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Formulation, sensory and nutritional evaluation of vitamin D enriched *Mathi*

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Abstract
The present study aimed to evaluate sensory properties, nutritional composition and shelf life of *Mathi* supplemented with vitamin D enriched button mushroom powder (produced by exposure of UV-B rays at 60cm distance for 30min time duration). Supplemented *Mathi* was highly acceptable at 10% level by panel members. *Mathi* supplemented with UV treated button mushroom powder contained high protein (p<0.05), ash (p<0.05) and fibre content (<0.01) as well as high *In-vitro* protein digestibility (p<0.01), total phenols (p<0.01) and high mineral content viz., iron, copper, phosphorus, potassium, zinc and selenium as compared to control *Mathi* (without button mushroom powder). After storage *Mathi* supplemented with vitamin D enriched button mushroom powder was acceptable upto 105 days of storage (3.5 months) and not showed any microbial growth (bacterial and fungal). Air tight glass container was best considered best packaging material than other packaging materials.

Keywords: Nutritional composition, *in-vitro* protein digestibility, total phenols, mineral content, microbial growth

Introduction
Diet with full of colorful fruits and vegetables is key of getting enough nutrients but lacks in vitamin D. Food which lacks in color also lacks in essential nutrients but in contrast, mushrooms commonly white in color contains abundance of essential nutrients. No other plant based foods produce vitamin D except mushrooms. Mushrooms are considered as vegetable but in actual they belongs to fungi kingdom instead of plant and also known as meat of vegetable world which is naturally low in fat, sodium and calories. Mushrooms are referred as functional food which provides basic nutrition and helps to prevent chronic diseases due to presence of beneficial dietary fibers (beta-glucans, chitin etc.) and antioxidants (Megan Ware 2017) [11]. Increasing consumption of whole, unprocessed foods, like mushrooms, appears to decrease the risk of obesity, diabetes, and heart disease. They also promote a healthy complexion and hair, increased energy, and overall lower weight. It is total diet or overall eating pattern that is most important in disease prevention and achieving good health. Mushrooms are also the only vegan, non-fortified dietary source of vitamin D. Dairy products are normally a good food source of vitamin D, but vegans do not consume any animal products, so mushrooms can offer an alternative source of this important vitamin (Food guide for health 2009) [5]. Ultraviolet light encompasses the 100-400 nm range of the electromagnetic spectrum and can be subdivided into three main regions. These include UV-A (315-400 nm), UV-B (280-315 nm) and UV-C (200-280 nm) (Koutchma 2009) [9]. While sunlight is a natural source of UV irradiation, there are several lamp technologies which emit artificial UV light. Ergosterol is precursor of vitamin D present in mushrooms (21-107mg/100g) which get enhanced and converted into ergocalciferols on exposure to sun rays or artificial UV rays either in growth phase or post-harvest period of mushroom (Roberts *et al*., 2008) [15]. *Mathi* is a rajasthani snack like flaky biscuit is now available at every sweet shop in India. It is made from flour, water, cumin seed (optional) etc. *Mathi* creation was influenced by need to have preserved food which stays edible for weeks in big jars at room temperature. It is popular snack mostly served in marriages, parties and poojas with pickles and even as tea-time snack. These days we see *Mathi* in different flavors like Masala Mathi, Cumin Mathi, Achari Mathi and Fenugreek leaves (methi) Mathi. Mushrooms inherent umami counter balances saltiness and allows for less salt to be used in recipes like *Mathi*. Vitamin D enriched mushroom based recipes are food based approach that would help to elevate the serum vitamin D levels of the deficient population which would have long term benefits with no side effects.

Thus vitamin D enriched mushroom powder was incorporated in the tea-time snack (*Mathi*) to enhance its nutritional composition and provide health benefits.

Material and methods

Procurement of raw materials

Ingredients required to develop *Mathi* like refined wheat flour, carom seeds, ghee, oil were procured from local market of Ludhiana whereas fresh Button mushroom (*Agaricus bisporus*) was procured from mushroom farm, PAU, Ludhiana in a single lot.

Processing of button mushroom treating with UV rays

Fresh button mushroom was cleaned, sliced longitudinally and exposed to varied UV rays such as UV-A, UV-B and UV-C using varied distance (30cm, 45cm, 60cm) at varied time durations (10min, 20min, 30min) for vitamin D enrichment followed by freeze drying at -40˚C and pulverized in fine powder further used for vitamin D estimation. Button mushroom powder with maximum vitamin D content was further used in development of *Mathi.*

Formulation and development of *Mathi*

Indian snack *Mathi* was prepared with incorporation of vitamin D enriched button mushroom powder replacing main ingredient (refined flour) listed in the standard method. The standard method used for product development was as follows:

Material Required

Refined wheat flour	-	90g
Button mushroom powder	-	10g
Ghee	-	20 g
Carom seeds (Ajwain)	-	¼ tsp
Salt	-	¼ tsp
Refined oil	-	50ml

Method

Refined flour, vitamin D enriched button mushroom powder, salt and carom seeds were mixed well. Ghee was added in mixture for shortening and mixed thoroughly. It was kneaded into a stiff dough and further divided into small balls. The balls were rolled into shape of *Mathi* and pricked with knife so that it remained flat even after frying. *Mathi* was deep fried till turn golden brown in colour.

Sensory evaluation

Mathi developed with vitamin D enriched button mushroom powder was evaluated for sensory attributes such as colour, appearance, aroma, texture, taste and overall acceptability by a panel of 10 trained members using 9 point hedonic scale. The judges were served each preparation with one control (without button mushroom powder) and three experimental samples (Ranganna 2002) [14]

Nutritional evaluation

Developed *Mathi* was further subjected to nutritional analysis for proximate and minerals using standard methods. *In-vitro* protein digestibility and total phenols were also analysed. Shelf life of *Mathi* was analyzed for 5 months using different packaging materials.

Proximate Analysis

Proximate composition such as Protein, Crude fat, Crude fibre, Crude ash, Carbohydrate and Energy were analyzed using standard methods suggested by AOAC (2000) [1].

In-vitro protein digestibility

The estimation was carried out by Macro kjeldahl method (Akeson and Stachman 1964) modified by (Singh *et al* 1989)

Total phenols (Singleton *et al* 1999) [17]

Extraction of Bioactive compound- phenolic compounds

The sample weighed as known quantity was taken in 100ml of the conical flask. Then add 15ml of 80% methanol acidified to pH 2.0 with 6N HCl (Hydrochloric acid) by vortex at room temperature for 30 minutes. The filtrate was decanted and re-extracted the residue for complete removal of phenolic compounds. This procedure was repeated for two times. The three supernatants were pooled, centrifuge at 6000rpm for 15min and filtered with the help of Whatman No.1 filter paper. Then the volume was made by solvent to 50ml. The sample was shifted to micro centrifuge tubes and stored at -20°C for total phenolic content (TPC) for which the known quantity of aliquot of sample was taken and volume up to 1.5ml with D/W was made. Then 0.5ml of FC reagent was added followed by adding 10ml of 7.5% Na_2CO_3 and incubated at 37°C for 60 minutes. Resulting blue color complex was read at 750nm. Total phenol content was calculated in (mg/100g) using equation as follows:

Total phenol (mg GAE/100 g) =

Std. Concentration/Std. O.D × Sample O.D/Aliquot taken × Vol made up/Sample taken × 100/1000 × Dilution factor

Mineral estimation

Minerals namely iron, copper, phosphorus, potassium, zinc and selenium were estimated by Inductively Coupled Plasma Optical Emission Spectrometry (ICP- OES) method using ICP optical emission spectrophotometer (ICP-OES Optima 2100 DV) using wet digestion.

Calculation

Mineral concentration (mg/L) = Sample concentration (ppm) – Blank concentration (ppm) x dilution factor.

Shelf life of *Mathi* using various packaging materials (Miles and Misra 1938) modified by (Thatcher and clark 1968) [18]

Different packaging materials such as zip lock polyethylene bags, glass container and plastic container were used for packing *Mathi* made by the incorporation of vitamin D enriched button mushroom powder with maximum overall acceptability and further evaluated for the bacterial and yeast/ mold growth.

The original sample contains the Colony Forming Units (CFU) /ml is measured as:

CFU/ml=Average number of colonies for a dilution x 50 x dilution factor.

Statistical Estimation

Independent sample t-test was applied for the comparison of nutritional parameters between control sample and experimental sample. The significant difference between organoleptic scores of samples were tested using One way ANOVA (Tukey's test) to compare three or more products with control sample. For storage studies, factorial ANOVA was applied to test the interaction of packaging and storage time on microbial growth.

Results and Discussions

Sensory evaluation of *Mathi*

The test samples were prepared with refined flour supplemented with vitamin D enriched button mushroom

powder and three levels such as 5%, 10% and 15% were prepared, along with one control sample. The mean scores for various sensory attributes were given by panelists are shown in Table 1.

Table 1: Sensory evaluation of *Mathi* supplemented with vitamin D enriched button mushroom powder

Treatment	Colour	Appearance	Aroma	Texture	Taste	Overall Acceptability
C	8.20^{a}±0.63	7.90^{a} ±0.57	8.05^{a} ±0.50	8.20^{a} ±0.63	8.40^{a} ±0.70	8.15^{a} ±0.41
T1	8.20^{a}±0.42	8.00^{a} ±0.47	8.05^{a} ±0.60	8.20^{a} ±0.42	7.80^{a} ±0.42	8.05^{a} ±0.32
T2	8.18^{a}±0.29	8.23^{a} ±0.30	8.18^{a} ±0.29	8.48^{a} ±0.45	8.48^{a} ±0.45	8.31^{a} ±0.17
T3	6.60^{b}±0.97	6.60^{b} ±0.97	6.60^{b} ±1.08	6.85^{b} ±1.33	6.45^{b} ±1.01	6.62^{b} ±1.01
F Value	15.886	13.732	12.134	8.385	18.578	18.420
p-Value	0.000 (<0.0001)	0.000 (<0.0001)	0.000 (<0.0001)	0.000 (<0.0001)	0.000 (<0.0001)	0.000 (<0.0001)

Means with different notation as (a and b) indicates significant difference C – control
T1 – 5% treated button mushroom powder
T2 – 10% treated button mushroom powder
T3 – 15% treated button mushroom powder

The data in above table illustrated that the average scores of all sensory attributes obtained by T2 treatment supplemented with 10% treated button mushroom powder were higher than control and other treatments. Among all sensory attributes there was no significant difference observed within T2 treatment followed by T1 treatment (5%) and control samples. On the other hand, T3 treatment (15% supplementation) scored least ranges from (6.45-6.85) and significant difference was observed as compare to control and other treatments. The reason might be the more oil absorbance with increased button mushroom powder supplemented *Mathi* which adversely effected its overall appearance, colour, taste and flavor. The mean score of overall acceptability was recorded in T2 treatment (8.31), control (8.15) and T1 treatment (8.05). The products prepared with incorporation of different nutritious foods had obtained significantly high acceptability as compared to preparation with basic ingredients.

Chauhan *et al* (2017) [3] reported that the *Mathi* prepared with the incorporation of 5% Beech mushroom (*hyposizygus tessellatus*) was observed highly acceptable than the other treatment levels. Verma and Jain (2012) prepared *Mathi* with the incorporation of dried powder of vegetables such as the mixture of spinach, mint, carrot and lotus stem respectively. The score for sensory attributes were found to be lower than *Mathi* prepared with the supplementation of fresh vegetables.

Gupta and Verma (2016) [6] revealed that with the *Mathi* supplemented with 10% of fresh fenugreek leaves scored higher than the *Mathi* prepared with the incorporation of dried fenugreek leaves whereas the scores were significantly higher than the control *Mathi*.

Bansal and Kochhar (2013) [2] prepared *Mathi* with the supplementation of 10% partially defatted peanut flour with the higher level of acceptance. Kaur and Brar (2017) [17] found that the *Mathi* prepared with 10% of mango seed kernel flour got higher level of acceptability than the other test samples.

Nutritional analysis of *Mathi*

Table 2: Proximate composition of *Mathi* supplemented with vitamin D enriched button mushroom powder (on dry weight basis)

Treatment	Protein (%)	Fat (%)	Ash (%)	Fibre (%)	Carbohydrate (%)	Energy (Kcal)
Control Mathi	5.88±0.19	34.65±0.47	1.53±0.06	0.29±0.08	52.58±0.32	545.67±4.95
Acceptable (10%TBMP)	7.77±1.11	34.61±1.11	2.97±0.62	2.81±0.04	47.25±1.79	531.57±4.76
|t|-Value	2.912	0.061	3.984	47.211	5.079	3.556
p-Value	0.044	0.954	0.016	0.000 (<0.0001)	0.007	0.024

TBMP- treated button mushroom powder at the distance of 60cm for 30min

The significant difference of proximate composition for treated button mushroom powder supplemented *Mathi* and control *Mathi* is shown in Table 2. The data revealed that *Mathi* supplemented with vitamin D enriched button mushroom powder contains significantly (p<0.05) high protein content at 7.77% in comparison of control *Mathi* at 5.88%. On the other hand, there was no significant difference observed in the fat content of supplemented *Mathi* and control *Mathi* at 34.61% and 34.65% respectively. The ash content of treated button mushroom powder supplemented *Mathi* was found to be significantly higher 2.97% (p<0.05) as compare to control *Mathi* at 1.53%. In case of fibre content, 2.81% fibre analyzed in supplemented *Mathi* which was significantly higher than the control *Mathi* as 0.29% (p<0.0001). The calculated value of carbohydrate was seen 47.25% in supplemented *Mathi* which was significantly lower than control *Mathi* 52.58% carbohydrate content was observed. Energy content was found to be 531.57Kcals in supplemented *Mathi* with treated button mushroom powder instead of control *Mathi* i.e. 545.67 Kcals.

According to Chauhan *et al* (2017) [3] *Mathi* was prepared with the supplementation of Beech mushroom (*Hypsizygus tessellatusi*) at the level of 5% and 10%. The protein content 15.23%, fat content 20.11%, ash content 2.44% followed by carbohydrate and energy content as 56.02% and 467.38 Kcals were observed at the level of 10% which were significantly higher than the control sample with 13.75% protein, 18.14% fat and 2.37% of ash whereas carbohydrate and energy were calculated at 63.18% and 470.98Kcals respectively.

The above data illustrates that the proximate composition of supplemented *Mathi* was enhanced with the incorporation of vitamin D enriched button mushroom powder which can help to improve the nutritional status of population.

***In-vitro* protein digestibility and total phenol content**

According to various studies, it was found that the processing of food enhance the digestibility of protein as well as phenolic content which improved the quality of food as compare to the raw food.

Table 3: *In-vitro* protein digestibility and total phenol content of *Mathi* supplemented with vitamin D enriched button mushroom powder (on dry weight basis)

Products	*In-vitro* protein digestibility (%)	Total phenol content (mg/100g)
Control Mathi	48.41±0.97	60.00±0.64
Acceptable Mathi (10%TBMP)	56.26±0.81	190.00±0.64
|t|-Value	10.792	143.295
p-Value	<0.0001	<0.0001

TBMP- treated button mushroom powder at the distance of 60cm for 30min

Results shown in Table 3 illustrated the *in-vitro* protein digestibility of *Mathi* prepared by adding vitamin D enriched button mushroom powder at 56.26% which was significantly higher than control *Mathi* at 48.41%. Dhanesh *et al* (2018a) [] prepared *Mathi* with incorporation of 10% defatted peanut cake flour and 1% fenugreek leave powder was observed significantly higher content of *in-vitro* protein digestibility at 60.22% and lower in control *Mathi* at 42.97%.

Similarly total phenols of *Mathi* supplemented with vitamin D enriched button mushroom powder was observed at 190mg/100gm which was significantly (p<0.0001) higher in comparison of control *Mathi* at 60mg/100gm. Kaur and Aggrawal (2015) [] prepared potato–rice based *Chkali* by using three different varieties of potato and analyzed total phenols content. Significantly higher content of total phenols was observed in *Chkali* supplemented with boiled mashed potato (k.Pukhraj) at 80.51mg GAE/100g in comparison of other two varieties i.e. Chipsona-1 and Chandramukhi at 51.56 and 56.01mg GAE/100g and control *Chkali* at 41.00mg GAE/100g.

The above data revealed that the incorporation of vitamin D enriched button mushroom powder enhanced the total phenols content of *Mathi* as compared to the standard products as well as freeze drying process also improved the total phenols content in mushroom powder.

Mineral content of *Mathi*

From Table 4 it can be seen that the mineral content of *Mathi* supplemented with vitamin D enriched button mushroom powder was significantly higher than that in control *Mathi*. Further, it was seen that the iron content of Supplemented *Mathi* was 4.72 mg/100g which was significantly (P<0.01) higher than that in control *Mathi* at 2.99mg/100g. The copper content of supplemented *Mathi* was found significantly higher at 2.46 mg/100g as compared to 0.86mg/100g in control *Mathi*. Significant variations of phosphorus content were observed in supplemented and control *Mathi* i.e. 636.76 and 396.01mg/100g respectively. The potassium content of supplemented *Mathi* was observed at 673.47 mg/100g which was significantly (P<0.01) higher than control *Mathi* at 339.53 mg/100g. In case of zinc and selenium, supplemented *Mathi* contained 1.82mg/100g and 0.63 mg/100g respectively as compared to their presence in control *Mathi* at 0.14mg/100g and 0.21 mg/100g respectively.

Chauhan *et al* (2017) [3] developed *Mathi* with the incorporation of freeze dried Beech mushroom powder (*hypsizygus tessellatus*) at various levels i.e. 5% and 10% respectively. The results revealed that T1 treatment (5% supplementation) showed significantly higher content of potassium at 1518mg/100g as compared to control at 1492.37mg/100g respectively. In the same way, phosphorus content at 89.34mg/100g was observed in T1 treatment in comparison with control at 82.12mg/100g. Sodium content in T1 treatment found to be 63.56mg/100g which was significantly higher than control at 60.30mg/100g. Similarly, T1 treatment contained 83.98mg/100g calcium in comparison with control at 78.32mg/100g. Significant variation was observed in iron content of T1 treatment and control at 7.29mg/100g and 5.35mg/100g respectively. Significantly higher content of zinc at 2.75mg/100g was estimated in T1 treatment in comparison with control at 2.10mg/100g.

Table 4: Mineral content of *Mathi* supplemented with vitamin D enriched treated button mushroom powder (on dry weight basis)

Treatment	Iron (mg/100g)	Copper (mg/100g)	Phosphorus (mg/100g)	Potassium (mg/100g)	Zinc (mg/100g)	Selenium (mg/100g)
Control Mathi	2.99± 0.34	0.86±015	396.01±1.09	339.53±1.26	0.14±0.01	0.21±0.01
Acceptable (10%TBMP)	4.72±0.11	2.46±0.21	636.76±0.63	673.47±0.67	1.82±0.05	0.63±0.03
|t|-Value	4.802	6.208	191.370	234.546	33.242	14.568
p-Value	0.009	0.003	0.000 (<0.0001)	0.000 (<0.0001)	0.000 (<0.0001)	0.000 (<0.0001)

Values are given in Mean ± SE

TBMP- treated button mushroom powder at the distance of 60cm for 30min

Kaur and Brar (2017) [17] prepared *Mathi* with supplementation of mango kernel seed flour at different levels i.e. 10%, 20%, 30% and 40% respectively where E1 treatment (10% supplementation) was found to be highly acceptable among other treatments and used for mineral estimation. Calcium content of E1 treatment was observed at 26mg/100g as compared to control at 25.42mg/100g. In case of iron content, significant variation was observed between E1 treatment and control at 3.34mg/100g and 2.08mg/100g respectively. Similarly, magnesium content of E1 treatment contained 35.33mg/100g which was significantly higher than control at 24.72mg/100g.

The present data in above table revealed that the supplemented *Mathi* with vitamin D enriched button mushroom powder contain enhanced mineral composition in comparison of control *Mathi.*

Shelf life of supplemented *Mathi* during storage for 5 months

Microbial growth (bacteria/ yeast and mold) of *Mathi* supplemented with vitamin D enriched button mushroom powder on basis of packaging materials after storage for 5 months

Factorial ANOVA was applied for find out the effect of packaging material and time duration on the stored *Mathi* on

microbial count (Table 5). The results described that there was a no significant effect of packaging material on bacteria count, F(2,24)=3.204, p=0.058 and on fungi count, F(2,24)=2.418, p=0.110. There was a significant effect of time on bacteria count, F(3,24)=13.408, p=<0.0001as well as on fungi count, F(3,24)=12.527, p=<0.0001. There was no significant effect of interaction between packaging and time on bacteria count, F(6,24)=1.000, p=0.448 and on fungi count, F(6,24)=0.891, p=0.517.

Our data observed the microbial growth (bacterial and yeast/mold) of *Mathi* prepared with vitamin D enriched button mushroom powder were in permissible limit till 5months under good storage conditions. The increase in microbial growth is due to presence of moisture during storage period which break down fat and carbohydrate and increase microbial growth. For best results it is advisable to consume *Mathi* within 3 months of storage. On the basis of results we have concluded that the glass container is best packaging material as compared to other packaging materials such as zip lock polyethylene bags and plastic container. Nagi *et al.* (2012) [12] studied the effect of storage period and packaging on the shelf life of cereal bran incorporated biscuits. The results revealed that the after 1 month of storage the microbial count was observed in defatted cereal based biscuits. In HDPE packaging the microbial count was higher at $16.96x10^2$cfu/g as compared to laminated packed biscuits at $15.68x10^2$cfu/g after 3 months of storage period. The microbial count was in permissible limit. Lohekar (2014) [10] studied the total bacterial count of cereal pulse based value added nutritious instant mixes. Value added products were stored in different packaging materials i.e. polythene pouch and laminated aluminum pouch for 180 days. The results revealed the total bacterial count for *Instant Sev Mix* was below detectable level on 1st day in both packaging materials whereas $5x10^2$cfu/g was recorded in polyethylene pouch which was quite higher than total bacterial count of sample packed in laminated aluminum pouch at $3x10^2$ cfu/g after 180 days of storage period.

Sachan (2017) [16] studied the yeast and mold growth of supplemented Mathi prepared with iron rich food supplements such as cauliflower leaves, garden cress seeds, rice flakes and lotus stem. The yeast and mold count was found nil on 0th day whereas with the increase of storage period the yeast and mold count was also increased at $53x10^4$ cfu/g on 30th day, $95x10^4$ cfu/g on 60th day and $127x10^4$ cfu/g on 90th day of storage period.

Raja *et al.* (2014) [13] observed the yeast and mold count in fish curls prepared with the incorporation of different flours (corn flour, black gram flour and peanut flour) during 28 days of storage period. In all stored samples yeast and mold growth was not detected from initial day till 14th day of storage period. The yeast and mold count was found higher in black gram flour based sample at 2.70 log cfu/g on 21st day and 3.78 log cfu/g followed by peanut flour based sample at 2.56 log cfu/g on 21st day and 3.63 log cfu/g on 28th day of storage period. Lower yeast and mold count was observed in corn flour based samples at 2.32log cfu/g on 21st day and 3.46 log cfu/g on 28th day of storage period.

Table 5: Microbial growth (bacteria/ yeast and mold) of *Mathi* supplemented with vitamin D enriched button mushroom powder on basis of packaging materials after storage for 5 months (10^3cfu/g)

Sample	Packaging Material	Bacterial count				Yeast/mold count		
		105 Days	120 Days	135 Days	150 Days	120 Days	135 Days	150 Days
Mathi Acceptable (10%TBMP)	zip lock polyethylene bag	2.00	2.67	4.00	6.00	1.97	2.41	3.75
	glass container	1.67	2.33	3.33	3.33	1.67	2.15	3.18
	plastic container	1.67	3.33	4.00	6.00	1.80	2.34	3.59

TBMP- button mushroom powder treated with UV-B at the distance of 60cm for 30minute Medium used: Nutrient Agar/Potato Dextrose Agar
Incubation Time: 24hrs
Incubation Temperature: 37˚C

Conclusion

Incorporation of vitamin D enriched button mushroom powder has showed considerable effect on sensory properties of Indian snack *Mathi*. The results of our study conclude that incorporation of vitamin D enriched button mushroom powder upto 10% level enhanced sensory properties as well as high *in-vitro* protein digestibility, high total phenols, enhanced protein, ash, fibre and mineral content of developed *Mathi.* Thus, vitamin D enriched button mushroom powder could also use as a potential source for different snacks and functional food ingredients.

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