



Integration of Oyster and Milky Mushroom Flour to Underutilized Pulses for the Development of Mushroom Analogues

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Mushrooms are considered to be a healthy food as they are low in fat, high in protein with good biological value and antioxidant properties. Mushrooms also contain appreciable amounts of dietary fiber. But their quality starts deteriorating immediately after the harvest. On the other hand, horse gram and cowpea have limited utilization due to the presence of anti nutritional factor. In order to extend the shelf life of mushrooms with added value and breakdown the limit of utilization of horse gram and cowpea, extrusion processing has been carried out to develop mushroom analogues through the combination of mushrooms with those underutilized pulses. A formulation comprising 50-75% of pulses and 25-50% mushrooms was made up and extruded with feed moisture content of 12%, an extrusion temperature of 120°C and a screw speed of 150 rpm. The

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developed products had protein content ranging from 23.01 to 24.56% with increased antioxidant and *in-vitro*- digestibility properties. Addition of mushroom flour increased the Water Absorption Index (WAI) and decreased the Water Solubility Index (WSI). Incorporation of oyster mushroom powder at 25% and 50% along with cowpea and horse gram flour was found most acceptable in terms of texture and other organoleptic attributes. Developed mushroom analogues can act as a novel vegetarian protein rich convenience product in the market to improve nutritional status of the population.

Keywords: Mushroom; pulses; extrusion; protein mushroom analogues; antioxidant.

1. INTRODUCTION

Since ancient times, mushrooms have been used as a food item. Mushrooms have always been preferred due to their unique taste and flavour making them a demand food for every man's plate [1]. Mushrooms contain 50-65% total carbohydrate, 19-35% proteins and 2-6% fat content in the form of polyunsaturated fatty acids of their dry matter [2]. In addition to that, mushrooms contain effective antioxidants, especially the phenolic compounds. Mushrooms are highly digestible and are considered as a remarkable substitute for muscle protein [3]. Mushroom cultivation is a reliable and effective way for poor resource cultivators to grow nutritious food in a short time. At present, the total mushroom production in India is approximately 0.13 million tons. From 2010-2017, the mushroom industry in India has an average growth rate of 4.3% per annum [4]. They are highly perishable in nature and they start deteriorating immediately after their harvest. Processing of mushrooms and developing ready to eat products can serve the dual purpose of prevention of post-harvest losses as well as utilization of mushrooms for the improvement of nutritional status of society [5].

Horse gram and cowpea are under-utilized pulses grown extensively in India. Pulses are valuable source of good quality proteins, carbohydrates, dietary fibers, vitamins and minerals used for culinary purposes but have limited utilization in food products due to the presence of anti-nutritional factors [6]. Among those thermal processing, extrusion is becoming popular since it is a high temperature short time (HTST) process with ability to improve product quality and acceptability by reducing anti-nutritional factors [7]. It is an important process in the manufacturing of ready to eat (RTE) food which involves shaping and cooking of foods through thermal, shear and pressure forces. Research has shown that extrusion processing

alters the structural integrity of nutritional components and make more biologically accessible to enzymes [8] for effective utilization. Studies on effect of extrusion and processing conditions on cereal products have been extensively studied but studies on mushroom and pulses combination are few. Keeping in view of the nutritional aspects of mushroom and pulses, the present study was undertaken for the development and standardization of mushroom analogues utilizing horse gram, cowpea, oyster and milky mushrooms.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Cultivated oyster (*Pleurotus florida*) and milky (*Calocybe indica*) mushrooms were purchased from farmers in Madurai, Tamil Nadu, India. Mushroom powder was prepared at Community Science College and Research Institute, TNAU-Madurai. Fresh mushrooms were steam blanched for 2 minutes. Immediately immersed in cold water for 30 seconds, reduced into pieces and dried at 50-60°C in a cabinet drier for 8-10 hours were ground into flour using an electric grinder. Horsegram (*Macrotyloma uniflorum*) and cowpea (*Vigna unguiculata*) were purchased from local market in Madurai, Tamil Nadu, India. Grains were cleaned and milled. The recovered fractions of both mushroom and pulses were passed through BS 20 sieve to obtain flour.

Level of mushroom flour and pulse flour in formulation was adjusted in pre-mix along with 2% Guar gum as shown in Table 1. Premix was dry-blended by passing through sieve (2 mm) and calculated water was sprayed over it to adjust 12% moisture in pre-mix. The mixture was again passed through BS 20 sieve and blended for 15 min to obtain uniformity. The pre-mix was packed in 1 kg Low Density Polyethylene bags (LDPE) and stored overnight (12 hours) for equilibration of moisture.

Table 1. Formulations for the development of mushroom analogues

Treatments	Pulse flour	Mushroom flour	Formulation ratio
T ₁	Cowpea	Oyster	(75:25)
T ₂	Cowpea	Oyster	(50:50)
T ₃	Cowpea	Milky	(75:25)
T ₄	Cowpea	Milky	(50:50)
T ₅	Horse gram	Oyster	(75:25)
T ₆	Horse gram	Oyster	(50:50)
T ₇	Horse gram	Milky	(75:25)
T ₈	Horse gram	Milky	(50:50)

2.2 Extrusion Processing

High temperature short-time (HTST) extrusion was conducted using Twin Screw extruder (SYSLG30-IV Model, Jinan Saibainuo Technology development co Ltd., China). Extrusion temperature employed was 120°C and screw speed (150 rpm) was kept constant. A circular die (5 mm diameter) at the exit of the barrel was used for extrusion. The analogues formed were collected, dried in cabinet tray drier at 50°C for 30 minutes to achieve 3-5% moisture content in the final product. It was sealed in Low Density Polyethylene bags (LDPE) till further analysis.

2.3 Proximate Composition

Moisture, Carbohydrate, ash, protein (N x 6.25), fat and fiber content were estimated by [9] methods.

2.4 Physical Properties

2.4.1 Water Solubility Index (WSI) and Water Absorption Index (WAI)

WAI and WSI were determined according to [10] method. Briefly, 2.5 gram of powdered sample and 30 mL of water was vigorously mixed in a 50mL centrifuge tube, incubated in a 37°C water bath for 30 min, and then centrifuged at 3500 rpm for 15 min. The supernatant was collected in a pre-weighed aluminum dishes and the residue with respect to the dry weight of sample powder used in the test was taken as water solubility index (WSI). The weight ratio of centrifuged precipitate to the dry weight of powder used in the test was taken as the water absorption index (WAI).

$$WAI (g/g) = W_p / W_d; WSI (\%) = W_{ds} / W_d$$

Where, W_p = Weight of precipitate, W_d = Weight of dry solids, W_{ds} = Weight of dissolved solids in supernatant.

2.4.2 Expansion ratio

The ratio of the diameter of product and the diameter of die used for preparing analogues was used to express the radial expansion of the sample [11]. The diameter of the sample was determined as the mean of 10 random measurements made with vernier calipers. The expansion ratio was calculated as follows:

$$\text{Expansion ratio} = \text{Analogues Diameter} / \text{Die diameter}$$

2.4.3 Bulk density

It was determined using the [12] method with slight modifications. Briefly, ten grams of mushroom analogues were filled into a 25 ml measuring cylinder. The cylinder was tapped on the bench top until no more settling was observed. The weight of the analogues was taken and bulk density was calculated with the given formulae,

$$\text{Bulk density (g/ml)} = (\text{Weight of sample} / \text{Volume of the sample after tapping}) \times 100$$

2.5 Colour Value

Hunterlab Colormeter (Lovi bond tinto meter) was used to measure the colour of the analogues. Data were received through the software in terms of L^* (lightness), ranging from zero (black) to 100 (White), a^* (Redness) + 60 (Red) to -60 (Green) and b^* (Yellowness) ranging from +60 (Yellow) to -60 (Blue) values of international (CIE) colour system.

2.6 Hardness

Instrumental hardness, maximum peak force for the analogues was measured in triplicate using a texturometer (Model JSV 1000-Japan). Analogue was compressed with a 36 mm probe of target mode distance 3 mm and trigger force of 100 kg

with Test-speed 60 mm/min and Maximum speed 100 mm/min [13].

2.7 Antioxidant Properties

Sample extractions were prepared for total phenolic content (TPC) and DPPH with methanol. One gram of ground sample was mixed with 40 mL of 70% methanol and kept overnight in a shaker for extraction.

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging was conducted as described in [14] with minor modifications. A 0.1 mM of DPPH stock solution was prepared with 100% methanol. To 0.5 mL of sample extract, 1 mL of DPPH and 1.5 mL of 100% methanol were added and the mixture was incubated for 30 min in the dark. The absorbance was read at 517 nm. The results were expressed as the % scavenging effect following this formula:

$$\% \text{ Scavenging effect} = (1 - (A_{\text{sample}} - A_{\text{sample blank}}) / A_{\text{control}}) \times 100$$

A_{control} is the absorbance of DPPH only

$A_{\text{sample blank}}$ is the absorbance of the sample only

TPC of the samples was measured using 0.2 N Folin-Ciocalteu (FC) reagent according to the method adapted from [15]. Gallic acid was used for standard curve. Results were expressed as gallic acid equivalent per gram sample (GAE/g sample).

2.8 Protein and Starch Digestibility

The *in-vitro* protein digestibility was carried out by following triple enzyme (pancreatic trypsin, bovine pancreatic chymotrypsin, and porcine intestinal peptidase) method as described by [16]. *In-vitro* starch digestibility was determined using the method followed by [17].

2.9 Sensory Evaluation

Ten untrained judges evaluated the analogues for appearance, flavor, texture, taste and overall acceptability using a nine-point hedonic scale (1 = dislike extremely and 9 = like extremely) for each sample [18].

2.10 Statistical Analysis

Statistical analysis was conducted using a SPSS Program version 20.0. Statistical significance of

the terms was done by analysis of variance (ANOVA) and significant differences were defined at $P < 0.05$. Each sample was analysed in triplicates and average values are reported.

3. RESULTS AND DISCUSSION

The proximate composition, on a dry basis, of flours and final product is depicted in Table 2. The moisture level of the raw material and mushroom analogues ranged from 3.96 to 9.60%. The protein, fat and fiber were found to be 21.92 to 27.81%, 1.09 to 2.53% and 6.42 to 16.80% respectively. The carbohydrate content was found to be 21.92-27.81% for raw materials and 24.56% for mushroom analogues.

3.1 Physical Properties

Extrusion cooking significantly ($P < 0.05$) affected all the physical parameters like WAI, WSI, expansion ratio and bulk density between all the treatments. WAI is an indicator of the ability of extruded analogues to absorb water due to starch gelatinization occurs during extrusion and its capacity in swelling of starch granules whereas WSI is an indicator of soluble molecules due to starch degradation during extrusion [19]. WAI and WSI values for analogues are shown in Table 3. The average value of WAI and WSI varied from 3.25 - 5.28 (g/g) and 22.78 - 32.43% respectively. Our study results shows that increasing the protein-rich mushroom flour concentration significantly ($P < 0.05$) increases WAI. These observed values are consistent with the results obtained in Whey protein concentrate incorporated samples [20]. The WSI of mushroom incorporated samples decreased significantly ($P < 0.05$) while increasing the concentration. This result was supported by [21] where the WSI decreased with increasing milk proteins.

Effect of extrusion parameters on expansion ratio is shown in Table 3. The expansion ratio values of analogues were in the range of 1.15 to 1.86. Ghumman et al. have studied and concluded that the expansion ratio of extruded products depends on amylose content [5] and the degree of starch gelatinization as well as protein and fibers which act as a diluent and reduces expansion of the product [22]. T_1 resulted in significantly ($P < 0.05$) higher expansion ratio 1.86 due to higher amylose content present in cowpea flour along with oyster mushroom flour containing higher protein content and lesser fiber content compared to milky mushroom flour. This result

was comparable with studies carried out in extrusion processing of black ear mushrooms [23].

Bulk density is an indication of compact and nonporous structure of extruded products which significantly affect the textural properties. Extrusion significantly ($P<0.05$) affected the bulk density of different treatments. Protein-starch and fiber-starch interactions increase the density of product [24]. So, in our study increase in the mushroom flour concentration significantly ($P<0.05$) increased the bulk density from 0.127-0.287 g/ml of sample. Moreover lower expansion ratio will lead to higher bulk density of product. Our observed results were comparable to barley and horse gram based extruded product [25].

3.2 Colour and Textural Properties

The hunter colour values of mushroom analogues are given in table 4. L^* values in extruded products are mainly due to the expansion rather than the chemical reaction of the analogues [26]. Higher L^* value, ranges from 36.05-45.42 were found in cowpea incorporated analogues as compared to horse gram added

analogues which ranges from 31.79-43.52. Increasing the concentration of milky mushroom flour significantly ($P<0.05$) reduced the L^* value which may be due to higher fibre content in milky mushroom reducing the porosity and expansion ratio of product. These results were comparable with brightness of lentil and horse gram extrudates [6]. Feed moisture and extrusion temperature significantly affect a^* and b^* values. In our study both the parameters are kept constant, so there was very less significant ($P<0.05$) difference in a^* value and no significant ($P<0.05$) difference in b^* values between the treatments are observed. Higher b^* value in oyster mushroom incorporated treatments (T_1, T_2, T_5, T_6) may be due to higher protein concentration in the formulation increases the yellowness of mushroom analogues [21].

The effect of extrusion on hardness of mushroom analogues is shown in Table 4. No significant ($P<0.05$) difference observed in between the treatments. In our results, the maximum force required to break the sample was non-significantly higher in milky mushroom incorporated treatments (T_2, T_3, T_7, T_8) due to higher fibre content which interferes with the starch-protein matrix formation [8].

Table 2. Chemical composition (g/100 g) of raw material and mushroom analogue

Proximate constituents	Oyster mushroom flour	Milky mushroom flour	Horse gram flour	Cowpea flour	Final product
Moisture (%)	9.21±0.3	9.45±0.8	9.60±0.5	9.05±0.1	3.96±0.2
Fat (%)	2.49±0.4	2.53±0.3	1.58±0.2	2.1±0.6	1.09±0.5
Crude Protein (%)	27.81±0.1	22.62±0.4	21.92±0.6	25.38±0.4	24.56±0.3
Carbohydrate (%)	40.33±0.1	41.5±0.5	56.56±0.5	53.44±0.5	61.81±0.6
Ash content (%)	7.6±0.2	7.1±0.6	3.04±0.2	3.61±0.3	3.8±0.4
Crude fiber (%)	12.56±0.1	16.80±0.1	7.3±0.6	6.42±0.2	7.78±0.1

Values are Mean ± S.D (n=3)

Table 3. Physical properties of mushroom analogues

Treatments	WAI (g/g)	WSI (%)	Expansion ratio	Bulk density (g/ml)
T ₁	3.76±0.09 ^c	28.36± 0.49 ^d	1.86±0.05 ^h	0.148±0.05 ^b
T ₂	4.55±0.08 ^d	24.18±0.26 ^b	1.55±0.05 ^d	0.201±0.05 ^c
T ₃	4.60±0.04 ^e	30.22±0.19 ^e	1.65±0.06 ^e	0.154±0.06 ^b
T ₄	5.28±0.09 ^h	22.91±0.35 ^a	1.3±0.08 ^c	0.163±0.04 ^b
T ₅	3.70±0.01 ^b	32.43±0.35 ^f	1.8±0.02 ^g	0.127±0.01 ^a
T ₆	4.75±0.05 ^g	22.81±0.94 ^a	1.27±0.02 ^b	0.287±0.04 ^e
T ₇	3.25±0.07 ^a	26.49±0.56 ^c	1.75±0.01 ^f	0.168±0.06 ^b
T ₈	4.63±0.05 ^f	22.78±0.65 ^a	1.15±0.11 ^a	0.220±0.07 ^d

^{a,b,c,d} Means with the different letter within column are significantly different ($P< 0.05$)

Values are Mean ± S.D (from 3 determinations)

Table 4. Colour values and hardness of extruded mushroom analogues

Treatments	L*	a*	b*	Hardness (N)
T ₁	36.05±1.52 ^a	6.46±0.60 ^{bc}	18.67±0.95 ^a	11.00±0.58 ^a
T ₂	35.56±1.74 ^a	2.77±0.24 ^a	17.17±0.48 ^a	12.93±0.92 ^a
T ₃	45.42±1.14 ^b	3.80±1.03 ^{ab}	18.28±1.00 ^a	13.24±1.52 ^a
T ₄	36.13±0.90 ^a	3.60±0.66 ^{ab}	13.96±1.15 ^a	14.68±1.95 ^a
T ₅	31.79±0.72 ^a	8.81±0.90 ^{ab}	17.96±1.65 ^a	11.67±0.32 ^a
T ₆	35.68±1.67 ^a	5.33±1.39 ^{ab}	17.74±0.69 ^a	12.90±0.85 ^a
T ₇	43.52±1.73 ^b	5.41±0.39 ^{ab}	12.57±4.51 ^a	13.43±2.27 ^a
T ₈	35.83±2.27 ^a	4.92±0.90 ^{ab}	17.46±1.22 ^a	14.51±1.07 ^a

^{a,b,c,d} Means with the different letter within column are significantly different ($P < 0.05$);
Values are Mean ± S. D (from 3 determinations)

3.3 Antioxidant Properties

Total phenolic content and free-radical scavenging activity of different treatments are significantly different from each other based on Duncan's test $P < 0.05$, $n=3$. Antioxidant activities of the samples are depicted in Table 5. In this study, free radical scavenging activity of the treatments varied from 74.25-90.36% DPPH inhibition whereas the total phenolic content of the samples ranged between 11.91-12.46 mg GAE/g of sample. Antioxidant potential (% DPPH inhibition) and total phenols increased with increasing the concentration of mushroom powder in the mushroom analogues. This can be attributed to inherent antioxidant activity of mushrooms. Oyster mushroom powder incorporated treatments resulted in higher DPPH inhibition (%) as compared to milky mushroom powder incorporated mushroom analogues. This results agreed with others reported value on antioxidant properties of commercially cultivated Indian edible mushrooms (button mushroom < milky mushroom < elm oyster mushroom) [1]. Incorporation of oyster mushroom in instant noodles improved the content of total phenols from 1.17 – 1.61 mg GAE/ g of sample and free radical scavenging activity 22.15-51.53% in comparison to control [27]. Likewise, addition of black ear mushroom in brown rice extrudates increased the antioxidant potential of the product

[23] Horse gram combinations resulted in significantly ($P < 0.05$) higher antioxidant properties when compared to cow pea combinations. The presence of higher phenolic indexes in horse gram when compared to chickpea and cowpea expresses comparably lower antioxidant properties [28].

3.4 Protein and Starch Digestibility

Extrusion processing is a high temperature short time process induces protein denaturation, starch gelatinization and elimination of different anti-nutritional factors like phytic acid trypsin inhibitor, thus significantly improve the *in-vitro* starch and protein digestibility [29]. The results showed in Table 5 clearly indicate that due to extrusion processing, the increase in digestibility was higher in starch than protein. Protein and starch digestibility were improved upto 78.93% and 86.52% respectively. The digestibility values obtained in our study were in agreement with those reported by [20]. Cowpea combinations (T₁ – T₄) resulted in significantly ($P < 0.05$) higher digestibility when compared to horse gram combinations. The presence of higher polyphenols, trypsin inhibitor activity and less oligosaccharides in horse gram when compared to chickpea and cowpea resulted in lower digestibility [30].

Table 5. Antioxidant properties and *in-vitro* digestibility activities

Treatments	DPPH inhibition (%)	Total phenols (mg GAE/g)	IVPD (%)	IVSD (%)
T ₁	82.91±2.04 ^c	12.19±0.03 ^d	75.31±0.60 ^c	86.52±0.75 ^{cd}
T ₂	85.60±1.65 ^d	12.15±0.04 ^c	78.93±0.60 ^d	77.37±0.15 ^d
T ₃	74.52±0.53 ^a	12.12±0.24 ^b	78.93±0.60 ^d	85.15±0.12 ^f
T ₄	76.62±1.82 ^b	11.91±0.04 ^a	77.12±0.60 ^d	80.13±0.09 ^e
T ₅	89.42±2.56 ^{ef}	12.46±0.31 ^f	68.07±0.60 ^a	76.04±0.05 ^a
T ₆	90.36±2.98 ^f	12.32±0.09 ^e	71.69±0.60 ^b	71.94±0.18 ^b
T ₇	78.05±1.42 ^b	11.92±0.06 ^a	68.07±0.60 ^a	77.20±0.22 ^d
T ₈	88.62±0.94 ^e	12.46±0.29 ^f	71.69±0.60 ^b	75.22±0.06 ^c

^{a,b,c,d} Means with the different letter within column are significantly different ($P < 0.05$)
Values are Mean ± S. D (from 3 determinations)

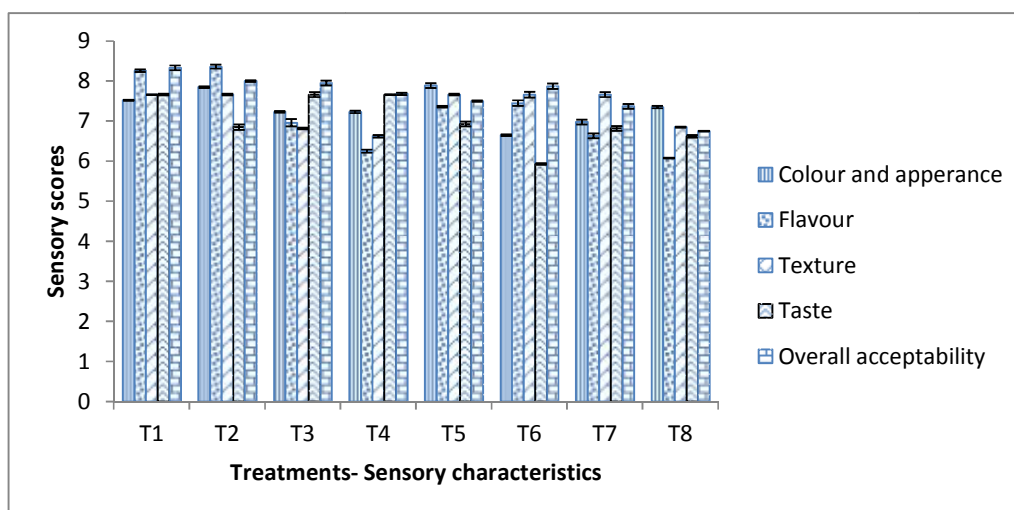


Fig. 1. Sensory characteristics of mushroom analogues. The data points are the mean \pm SD from three replicates

3.5 Sensory Evaluation

The results of sensory analysis are shown in Fig. 1. The cooked analogues were evaluated for colour & appearance, flavour, texture, taste and overall acceptability. The sensory scores for overall acceptance (OA) obtained for analogues ranges from 6.75-8.33. Among all the samples, T₁ (8.33) and T₂ (8.00) scored higher OA may be due to higher flavour score obtained in oyster mushroom flour incorporated samples. Mushroom analogues with cowpea and oyster mushroom combination were found to be more acceptable in sensory evaluation using 9-point hedonic scale.

4. CONCLUSION

Incorporation of mushroom flour improved protein, fiber content and antioxidant properties of the analogues. Water absorption of the analogues increased with simultaneous reduction of soluble loss desirable for cooking with minimum solid loss. Analogues with good acceptability were developed keeping the mushroom concentration 25 and 50% in combination with cowpea and horse gram respectively in final formulation. Resulted mushroom analogues by extrusion can be promising source during scarcity of mushrooms with extended shelf-life for developing countries.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our

area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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