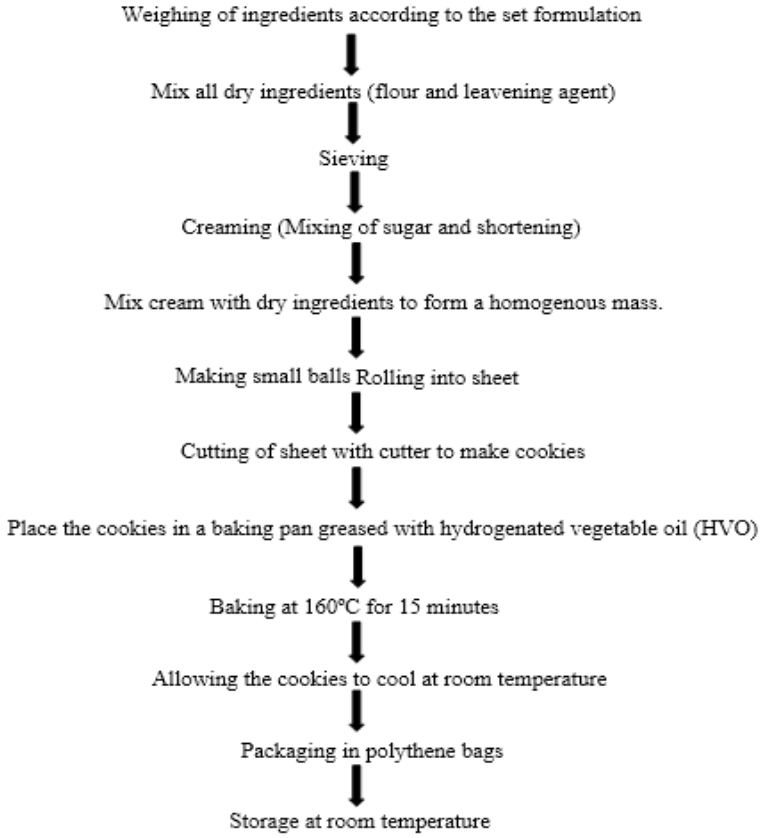

Fig 1: Flow sheet for preparation of cookies

For test samples, the refined wheat flour in the formulation was replaced with flours of soybean, moth bean and chick pea @ 10, 15, 20 and 25 percent (w/w) as shown in Table 1, 2 and 3. All the samples were baked as above (Fig.1). Each sample was prepared in duplicate.

Table 1: Standardization of recipe for preparation for preparation of cookies by incorporating soybean flour

Ingredients (g)	Control	T1	T2	T3	T4
Refined Wheat Flour	100	90	85	80	75
Soybean	--	10	15	20	25
Shortening	60	60	60	60	60
Sugar	50	50	50	50	50
Leavening Agent	1	1	1	1	1
Water (ml)	5	5	5	5	5
Flavor(custard powder)	0.5	0.5	0.5	0.5	0.5

Table 2: Standardization of recipe for preparation for preparation of cookies by incorporating moth bean flour

Ingredients (g)	Control	T1	T2	T3	T4
Refined Wheat Flour	100	90	85	80	75
Mothbean	--	10	15	20	25
Shortening	60	60	60	60	60
Sugar	50	50	50	50	50
Leavening Agent	1	1	1	1	1
Water (ml)	5	5	5	5	5
Flavor(custard powder)	0.5	0.5	0.5	0.5	0.5

Table 3: Standardization of recipe for preparation for preparation of cookies by incorporating chickpea flour

Ingredients (g)	Control	T1	T2	T3	T4
Refined Wheat Flour	100	90	85	80	75
Chickpea	--	10	15	20	25
Shortening	60	60	60	60	60
Sugar	50	50	50	50	50
Leavening Agent	1	1	1	1	1
Water (ml)	5	5	5	5	5
Flavor(custard powder)	0.5	0.5	0.5	0.5	0.5

Sensory evaluation

The samples of cookies so prepared were further subjected to sensory evaluation by trained panel consisting of 6 persons using 9-point hedonic scale (Ranganna, 1986) [15]. Ranking was noted on the sensory score card. Based on sensory score the cookies sample was optimized for the best pulse which is suitable for increasing protein content of cookies.

Physico-chemical analysis

The prepared samples of cookies incorporated with soybean, mothbean and chickpea flour at various levels by replacing refined wheat flour @ 10, 15,20 and 25 per cent were analyzed for physical properties i.e. diameter, thickness, spread factor and texture profile. Chemical analysis of the cookies sample prepared using optimized formulation based on maximum sensory score was carried out using standard methods.

Determination of diameter (width), thickness and spread factor of cookies were carried out by the standard methods described by Zucco *et al.* (2011) [21].

Diameter

To determine the diameter (D), six cookies were placed edge to edge. The total diameter of the six cookies was measured in mm by using a ruler. The cookies were rotated at an angle of 90^{O} for duplicate reading. This was repeated once more and average diameter was reported in millimeters.

Thickness

To determine the thickness (T), six cookies were placed on top of one another. The total height was measured in millimeters with the help of ruler. This process was repeated get an average value and results were reported in mm.

Spread factor

Spread factor (SF) was determined from the diameter to thickness ratio.

Textural analysis

Different samples of cookies as prepared above were studied for the effect of replacement of refined wheat flour with flours of soybean, moth bean and chickpea for its breaking strength, using Texture Analyzer (Make: Lloyd) with the test settings reported in Table 4. The measurement of each sample was done in triplicate. The texture was quantified as total load required for rupturing the cookies. Snap strength test was conducted for the same.

Table 4: Machine settings for texture analysis of cookies

Specifications	Values
Probe	Three point bend rig
Mode	Return to start
Load cell (kg)	5
Pre-test speed (mm/s)	1
Test speed (mm/s)	3
Post test speed (mm/s)	10
Test distance (mm)	5
Trigger force (g)	5
Trigger type	Auto
Tare mode	Auto
Data acquisition rate (pps)	200

Proximate analysis

Determination of Moisture content

Sample of 5g was weighed in an aluminum dish and allowed to dry in a hot air oven maintained at 100 ^{0}C for 4 hours. It was cooled in a desiccators to room temperature. Until constant weight achieved, it was placed in oven and desiccator. Difference in weight in percentage was reported as moisture content (A.O.A.C., 2012) [2].

Determination of ash content

Sample of 5g was weighed into silica crucible and placed in muffle furnace. It was kept in muffle furnace maintained at 550 ^{0}C for 4 hours. It was cooled in desiccators and weighed. This was repeated until two consecutive weights were constant. The percent ash was calculated using the difference between the initial and final weight. (A.O.A.C., 2012) [2].

Determination of fat content

Ground 5g sample was weighed accurately in thimble and defatted with petroleum ether using Soxhlet apparatus for 6-8 hours at 70 ^{0}C. The resultant ether extract was evaporated to remove traces of ether and lipid content was calculated (A.O.A.C., 2012) [2].

Determination of protein content

It was determined using micro-Kjeldahl method where 0.5 g of sample was digested with concentrated sulphuric acid (H_2SO_4) containing pinch of catalyst mixture (K_2SO_4: HgO: CuSO4, 99:4.1:0.8). Then digested solution was distilled with 40% NaOH and liberated ammonia was trapped in 4% boric acid, using mixed indicator (methyl red: bromocresol green, 1:5). The per cent nitrogen was calculated using following formula and protein percentage was quantified in the sample by multiplying with a factor of 6.25 (AOAC, 2012) [2].

$$\% N = \frac{\text{Titre (ml) x N of HCl x 0.014 x 25 x 100}}{\text{Aliquot taken x Weight of sample}}$$

% Protein = % N x 6.25

Determination of carbohydrate content

The total sugar content was estimated by Phenol - H_2SO_4 method using glucose as standard as described by Wankhede and Tharanathan (1976) [20]. The sample (0.5 g) was weighed accurately in test tube and kept in ice water bath for few minutes followed by addition of cold H_2SO_4 (72%) with gentle stirring. It was refluxed at 98^{O}C for 3 – 4 h to achieve the complete hydrolysis. The total sugar content was estimated by Phenol - H_2SO_4 method using glucose as standard. The orange yellow colour was read at 480 nm on spectrophotometer (Thermo Electron Corporation, Model: Spectronic 20D+). From the calibrated curve the concentration of sugar in hydrolytic solution was calculated and per cent total sugar in the sample was quantified.

Results and Discussion

Effect of defatting and sprouting on nutritional composition of pulses

The data presented in Table 5 illustrates that after sprouting the pulses significant variation in the ash, protein, fat and carbohydrate content was observed. Protein content of soybean, mothbean and chickpea was significantly increased due to the activation of natural enzymes. Increase in protein content of sprouted pulses further helped in increasing the protein content of cookies. Moreover, it was also observed that carbohydrate content was decreased in respective pulses after sprouting. Further sprouting of pulses was found to increase the biological value of protein. Similar results were obtained by Naik and Sekhon (2014) [12] & Rai *et al*. (2014) [14].

Table 5: Effect of sprouting on nutritional composition of pulses

Flour	Ash (%)		Protein (%)		Fat (%)		Carbohydrate (%)	
	Raw	Treated	Raw	Treated	Raw	Treated	Raw	Treated
Soy bean	2.98	4.33	18.22	33.54	9.8	4.48	67.42	56.08
Moth bean	4.3	2.01	6.41	10.2	2.31	10.74	85.55	75.63
Chickpea	2.29	1.23	15.77	16.04	5.31	2.86	74.96	74.44

Data are mean values of triplicate determination

Physical analysis

The physical parameters like thickness, diameter was measured and subsequently spread factor was calculated accordingly. The data presented in Table 6 illustrates that no significant change in the spread factor with increase in replacement levels from 0 to 25 per cent was observed. The results obtained are in good agreement with the results reported by McWatters (1977) [11].

Table 6: Physical characteristics of different blends of cookies

Parameters	Control	Soybean				Mothbean				Chickpea			
		T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
Thickness (mm)	8	7	7	7	7	6	7	9	7	7	7	7	7
Diameter (mm)	50	49	50	50	50	50	52	53	54	50	50	55	52
Spread Factor	6.25	7	7.14	7.14	7.14	8.33	7.42	5.88	7.71	7.1	7.1	7.8	7.4

Data are mean values of triplicate determination

Effect of incorporation of different pulses on textural quality of cookies

The textural characteristics of cookies incorporated with different flours namely soybean, mothbean and chickpea with different ratios are given in table 7. Varying the incorporation ratio of different pulses changed the textural characteristics of cookies significantly over the control sample. The data reported in table 7 revealed that there is variability in the breaking strength of cookies prepared with different blends of soybean. The breaking strength increased from 6.229 to 7.102 Kg.f. In case of Mothbean the breaking strength of cookies increased with increase in replacement level but at 20% replacement level the breaking strength increased marginally. In case of Chickpea, the breaking strength increased from 10 pere cent to 25 percent marginally from 4.2301 to 4.9862 Kg.f, respectively. Higher protein content in treated flours than wheat flour may be attributed to the increased breaking strength of the cookies in proportion to the wheat flour. Although the protein content might have increased in blends of cookies but they were not found acceptable on sensory grounds. The results are in good agreement with the results reported by Kulkarni and Joshi (2013) [8] and Arshad *et al.* (2012) [3].

Table 7: Effect of different ratios of different pulse flours on textural quality of cookies

Cookies sample	Breaking Strength(kg.f)	Deformation(mm)
Control	4.1409	0.46787
Soybean		
T1	6.229	1.247
T2	6.288	0.82787
T3	7.04	1.3492
T4	7.102	0.5358
Mothbean		
Mothbean (T1)	4.0749	0.57082
Mothbean (T2)	4.7507	0.60149
Mothbean (T3)	5.8566	0.78102
Mothbean (T4)	5.9691	0.42718
Chickpea		
Chickpea (T1)	4.2304	0.51554
Chickpea (T2)	4.2593	0.55962
Chickpea (T3)	4.4251	0.60891
Chickpea (T4)	4.9862	0.90148

Data are mean values of triplicate determination
Where: T1: 10 %, T2: 15%, T3: 20, T: 25%

Sensory characteristics of cookies

The cookies prepared by incorporation of different pulse flours at different ratios were subjected to the sensory

evaluation by six trained panelist using hedonic scale. The data was analyzed and the data of most preferred blends of different flours were presented in table 8. Each blend of cookies was evaluated against control and out of which one blend was selected. The preferred blends were soybean 20 per cent, mothbean 15 per cent and chickpea 20 per cent. These blends were again evaluated to get optimized cookie with high protein content up to most acceptable level. The result was obtained by rank sum method revealed that cookies incorporated with soy flour at the 20 per cent exhibited poor rankings for all the sensory attributes. Chickpea incorporated cookies was judged best for texture among all the samples due appropriate protein content as compared to the soybean and mothbean. Colour was found to be better in chickpea cookies over soybean and mothbean cookies. Marked variation in the flavor of mothbean cookies was observed over other samples This may be due to the typical flavor component, which lead to decrease in score of mothbean cookies. Significant after taste was observed in soybean and mothbean cookies. The results showed that increase in incorporation level from 0 to 25.0 % (w/v) of soybean, mothbean and chickpea flour had a significant influence on appearance, texture colour, flavour, after taste and over acceptability. The cookie prepared with a blend of 20 per cent Chick pea and 80 per cent refined wheat flour were the most preferred in terms of overall acceptability. Although the protein content might have been more in other blends of cookies but they were not found acceptable on sensory ground. Hence, incorporation level of 20 per cent of chickpea flour in cookies can be optimized for the further study on the basis of results revealed by sensory evaluation. Similar trend of results were obtained by Okpala *et al*. (2013) [13] and Ranhotra *et al*. (1980) [16].

Table 8: Mean sensory mean scores of preferred blend of cookies samples with different flours of pulses.

Preferred blends	Appearance	Texture	Colour	Flavour	After taste	Overall acceptability
Control	6.45^{a}	7.52^{b}	7.28^{b}	6.25^{a}	6.051^{b}	6.48^{b}
Soybean (T3)	5.66^{a}	6.25^{a}	5.41^{a}	6.33^{a}	5.75^{a}	6.16^{a}
Mothbean (T2)	6.5^{b}	7.35^{b}	5.64^{a}	5.15^{b}	5.68^{a}	6.51^{b}
Chickpea (T3)	7.16^{c}	7.66^{b}	7.33^{b}	6.14^{a}	6.21^{b}	6.82^{c}

The values denoted by different letters in the same column are significantly different ($p \leq 0.05$).

Chemical composition of optimized cookies

The cookies were optimized with help of sensory analysis. Proximate composition of control cookie sample and optimized cookie sample i.e. Mothbean cookies (T3, 80:20) were analyzed (Table 9). The moisture and ash content remained same in the control as well as optimized cookies sample and slight decrease in fat content was observed. There was significant rise in the protein content of cookies with 6.83. In the present study protein content was found to increase more than two fold from 2.91 to 6.83 per cent. This trend of increase in protein content in the cookies as compared to control was supported by studies carried out by Kulthe *et al*. (2011) [9]. This increase could be due to increase in the proportion of chickpea flour in the flour blend as it contains good amount of protein and an excellent complement to lysine limited cereal protein. Reports have stated that soy flour replacement in product will increase the fat content (Farzana *et al*. 2015) [4] and in comparison chickpea has lower fat content. (Aleem *et al.* 2012 and Sreerama *et al*. 2010) [1, 19]. Subsequently carbohydrate content was decreased with increase in level of chickpea flour. This may be attributed due to the replacement of cereal with pulse which are rich in protein. Results are in good agreement with the results reported by Singh *et al*. (1993) [18].

Table 9: Chemical composition of Control and Optimized Cookies sample

Sample	Moisture Content (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)
Control	1.30	1.03	2.91	22.05	72.73
Chickpea(T3)	1.33	1.14	6.83	21.51	69.38

Data are mean values of triplicate determination

Conclusions

Protein level of cookies can be increased by partially replacement of refined wheat flour with potential protein rich pulses. Soybean, Chickpea, and Mothbean were identified as potential protein rich substitutes for preparing cookies. Treatments like defatting and germination were found to be helpful in increasing the protein content of pulses. Cookies of different blends upto 25% partial replacement levels with refined wheat flour were developed. Physical, Chemical, Textural & Sensory analysis were conducted and the results of Sensory evaluation of all the developed cookies obtained by rank sum method indicates that cookies of blend prepared with 20% Chickpea and 80% refined wheat flour were the most preferred cookies out of all evaluated samples and the same was optimized. Proximate analysis of optimized and control cookie showed that more than two fold increase in protein was achieved which fulfill the objectives of the study. The outcome of the present research can be used as valuable information for the development of high protein cookies.

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