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Effect of Different Pretreatment on Trypsin Inhibitor Activity and Nutritional Composition of Moth Bean and its Utilization in Fortified Cake

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Abstract: In the present investigation, efforts were made to study the effect of different pretreatments on trypsin activity of moth bean. The results revealed that, pretreatment reduced trypsin inhibitor activity and enhanced nutritional profile of moth bean. Amongst different investigated treatments, germination found to be statistically superior over other treatments and hence germinated moth bean flour was further utilized in preparation of fortified cakes. Further, the pretreated moth bean flour was used in fortification of cakes to study its effect on quality and nutritional properties of cakes. Fortification of cake flour with moth bean flour was carried out at different proportions and its effects on sensorial quality characteristics of cakes were studied. The results revealed that germinated moth bean flour incorporation in cakes up to level of 5% of Maida could enhance the sensorial as well as nutritional quality characteristics of cake.

Key words: Moth bean · Soaking · Germination · Trypsin inhibitor activity · Cake

INTRODUCTION

Legumes are widely grown throughout the world and their dietary and economic importance is globally appreciated and recognized. They are important sources of proteins, carbohydrates including fiber, certain minerals (Ca, Mg, Zinc, Iron, Potassium and Phosphorus). Moth bean (*Vigna aconitifolia* L.) is the most drought-tolerant pulse crop grown in arid and sandy tracts of Rajasthan, India's driest state [1],. It is an exceptionally hard legume thrives in South Asia in hot, dry, tropical conditions. A legume of subfamily *Papilionoideae*, moth bean is also known by various other names including *mat*, *matki*, *math*, or mout bean.

Consumption of Moth bean is limited to some states in India. Though extensive information is available on the nutritive value of moth bean is limited. Moth beans are normally consumed in India as cooked and seasoned (with spices) beans (whole as well as dhal) or sprouted and cooked (usually stir-fried with suitable spices) beans, prior to consumption. Alternatively, the sprouted beans or *dhal* prepared from the unsprouted seeds may be deep-fat fried (*dal muthia*) and mixed with other savory snacks to add nutty flavor and a crunchy texture to a variety of savory snack dishes as well as ready-to-eat packaged snack mixes.

Moth bean seeds contained 24.1% protein, 0.8% crude fiber, 1.3% fat and 3% ash [2]. It is rich source of iron which is about 9.6mg/100 mg. The minimum solubility of moth bean proteins exhibited at pH 4.5 and maximum at pH 7.5 [3]. The water, oil absorption and foaming capacities of moth bean flours were 2.0g/g, 1.6 g/g and 27.6%, respectively [4]. Presence of anti-nutritional factors is one of the main drawbacks limiting the nutritional and food qualities of moth bean. The present investigation was undertaken to study the effect of different pretreatments on trypsin inhibition activity and to utilize pretreated moth beans in cakes to enhance its nutritional properties.

MATERIALS AND METHODS

Materials: Moth beans, butter, eggs, maida, sugar, sodium bicarbonate were purchased locally and stored at room temperature in closed container prior to further use. Trypsin was procured from Faizyme's (Cape Town, South Africa). All chemicals were purchased from Merck (Darmstadt, Germany) and were analytical grade.

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Chemical Analysis: The chemical constituents viz. carbohydrate, moisture, fat, crude protein and ash content were determined by standard method [5].

Preparation of Moth Bean Flour: Moth bean seeds were first cleaned, make free from dust and other foreign materials, then these seeds were soaked in water, 2.5% salt and 1.5% sodium bicarbonate for 12hr at 37°C. Seed to water ratio was 1:3 (w/v) used. The soaked seeds were subsequently removed from the soaking medium and rinsed with distilled water. The soaked seeds were germinated in muslin cloth for 36 to 48 hr at 25°C with frequent watering. The germinated seeds were ground in Hammer mill to pass through 60 mesh sieve and store in air tight glass bottles at 4°c for further analysis.

Estimation of Trypsin Inhibitor Activity: The trypsin inhibitor activity (TIA) of moth bean samples was measured by standard method [5]. The reagents like 0.1M phosphate buffer, 2per cent casein solution, 5per cent trichloroacetic acid and trypsin solution (5 mg. of trypsin in 100ml of 0.001 N HCl) were prepared. The 1% extract was prepared by blending 1g of the moth bean flour sample with 100ml of phosphate buffer for about 10min. Then suspension was centrifuged at 3000 rpm for 15min. The 0.2 per cent solution was prepared by diluting 20 ml of the supernatant to 1000 ml with Phosphate buffer.

Pipette out 0.2, 0.4, 0.6, 0.8 and 1.0 ml aliquots of the extract in test tubes all in triplicate. Add the chosen level of trypsin solution to all the test tubes. Make up the solution in each test tube to 2.0 ml with phosphate buffer. Place all the tubes in a water bath at 35°C for 5 min. for temperature equilibrium. To one tube in each triplicate set, add 6.0 ml of trichloroacetic acid solution, these tubes serves as blanks. Add 2ml of casein solution, previously brought to 35°C to the first tube and at 30 sec intervals, add 2ml of casein solution to each of the remaining test tubes. After exactly 20 minutes of incubation at 35°C, add 6.0ml of trichloroacetic acid solution to the remaining experimental tubes of each triplicate set, at 30 sec. intervals, Remove the test tubes from the water bath, stopper them and shake vigorously to mix the contents. Set aside for 30min to attain room temperature, centrifuge or filter the contents of the test tubes. Read optical density of the filtrate at 280 nm against the corresponding blank of each and raise the absorbance values (A) to (A) 3/2. Then trypsin inhibitor activity per ml of the extract was calculated as per the values given in table 1.

The trypsin units inhibited per g. of sample (on dry basis) was calculated by using dilution factor of the extract used in the assay.

Control Cake Preparation: Control cake sample was prepared by using Maida 200g, Sugar 150g, Fat 75g, Butter 75g, Baking powder 2g and 2 Eggs. A single-bowl mixing procedure was used for sponge cake. First the eggs were beaten to form foam in a separate vessel then sugar and butter were mixed to form cream till light color paste then egg foam and Maida mixture (Maida and baking powder) were added in cream to form batter and batter was mixed in one direction. The Kitchen Aid Professional mixer KPM5 (St. Joseph, Michigan, USA) was used. The cake batter (250 g for the sponge cake) were placed into aluminium pans 200 mm long, 150 mm wide and 68mm high. Once batters were prepared and transferred to the pans, they passed directly to the baking phase. The batters were baked in an electric oven for 25 min at 200 °C. After baking, cakes were removed from the pan, left to cool for 1 h at room temperature and packed into hermetically sealed plastic bags to prevent drying. All quality measurements were performed 4 h after baking.

Moth Bean Flour Fortified Cake Preparation: The moth bean fortified cake was prepared by incorporating the moth bean flour about 5, 7.5 and 10% with Maida (200g) and Sugar 150g, Fat 75g, Butter 75g, Baking powder 2g

Table 1: Trypsin inhibitor activity

Level of extract (ml)	Absorbance	A 3/2	TU+	TU+
0.00	0.542	0.399	40.00	-
0.20	0.497	0.331	33.10	6.90
0.40	0.394	0.297	24.70	15.30
0.60	0.279	0.148	14.80	2.20
0.80	0.179	0.076	7.60	32.40
1.00	0.099	0.031	3.10	36.90

T.U. = Trypsin unit.

T.U.I. = Trypsin Units Inhibited.

and 2 Eggs were used. First the eggs were beaten to form foam in a separate vessel then sugar and butter were mixed to form cream till light color paste then egg foam and Maida mixture (Maida, moth bean flour and baking powder) were added in cream to form batter and batter was mixed in one direction. The Kitchen Aid Professional mixer KPM5 (St. Joseph, Michigan, USA) was used. The cake batter (250 g for the sponge cake) were placed into aluminium pans 200 mm long, 150 mm wide and 68mm high. Once batters were prepared and transferred to the pans, they passed directly to the baking phase. The batters were baked in an electric oven for 25 min at 200 °C. After baking, cakes were removed from the pan, left to cool for 1 h at room temperature and packed into hermetically sealed plastic bags to prevent drying. All quality measurements were performed 4 h after baking.

Organoleptic Analysis of Cake: Prepared cakes were subjected to sensory analysis based on 9-point hedonic scale for color, taste, texture, external mouth feel and overall acceptability using a panel of 10 members who are familiar with the product since childhood. Panel members were advised to use verbal descriptions and convert them into scores. The scores were based on the following criteria: Like extremely: 9; Like moderately: 7-8; like slightly: 5-6; dislike slightly: 3-4; and dislike extremely: 0-2. The scores were averaged and rounded to the nearest whole umber.

Statistical Analysis: The data obtained were subjected to analysis of variance (ANOVA) using complete randomized designe [6]. The critical difference at $P<0.05$ was estimated and used to find significant difference if any.

RSULTS AND DISCUSSION

Proximate Composition of Moth Bean: The representative moth bean flour samples were studied to find out the proximate composition. The results on the proximate composition are presented in table 2. The results revealed that the moisture content of two different varieties of moth bean was in the range of 8.40 to 8.90%. The maximum being observed in local cultivar (8.9%) and minimum was observed in Gujrat (8.40%). The total ash present in the local cultivar and Gujrat was 2.80 and 2.96%, respectively. The fat content, highest being observed in local cultivar of moth bean (4.10%) and Gujrat cultivar was found to be 3.46%. The protein content was in the range of 23.0 to 23.59% in which the Gujrat cultivar showed the maximum protein content (23.59%) regarding the percent carbohydrate content, local cultivar had the maximum value (58.51%) while Gujrat cultivar had the lowest (57.08%).

These results are in good agreement with results, reported by Siddhuraju [7] and Kadam [8] for various moth bean cultivars.

Effect of Soaking and Germination of Moth Bean Seeds on Proximate Composition: Pretreatments such as soaking in water, 2.5% salt and 1.5% sodium bicarbonate for 12hr at 37°C and germination were observed and given in table 3. At the end of 12hr soaking period the dry weight was reduced to 98.9 and 98.3% in case of water soaked and salt soaked moth bean seeds respectively. A gradual decrease in total ash content throughout the soaking period was observed. The loss in ash content of moth bean during overnight soaking in water may be probably because of leaching of total minerals in the soaking medium. The protein content decreased from 23.02 to 22.9% in 12hr water soaked and 22.84% in 12hr salt soaked moth bean seeds. Crude fat content was also found decreased during soaking [9]. It is also possible thought that the increase in protein was due to changes resulting from the uptake of water during sprouting. The decrease in carbohydrate content in moth bean during soaking and germination, this was because of active respiration process. The decrease in fat content in moth bean during germination could be due to total solid loss during soaking prior to germination [9] or use of fat as an energy source in sprouting process.

Table 2: Proximate composition of two different cultivars of Moth bean

Parameters	Local variety	Gujrat Variety
Moisture	8.90±0.14	8.40±0.10
Fat	4.10±0.02	3.46±0.06
Ash	2.80±0.03	2.96±0.06
Protein	23.02±0.03	23.59±0.07
Carbohydrate	59.51±0.06	57.08±0.05

*Each values of represents the average of three determinations

Table 3: Effect of different pretreatments on Chemical composition of Moth bean from Local Cultivar

Varity and Treatments	Moisture	Ash	Fat	Protein	Carbohydrates
(I) Local Variety					
Controlled	8.90±0.04	2.80±0.01	4.10±0.04	23.02±0.04	58.51±0.04
water soaked	9.12±0.05	2.67±0.02	4.08±0.02	22.09±0.04	58.46±0.05
salt soaked	9.93±0.06	2.50±0.02	3.92±0.04	22.84±0.03	58.44±0.06
Sodium bicarbonate soaked	10.11±0.04	2.40±0.01	3.86±0.05	22.80±0.03	58.41±0.05
Germination	11.16±0.04	2.40±0.03	3.68±0.04	23.12±0.04	58.79±0.04
S.E.	0.026	0.026	0.057	0.151	0.005
C.D.	0.082	0.082	0.018	0.476	0.018
(II) Gujrat Variety					
Control	8.40±0.05	2.96±0.02	3.46±0.02	23.57±0.06	57.68±0.06
Water soaked	8.40±0.04	2.94±0.05	3.38±0.04	23.51±0.05	57.00±0.05
Salt soaking	9.20±0.06	2.80±0.04	3.20±0.03	23.48±0.08	56.98±0.06
Sodium bicarbonate soaked	9.60±0.07	2.43±0.03	3.40±0.02	23.45±0.06	56.94±0.04
Germination	11.23±0.10	2.41±0.02	2.98±0.02	23.63±0.03	56.87±0.04
S.E.	0.051	0.03	0.03	0.005	0.25
C.D.	0.16	0.11	0.11	0.01	0.81

*Each values of represents the average of three determinations

An increase in moisture and ash content was observed due to germination. Increase in moisture content may be due to gradual absorption of moisture by the beans. The total ash content decreased gradually throughout the germination period. Leaching out of solid matter during pre germination soaking process could be the reason for significant reduction of mineral matter on germination [10]. Ghavidel and Prakash [10], studied the impact of germination and dehulling on nutrients, antinutrients, *in vitro* iron and calcium bioavailability and *in vitro* starch and protein digestibility of green gram, cowpea, lentil and chickpea and observed a significant increase in protein, thiamin, *in vitro* iron and calcium bioavailability and *in vitro* starch and protein digestibility contents of all the legume samples due to germination.

Germination had very little effect on crude protein content except little increases, which could be due to biosynthesis during germination [11, 12].

Effect of soaking and germination on trypsin inhibitor activity of moth bean: It can be observed from table 4 that trypsin inhibitor activity (TIA) decreased from 122.1 TUI/g to 102.92 TUI /g in salt in local cultivar where as in case of Gujrat cultivar it decreases from 135.6 to 126.30 TUI /g of flour. Salt solution removed TIA of moth bean to a greater extent than plane water. Since trypsin inhibitors are low molecular proteins, their extraction from the seed to the soaking medium is quite possible. The sodium salts present in soaking medium are likely to increase the porosity of seed coat. This is one of the reasons for greater loss of TIA from the seed in salt soaking. This was because of Sodium salts present in the soaking medium likely to increase the porosity of seed coat. In case of salt soaking medium, salts help leaching the soluble into the soaking. Marquez and Alonso [13] observed a large decrease in the TIA with some losses in soluble protein content due to soaking the chickpeas in a 1 w/v sodium carbonate solution. Kadam *et al.* [8] had reported complete inactivation of moth bean TIA in 20 min heating of the beans in boiling water (100 °C) and subsequent improvement in susceptibility of 30 min moist heat denatured moth proteins.

Chemical Composition and Nutritive Value of Moth Bean Fortified Cake: Proximate analysis of cake which was prepared by different levels of incorporation of germinated moth bean flour and also control sample are presented in the Table 5.

The results of the analysis showed that moth bean flour can certainly be used for incorporating into wheat flour for the manufacture of fortified cake, which enhances the protein and fat content of cake. Though there is a reduction in total carbohydrate but there is a significant increase in the protein content of cake. From result it was observed that the cake prepared by incorporation of 10% moth bean flour contained higher amount of fat and protein 23.94 and 6.66% respectively than other samples.

Table 4: Effect of soaking and germination on trypsin inhibitor activity of Moth bean from two different cultivars

Sr. No.	Treatments and varieties of Mouth bean	Trypsin Inhibition Activity TIA (TUI/g)	% Retention of Trypsin Activity TIA (TUI/g)
1	Control sample		
	Local	122.10±0.01	100.00±0.01
	Gujrat	135.60±0.02	100.00±0.00
2	Water soaked		
	Local	111.10±0.02	99.99±0.01
	Gujrat	129.54±0.03	95.53±0.02
3	Salt Soaked		
	Local	113.51±0.02	92.96±0.01
	Gujrat	126.63±0.03	93.36±0.02
4	Sodium Bicarbonate soaked		
	Local	109.23±0.01	89.45±0.02
	Gujrat	122.18±0.02	90.10±0.03
5	Germination		
	Local	102.92±0.02	84.29±0.04
	Gujrat	116.61±0.04	85.99±0.03

*Each values of represents the average of three determinations

Table 5: Proximate analysis of cake

Sample Codes	Moisture	Ash	Fat	Protein	Carbohydrates
Control	17.2±0.05	1.00±0.01	23.90±0.05	6.01 ±0.04	50.32±0.04
A	17.8±0.04	1.20±0.02	23.87±0.02	6.44 ±0.03	49.73±0.05
B	18.1±0.06	1.40±0.01	23.91±0.04	6.52 ±0.04	49.44±0.04
C	18.5±0.07	1.50±0.03	23.94±0.02	6.66 ±0.02	49.14±0.03
SE	0.057	0.050	0.005	0.005	0.0198
CD	0.18	0.162	0.018	0.018	0.064

*Each values of represents the average of three determinations

A- 10% moth bean flour B- 15% moth bean flour C- 20% moth bean flour Control-100% wheat flour

Table 6: Effect of different levels of germinated Moth bean four on sensory quality of cake

Sample	Color	Appearance	Taste	Texture	Overall acceptability
Control	9	8	9	8	8
A	9	9	9	9	9
B	8	8	8	8	8
C	8	8	8	7	8
SE	0.18	0.057	0.057	0.057	0.31
CD	0.59	0.187	0.18	0.18	1.01

*Each values of represents the average of three determinations

A- 10% moth bean flour B- 15% moth bean flour C- 20% moth bean flour Control-100% wheat flour

The nutritive value of cake was calculated and the result revealed that the cake prepared by incorporation of 10% moth bean flour had maximum energy (733 kcal/100g) than that of control sample. Control sample contained 707 kcal/100g; there was a significant increase in energy or nutritive value as increasing incorporation level of moth bean flour. 5% and 7.5% moth bean flour fortified cake had 723 kcal /100g and 728kacal/100g, respectively.

To perform various functions in the body and to lead a healthy life, wide range of nutrients are needed. The nutrients include protein, fat, carbohydrate, vitamins and minerals. It provides maximum energy to body and higher nutrients in diet, due to the addition of egg; they are highly digestible because of good quality proteins. It would be concluded that moth bean flour fortified cake is nutritionally important; it improves nutritional quality of product. Hence germinated moth bean flour was used for the nutritional enrichment of cake.

Organoleptic evaluation of moth bean fortified cake: Cake was prepared by using germinated and non-germinated moth bean flour at the level of 5, 7.5 and 10%, sensory quality parameters were obtained by hedonic

Table 7: Effect of different levels of non germinated Moth bean flour on Sensory quality of cake

Sample	Color	Appearance	Taste	Texture	Overall acceptability
Control	9	9	9	9	9
A	8	9	9	8	9
B	7	6	6	7	8
C	7	7	6	6	7
SE	0.057	0.057	0.057	0.057	0.057
CD	0.187	0.187	0.18	0.18	0.187

*Each values of represents the average of three determinations

A- 10% moth bean flour B- 15% moth bean flour C- 20% moth bean flour Control-100% wheat flour.

scale and the average of results given by 10 panel who are familiar with the product since childhood were presented in the Table 6 and 7. The organoleptic evaluation was done with respect to color, appearance, taste, texture and overall acceptability.

The incorporation of moth bean flour at various levels indicates that the addition of moth bean flour increase the nutritional quality in terms of carbohydrate and fat % increase but the change in sensory parameters shows a different trend. Regarding the internal sensory parameters i.e, taste decline as the level of incorporation increased in both i.e, germinated and non germinated moth bean flour fortified cake. The texture was best with 5% incorporation in case of germinated fortified cake resulted in satisfactory texture.

Regarding the overall acceptability, it was found to be best in case of 5% incorporation while there was a sudden decline in the acceptability as the level of incorporation was increased to 10%. This is due to higher proportion of moth bean flour. The appearance and color decreased with different levels of incorporation moth bean flour. Data presented in tables 6 and 7 indicates that the average score of overall acceptability of germinated moth bean fortified cake was found to be comparatively higher than that of control as well as non-germinated moth bean fortified cake. But the sample with 5% incorporation received better ranking as compared to others.

The germinated moth bean flour is, rich in protein and carbohydrate. Also the *in vitro* protein digestibility of moth bean protein increases significantly during germination. Non germinated moth bean flour contains comparatively higher trypsin inhibitor activity than that of germinated moth bean flour (Table 3). Trypsin inhibitors when ingested by man in significant amounts disturb the digestive process and may lead to undesirable physiological reactions [14]. For effective utilization of moth bean in human nutrition elimination of this undesirable attribute is necessary and it can be done by germination and cooking. Germination of grain legumes is very effective treatment to remove most of tryspin inhibitor activity. Ramakrishna *et al.* [15] studied anti-nutritional factors during germination in Indian bean (*Dolichos lablab L.*) seeds and observed a considerable decrease in antinutritional factors-trypsin inhibitory activity with germination. So for that purpose germinated moth bean flour is utilized in various products than non-germinated moth bean flour to improve their nutritional importance. Awan *et al,* [16] prepared biscuits from composite flour containing moth bean flour at a level of 10 to 25% and observed good sensory score for 10% and 15% incorporated biscuits as compared to white flour. Siddique [17] used eight different legume flours, as well as moth bean, for the production of *chapatti* and observed noticeable improvements in the quality of the fortified wheat flours.

This result clearly shows that there is no significant effect on the external quality parameters with the incorporation of moth bean flour up to 5%. From result it was observed that the sample with 5% germinated moth bean flour incorporation had better external as well as internal attributes and thus had better overall acceptability. It helps in improving the nutritional quality.

CONCLUSION

In the present investigation sincere efforts have been made to study the effect of different treatments on chemical composition of moth bean seeds and its utilization into cake. Seeds of moth bean were soaked, germinated and utilized in the preparation of fortified cake. It is evident from the results that moth bean is rich in carbohydrate (58.51%) and protein (23.02 %). It was observed that on soaking of moth bean, there was decrease in protein, fat, carbohydrate and ash content. Whereas slight increase in protein, carbohydrate and moisture content was observed due to germination.

The trypsin inhibitor activity was significantly decreased on soaking seeds in salt, it may be due to increased seed coat permeability which might have allowed to trypsin inhibitor loss. The method of

germination and cooking is very effective to eliminate trypsin inhibitor activity and tannins. Germination and cooking would improve the utilization of moth bean in different bakery product.

By fortifying this moth bean flour, in batter of cake, the nutritional and sensory characteristics of the final product were analyzed. This varies with respect to the different levels of moth bean flour. Quality of the cake could be improved by fortification of the moth bean flour. Moth bean fortified cake was having better color and taste. The results of the analysis and sensory evaluation show that the incorporation of germinated moth bean flour up to 5% was found to be most acceptable to obtain cake with improved nutritional quality and good sensorial attributes.

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