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Development of Vitamin D and Protein Rich Energy Bar with Mushroom

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ABSTRACT

Background: Vitamin D and protein deficiencies are amongst the most prevalent malnutrition problems of India and several other developing nations. In the present study an attempt has been made to develop a Vitamin D and protein rich energy bar utilizing mushroom powder. Among the vegetarian foods, mushroom is a very good source of Vitamin D and also rich in good quality protein.

Methods: Twelve formulations of energy bar prepared with varying proportions of mushroom powder (0-30%), sweeteners (40-50%) and cereals (20- 45%) were analyzed for nutritional, anti-oxidative, sensory, color and textural properties.

Result: A significant improvement in vitamin D, protein, crude fiber, ash and antioxidant activity contents of energy bars with increasing proportions of mushroom powder was observed. Energy bar prepared with 20% mushroom powder, 40% sweetener, 25% cereals, 10% peanuts and a 5% dry fruits showed good sensory acceptability along with a good amount of vitamin D (423.99 IU/100 g), protein (15.18 g/100 g), antioxidant activity (78.52% scavenging of DPPH (2,2-diphenyl-1-picrylhydrazyl) crude fiber (4.76 g/100 g) and ash content (2.60 g/100 g). Single bar (50 g) of this snack can provide 53% of recommended dietary allowance (RDA) of Vitamin D and 12.7% of RDA of protein for an adult.

Key words: Bar, Energy, Mushroom, Protein, Vitamin D.

INTRODUCTION

Vitamin D deficiency is very common due to life style changes during present days. According to various reports almost 70-100% of Indian population of different age groups is either deficient or insufficient in vitamin D (Sanwalka, 2015). Vitamin D deficiency often results in bone related disorders such as rickets and osteoporosis, cardiovascular diseases, diabetes, cancer and infections such as tuberculosis. Protein deficiency is also quite prevalent and can lead to growth problems, mental and physical development (Sharma and Devi, 2021). Natural sources of Vitamin D are very few and most of them are non-vegetarian. Among the vegetarian foods, mushrooms are fair source of vitamin D and concentrated source of ergosterol (vitamin D_2 precursor) (Urbain *et al.,* 2011).

Mushrooms also contain good quality proteins rich in essential amino acids like leusine, methionine, tryptophan and valine (Longvah and Deosthale, 1998; Diez and Alvarez, 2001; Agrahar-Murugkar and Subbulakshimi, 2004). Being rich in good quality proteins, mushrooms can be useful infighting the protein malnutrition in the country. Low sugars and lipid levels with no cholesterol and higher proportion of polyunsaturated fatty acids is an added advantage. Mushrooms are also good for diabetic and heart patients as they don't have any starch or cholesterol content (Wani *et al.,* 2010). Moreover, mushrooms are also rich in dietary fiber, several other vitamins (folic acid, thiamine, riboflavin and niacin) and minerals (potassium, sodium, copper, selenium, phosphorous *etc*). Mushrooms are also good source of many antioxidants (Mau *et al.,* 2004; Puttaraju *et al.,* 2006; Ferreira *et al.,* 2007; Oyetayo, 2007).

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Keeping in mind the widespread Vitamin D and protein malnutrition in India and the demand of a nutritious, healthy and convenient snacking item, the present study was planned to develop a Vitamin D and protein rich energy bar utilizing mushrooms powder along with cereals, natural sweeteners such as honey and jaggery, peanuts and dry fruits which will help in fighting the Vitamin D and protein deficiencies prevalent among the population.

MATERIALS AND METHODS

The present study was carried out at ICAR-Directorate of Mushroom Research, Solan during the year 2018.

Raw materials

Rolled oats, puffed rice, jaggery, honey, peanuts, nuts (including almond, cashew nuts and raisins) were procured from the local market of Solan, Himachal Pradesh, India.

For preparing mushroom powder, fresh white button mushrooms (*Agaricus biosporus*) procured from ICAR-Directorate of Mushroom Research, Solan were washed properly in potable water, cut into slices and dried in a cabinet or trey dryer at 50°C for 20 hours to obtain a moisture content of less than 5%. The dried mushroom slices were then ground in a grinding mill (Retsch, Model No. SM100 Standard, Germany) followed by sieving through sieve of 0.5 mm size (ISS No. 50) to convert into a fine powder.

Energy bar preparation

Twelve formulations of mushroom fortified energy bar were prepared with varying levels of mushroom powder, sweeteners and cereals (Table 1). Honey and jaggery were used as sweeteners instead of sugar to make it healthier. Cereals (oats and puffed rice), peanuts and nuts (almonds and cashew nuts) were dry roasted in a pan for 2 min to give them a crunchy texture and good aroma and then ground coarsely for the preparation of energy bar. A syrup of 80°Brix total soluble solid (TSS) was prepared by melting sweeteners (jaggery and honey in 1:1 ratio). Energy bar was prepared by cold mixing of mushroom powder, cereals, peanuts and dry fruits with sweeteners in the mixer (Hevells, Supermix 500, India). The mixture was then put into tray measuring 18×10×2 cm pre-greased with vegetable oil and sheeting was done with the help of rolling pin. After keeping this tray in the refrigerator for an hour, the bars of approximately 50±2 g weight were cut with the help of knife. These energy bars were then wrapped in butter paper and packed individually in laminated aluminium foil pouches of suitable size and stored for quality analysis (Mridula *et al.,* 2010).

Proximate composition and energy value

All the energy bar samples were analyzed for moisture, crude fat, ash and crude fibre content by following standard AOAC protocols (AOAC, 2010). Protein content of the bars was analyzed using Micro Kjeldahl method (Kelplus Classic Dx, Pelican Equipments). Total carbohydrate content was calculated by difference method (Total carbohydrate = 100-[Moisture + Protein + Fat+ Ash content]). Energy values of the bar were obtained by multiplying protein, carbohydrates and fat content by the factor 4, 4 and 9, respectively.

Table 1: Formulations of snack bar using mushroom (g/100 g)*.

Formul-ations	Mushroom powder	Sweeteners (1:1)		Rolled oats	Puffed rice
		Honey	Jaggery		
F1	0	20	20	22.5	22.5
F2		22.5	22.5	20	20
F3		25	25	17.5	17.5
F4	10	20	20	17.5	17.5
F5		22.5	22.5	15	15
F6		25	25	12.5	12.5
F7	20	20	20	12.5	12.5
F8		22.5	22.5	10	10
F9		25	25	7.5	7.5
F10	30	20	20	7.5	7.5
F11		22.5	22.5	5	5
F12		25	25	2.5	2.5

* Levels of other ingredients: Roasted peanuts 10 g and dry fruits (equal amount of almond, cashew nuts and raisins) 5 g per 100 g in each sample.

Vitamin D

For determination of Vitamin D, one gram of powdered sample was extracted thrice with 25 ml of methanol/ dicholoromethane (75:25 v/v) solution and the extract was evaporated to dryness. The residue was then dissolved in 2 ml of methanol/dicholoromethane, 75:25 v/v and filtered through a 0.45 µm nylon syringe filter. The absorption of filtrate was taken at 264 nm through a UV-VIS spectrophotometer. A standard curve was also plottedwith 100 to 1000 µg concentration of vitamin D_2 at A_{264} (Kumar and Rajput, 2011).

Antioxidant activity

Antioxidant activity of energy bar samples was determined by 2,2-diphenyl-2-picryl-hydrazyl (DPPH) inhibition method (Shimada *et al.,* 1992).

Texture analysis

Texture measurements of energy bar samples were doneby using the texture analyzer (TA-XT 2, Stable Microsystems, load cell 5 kg). Three piecesof each snack bar sample of size 2.5×2.5×2 cm were then subjected to hardness testing and the results were expressed in N.

Color analysis

Hunter Colorimeter was used to determine the color values (L, a and b) of theenergy bar samples. Hue angle (h°) and chroma (C*) are calculated by using the following formula:

$$h^\circ = \tan^{-1}(b/a)$$

$$C^* = [a^2 + b^2]^{1/2}$$

Where,
b= b values,
a= a values.

Sensory qualities

Energy bar samples were evaluated by a group of eleven panelists using nine point hedonic scale ratings for different organoleptic characteristics such as colourand appearance, flavor and taste, texture and mouth feel and overall acceptability. Nine point hedonic scale is as follows; like extremely - 9, like very much - 8, like moderately -7, like slightly - 6, neither like nor dislike - 5, dislike slightly 4, dislike moderately - 3, dislike very much - 2, dislike extremely - 1 (BIS, 1971).

Statistical analysis

The data obtained in the present study were subjected to one factor statistical analysis of variance (ANOVA) technique using completely randomized design (CRD). The critical difference value at 5 per cent levels of significance was calculated and used for making comparison among different formulations. Statistical ranks based on significant difference by Duncan's multiple range tests are given in the form of superscript over corresponding data values.

RESULTS AND DISCUSSION

Proximate composition and energy value

Proximate composition and energy values of bars prepared by following twelve formulations are given in Table 2. The energy bars showed a significant increase in moisture content (from 8.82 to 14.14 g/100 g) with increasing levels of mushroom powder and sweeteners. This could be due to hygroscopic nature of honey, jaggery and mushroom powder. Protein, fibre and ash content of energy bars also increased significantly (from 9.24 to 17.89 g/100 g, 0.94 to 6.57 g/100 g and 1.68 to 3.51 g/100 g, respectively) with increasing levels of mushrooms powder, which can be attributed to high protein, minerals and fiber content of mushrooms (Reis *et al.,* 2012; Bernaś *et al.*, 2006; Manzi *et al.,* 2001; Manzi *et al.,* 2004). Similar trends were obtained during energy bar formulation using flaxseed (Mridula *et al.*, 2013) and potato extrudates (Giri and Mridula, 2016). Fat, carbohydrate and energy values of energy bars have shown a reverse trend and decreased significantly (from 10.18 to 5.29 g/100 g, 68.72 to 61.14 g/100 g and 408.38 to 357.48 kcal/100 g) with increasing incorporation of mushroom powder as mushroom is a low calorie food with low fat and carbohydrate contents (Farzana *et al.,* 2017).

Per cent RDA of Vitamin D and protein from energy bars

During the present investigation, vitamin D content of different formulations of energy bars ranged between 92.61 to 478.04 IU/100 g (Table 3). Significant increase in vitamin D content of energy bar with increased incorporation of mushroom powder might be due to presence of provitamin D_2 (ergocalciferol) in mushrooms (Phillips *et al.,* 2011). As per the recommended dietary allowance (RDA) of Vitamin D and protein it was found that the bar incorporated with 20% mushroom powder can provide upto 53% of RDA of Vitamin D and 12.7% of RDA of protein from single snack

Table 2: Proximate composition and energy value of snack bars*.

Snack bar formulation		Moisture	Fat	Protein	Carbohydrate	Ash	Crude fibre	Energy
Mushroom %	Sweeteners %	(g/100 g)	(g/100 g)	(g/100 g)	(g/100 g)	(g/100 g)	(g/100 g)	(kcal/100 g)
0	40	8.82^{e}	10.18^{a}	10.92^{fg}	68.27^{ab}	1.81^{g}	1.12^{d}	408.38^{a}
	45	10.10^{d}	10.04^{a}	9.83^{gh}	68.31^{ab}	1.72^{h}	1.03^{d}	402.91^{b}
	50	10.17^{d}	9.73^{a}	9.70^{gh}	68.72^{a}	1.68^{h}	0.94^{d}	401.26^{b}
10	40	10.00^{d}	9.00^{b}	13.61^{cd}	65.15^{de}	2.24^{e}	2.94^{c}	396.05^{c}
	45	11.40^{c}	8.57^{bc}	10.81^{fg}	67.06^{bc}	2.17^{ef}	2.85^{c}	388.56^{d}
	50	12.87^{b}	8.13^{c}	9.24^{h}	68.01^{ab}	1.75^{gh}	2.75^{c}	382.18^{e}
20	40	10.17^{d}	7.34^{d}	15.18^{b}	64.71^{de}	2.60^{d}	4.76^{b}	385.60^{de}
	45	10.91^{cd}	7.13^{d}	12.63^{de}	67.10^{bc}	2.23^{e}	4.66^{b}	383.08^{e}
	50	13.87^{a}	6.03^{e}	11.87^{ef}	66.08^{cd}	2.14^{f}	4.91^{b}	366.10^{g}
30	40	11.24^{c}	6.21^{e}	17.89^{a}	61.14^{f}	3.51^{a}	6.57^{a}	372.04^{f}
	45	12.90^{b}	5.37^{f}	14.44^{bc}	63.93^{e}	3.35^{b}	6.48^{a}	361.85^{h}
	50	14.14^{a}	5.29^{f}	12.77^{de}	64.69^{de}	3.11^{c}	6.39^{a}	357.48^{i}
C.D. (0.05)		0.947	0.516	1.347	1.582	0.077	0.277	3.643

* Means in a column with common superscript are not significantly different at the 0.05 level of probability by Duncan's multiple range test.

Table 3: Per cent RDA of vitamin D and protein from snack bars*.

Snack bar formulation		Vitamin D (IU/100 g)	% RDA of Vitamin D obtained from a snack bar (50 g)‡	% RDA of protein obtained from a snack bar (50 g)¶
Mushroom %	Sweeteners %			
0	40	99.90^{e}	12.5	9.1
	45	92.61^{e}	11.6	8.2
	50	95.63^{e}	12.0	8.1
10	40	280.31^{d}	35.0	11.3
	45	292.14^{d}	36.5	9.0
	50	282.47^{d}	35.3	7.7
20	40	423.99^{bc}	53.0	12.7
	45	387.98^{c}	48.5	10.5
	50	407.12^{bc}	50.9	9.9
30	40	418.33^{bc}	52.3	14.9
	45	478.04^{a}	59.8	12.0
	50	445.82^{ab}	55.7	10.6
C.D. (0.05)		46.829		

‡RDA of vitamin D= 400 IU; ¶ RDA of protein = 60 g.

* Means in a column with common superscript are not significantly different at the 0.05 level of probability by Duncan's multiple range test.

bar (50 g) (Table 3). This mushroom fortified energy bar can be a good source of Vitamin D and protein and thus by contributing significantly to RDA of both these nutrients can help in alleviating protein and Vitamin D deficiencies prevalent in many developing countries.

Antioxidant activity

Antioxidant activity of energy bar also varied significantly with varying concentrations of mushroom powder and sweeteners (Fig 1). The general trend was an increase in antioxidant activity (from 39.22 to 78.52% scavenging of DPPH) of the bar with increasing concentration of mushrooms powder. Higher antioxidant properties of energy bar with more mushroom powder might be due to presence of several bioactive compounds, such as polyphenols, polysaccharides, vitamins, carotenoids and minerals in mushrooms resulting in significant antioxidant properties (Kozarski *et al.,* 2015).

Sensory quality

Sensory score for color and appearance decreased with increasing level of mushroom powder (from 7.27 to 6.09) but it remained acceptable for all the formulations (>6.0). Whereas, the sensory scores for taste and flavor, texture and mouth feel and overall acceptability were significantly higher for the bars prepared with 20% mushroom powder which might be due to a positive effect of mushroom powder on the flavor and texture of the energy bar upto this point (Table 4). In a similar study, no significant difference in sensory characteristics (color, taste, aroma, texture, flavor and overall acceptability) of cake samples was recorded up to the level of 20% replacement of refined wheat flour (*maida*) with mushroom powder (Arora *et al.,* 2017).

Table 4: Sensory evaluation of snack bar (Based on 9-point hedonic scores)*.

Snack bar formulation		Color and appearance	Taste and flavor	Texture and mouthfeel	Overall acceptability
Mushroom %	Sweeteners %				
0	40	7.27^{ab}	5.82^{d}	6.09^{bcd}	6.59^{bcd}
	45	7.32^{ab}	6.45^{bcd}	6.45^{abcd}	6.73^{abc}
	50	7.45^{a}	6.64^{abc}	6.55^{abc}	6.82^{abc}
10	40	6.50^{bc}	6.73^{abc}	6.36^{abcd}	6.55^{bcd}
	45	6.64^{abc}	6.64^{abc}	6.64^{abc}	6.82^{abc}
	50	6.45^{bc}	6.91^{abc}	6.73^{ab}	7.00^{ab}
20	40	6.82^{abc}	7.18^{ab}	7.09^{a}	7.18^{ab}
	45	6.55^{bc}	7.09^{ab}	7.00^{a}	7.00^{ab}
	50	6.73^{abc}	7.23^{a}	7.00^{a}	7.36^{a}
30	40	6.36^{c}	6.27^{cd}	6.09^{bcd}	6.18^{cd}
	45	6.27^{c}	6.23^{cd}	5.73^{d}	6.00^{d}
	50	6.09^{c}	6.27^{cd}	5.91^{cd}	5.95^{d}
C.D. (0.05)		0.876	0.771	0.811	0.676

* Means in a column with common superscript are not significantly different at the 0.05 level of probability by Duncan's multiple range test.

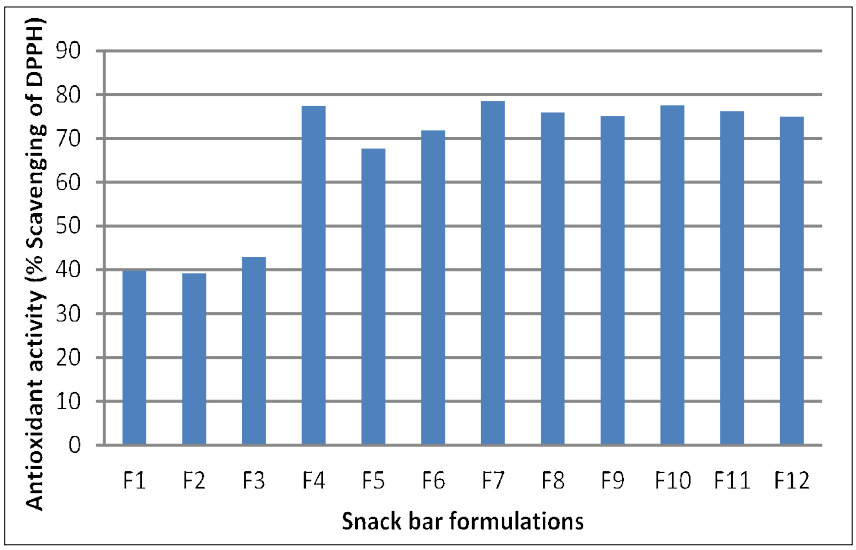


Fig 1: Antioxidant activity (% Scavenging of DPPH) of different snack bar formulations.

Colour characteristics

Although, both mushroom powder and sweeteners significantly affected the color value (L, a, b, hp and C*) of energy bar (Table 5 and Fig 2), the bar samples even at the 20% level of mushroom powder were accepted well based on organoleptic scores (Table 4). Eissa *et al.* (2007) have also reported that bread incorporated with mushroom flour (5-15%) showed darker crust hence lower crust L values as compared to control.

Textural quality

Hardness of bar represents the force required for biting into it. A significant increase in the hardness (from 27.56 to 66.5 N) of energy bars with increasing concentration of mushroom powder was observed during the present investigation (Fig 3). This might be because of compacting effect of mushroom powder on the texture of snack bar making it more dense. In similar studies, an increase in hardness of mushroom fortified bread and cake samples with increasing level of mushroom fortification have also been reported earlier (Eissa *et al.,* 2007; Majeed *et al.,* 2017). Whereas, on the contrary, legume flour incorporation has reported to cause a decrease in hardness of cereal bars (Yadav and Bhatnagar, 2017). However, as per the sensory evaluation, the scores for texture and mouth-feel of energy bars were recorded to be most acceptable for 20% level of mushroom powder incorporation (Table 4).

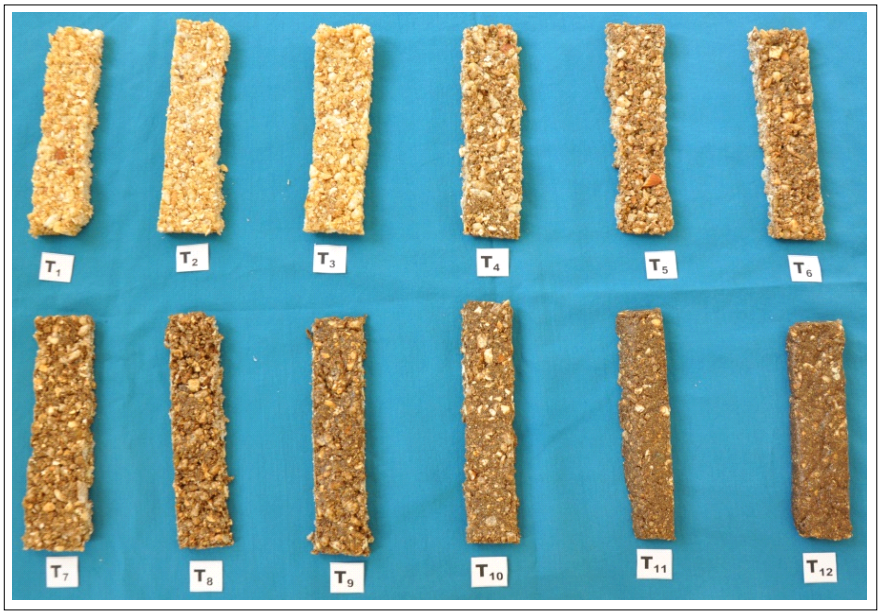


Fig 2: Different formulations of snack bars with varying mushroom and sweetener concentrations.

Table 5: Color characteristics of snack bars*.

Snack bar formulation		L	a	B	h°	C*
Mushroom %	Sweeteners %					
0	40	36.99^{b}	8.75^{a}	17.71^{e}	63.70^{l}	19.76^{d}
	45	38.54^{a}	3.71^{h}	20.74^{c}	79.85^{c}	21.07^{c}
	50	38.95^{a}	4.05^{g}	25.65^{b}	81.03^{b}	25.97^{b}
10	40	30.71^{e}	5.40^{c}	26.75^{a}	78.59^{d}	27.29^{a}
	45	31.59^{d}	2.81^{i}	19.32^{d}	81.73^{a}	19.53^{e}
	50	33.44^{c}	4.30^{f}	13.20^{g}	71.97^{e}	13.88^{g}
20	40	22.76^{hi}	4.66^{e}	12.50^{i}	69.58^{g}	13.34^{h}
	45	23.23^{gh}	5.70^{b}	14.51^{f}	68.54^{h}	15.59^{f}
	50	23.58^{g}	5.24^{d}	12.86^{h}	67.83^{i}	13.88^{g}
30	40	25.34^{f}	4.00^{g}	11.57^{k}	70.95^{f}	12.24^{i}
	45	20.34^{j}	4.09^{g}	9.05^{l}	65.69^{k}	9.93^{j}
	50	22.35^{i}	5.16^{d}	12.28^{j}	67.20^{j}	13.32^{h}
C.D. (0.05)		0.602	0.142	0.160	0.557	0.150

* Means in a column with common superscript are not significantly different at the 0.05 level of probability by Duncan's multiple range test.

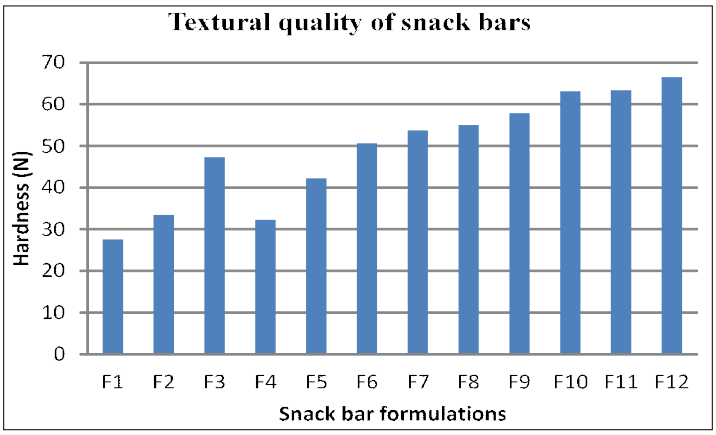


Fig 3: Textural quality of different snack bar formulations.

CONCLUSION

Mushroom fortification improved the level of Vitamin D, protein, antioxidant activity and fiber in the bars while lowering the fat content and calorific value. Energy bar prepared with 20% mushroom powder, 40% sweetener, 25% cereals, 10% peanuts and a 5% dry fruits showed good sensory acceptability along with a good amount of vitamin D (423.99 IU/100 g), protein (15.18 g/100 g), antioxidant activity (78.52% scavenging of DPPH (2,2-diphenyl-1-picrylhydrazyl), crude fiber (4.76 g/100 g), ash (2.60 g/100 g) and phenols (383.45 mg Gallic acid equivalent/100 g). Single bar (50 g) of this mushroom fortified energy can provide 53% RDA of Vitamin D and 12.7% RDA of protein for an adult. This mushroom fortified energy bar can be a healthy and nutritious snacking option for people of all age groups and will help in alleviating protein-energy malnutrition as well as vitamin D deficiency prevalent in the country.

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Conflict of interest: None.

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