

## Evaluation of *Agaricus bisporus* Lange (Sing.) Strains and their Steeping for Improved Shelf life

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*Agaricus bisporus* strains, S11 and U3 were grown on four set of compost formulations made from wheat straw (WS) and paddy straw (PS) to show minimum spawn run period with WS+PS (50:50) formulation. Number of fruit bodies (726-2468/qlts compost) and their average weight (9.2 g-14.0 g) was higher in U3 in all the substrate formulations as compared to S11. The strain U3 gave yield potentials of 22.83 and 23.70 kg/qlts compost, with WS+PS (50:50 and 25:75) respectively as compared to 26.49 kg/qlts WS compost which were statistically at par with WS alone. This suggested partial replacement of wheat straw with paddy straw for mushroom cultivation to reduce production cost. Mushrooms being highly perishable product require preservation techniques for good market price. Mushrooms were blanched and steeped in six different solutions comprising of potassium metabisulfite (KMS), citric acid, sucrose and sodium chloride (NaCl) for one month at room temperature ( $24 \pm 2^\circ\text{C}$ ) to extend the shelf life. The steeping solution III (0.2% KMS+0.2% citric acid+6% sucrose+3% NaCl) proved better for U3 strain while steeping solution V (0.2% KMS+0.2% citric acid+6% sucrose+3% NaCl) was effective for S11 strain. Carbohydrate, protein and fat analysis indicated effectiveness of steeping solutions in maintaining nutritional quality of mushrooms.

**Key words:** *Agaricus bisporus*, wheat straw, paddy straw, steeping.

Mushroom has been considered a delicacy food all over the world. It is rich in good quality proteins with lysine and tryptophan that are normally deficient in cereals. *Agaricus bisporus* (button mushroom) is the most popular variety which belongs to the family Agaricaceae in the order Agaricales of class Basidiomycetes. It accounts for 90 per cent of total country's production while its global share is about 40 per cent. It is a saprophytic fungus which requires a well composted substrate containing carbon, nitrogen and other essential elements, such as phosphorous, sulphur, potassium and iron,

vitamins such as thiamine, biotin, etc. The C:N ratio of 17:1 is optimum for the cultivation of mushroom<sup>1</sup>. In India, a number of commercial strains like S-11, S-130, S-140, S-649, S-791, CM-1, CM-5, CM-10 (hybrid), A-15 (sylvan, hybrid), U-3, X-13, Delta, etc. have been used for cultivation whereas in Punjab, S-11 and U3 are preferably cultivated<sup>2</sup>.

India produces huge quantity of agricultural wastes which can be utilized for preparation of compost. Straw of cereals are used in most countries as a basic compost material where rice straw is largely used in Asian countries<sup>3</sup>. Several formulations have been used by different workers for production of *Agaricus* spp. depending upon the availability and cost of substrates. The compost for *A. bisporus* was produced from wheat straw, straw-bedded horse manure, chicken manure and gypsum by Straatsma et al.<sup>4</sup> Straws (rice, wheat, oat and barley), by products of sugarcane (bagasse), horse and

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chicken manure are the important lignocellulosic substrates used for composting<sup>5</sup>.

In Punjab, wheat straw is the basic substrate used for cultivation of *Agaricus bisporus*. However, the continuously increasing cost of wheat straw due to utilization as feed discouraging its use as substrate. On the other hand, paddy straw is one of the most abundantly available lignocellulosic agricultural by-product. Its burning in the open fields is a conventional practice for its disposal leading to environmental pollution. So, its utilization in compost production would prove an eco-friendly approach while making the technology cost effective.

Shelf life of mushrooms is the most important factor in determining the market price. Therefore, mushrooms need special care to retain freshness during shipping and marketing. A large number of methods have been reported in literature viz., modified-atmosphere packaging, vacuum cooling,  $\alpha$ -irradiation, chemical washing, steeping, drying, canning, freezing and value-addition etc. to improve shelf life of mushrooms. Most mushroom crops are preserved by canning and only a small portion treated by other methods such as freeze-drying<sup>6</sup>. However, its shelf-life can be enhanced for a longer period by processing. Among these, steeping of mushrooms is one of the convenient as well as an economical method to extend the shelf-life of mushrooms and retaining whiteness<sup>7</sup>.

Keeping in view the above mentioned points, present study was planned to increase paddy straw utilization for cultivation of *Agaricus bisporus* strains (U3 and S11) and improving shelf life of mushrooms through steeping.

## METHODS AND MATERIALS

### Procurement of cultures and spawn preparation

*Agaricus bisporus* Lange (Sing.), strains U3 and S11 were procured from germplasm collection bank of the Department of Microbiology, Punjab Agricultural University, Ludhiana. The cultures were maintained on potato dextrose agar (PDA) medium at 4°C. The spawn was prepared on boiled wheat grains mixed with 4 per cent  $\text{CaCO}_3$  and 2 per cent  $\text{CaSO}_4$  powders, autoclaved at 1.8 kg cm<sup>-2</sup> for 1.5 hr<sup>8</sup>.

### Compost preparation

The selected strains of button mushrooms were evaluated in winter season (October-March). The cultivation trials were conducted indoor under natural climatic conditions using standard methodology<sup>9</sup>. Four different combinations of wheat straw and paddy straw as substrate (100:0, 50:50, 25:75, 10:90) were used in compost preparation using long method of composting given by Garcha and Khanna<sup>9</sup>.

### Mushroom production and Harvesting

The compost (10 kg) was spawned with 70 g of spawn and after thorough mixing compost was filled into polythene bags (20"x 24"). Casing soil consisting of 6-8 months old FYM and sandy soil (4:1 v/v) was used to cover mycelial impregnated compost. Adequate humidity (=RH 70-90%) was maintained by spraying water on the bags twice a day. Very little or no ventilation was provided until the first appearance of the pinheads. Thereafter, intermittent cross-ventilation was given for total 4-8 hour/ day. The mushrooms were harvested by gentle twisting of the fruit body. The soil end parts of the harvested bodies were trimmed off and yield was recorded as percent biological efficiency (B.E), which was calculated by the formula:

$$\text{B.E (\%)} = \frac{\text{Fresh wt. of mushrooms} \times 100}{\text{Fresh wt. of compost}}$$

### Effect of Blanching and Steeping

#### Blanching

For studying the effect of blanching and steeping, *Agaricus bisporus*, strains S11 and U3 were harvested at egg stage and divided into 12 lots each weighing 50 g. Fresh mushrooms were washed with 0.1% KMS and blanched for three minute in boiling water.

#### Steeping

The blanched mushrooms were then stored in six different steeping solutions comprised of potassium metabisulfite (KMS), citric acid, sucrose and sodium chloride (NaCl) having varied concentrations of KMS and citric acid to study their effect on shelf life. The concentration of different components were I-0.05%KMS+0.2% Citric acid+6% Sucrose+3% NaCl, II- 0.1% KMS+0.2% Citric acid+6% Sucrose+3% NaCl, III- 0.2%KMS+0.2% Citric acid+6% Sucrose+3% NaCl, IV- 0.1%KMS+0.1% Citric acid+6% Sucrose+3%

NaCl, V- 0.1% KMS+0.2% Citric acid+6% Sucrose+3% NaCl, VI- 0.1% KMS+0.3% Citric acid+6% Sucrose+3% NaCl. Observations were made on physical (color and texture), biochemical (carbohydrates, proteins, fats) parameters and microbial count after one month of storage in steeping solution.

### Physical

#### Color analysis

Color of the mushroom pileus was estimated using the CIELAB scale at an observer angle of 10° with a Mini scan XE plus Hunter Lab Colorimeter. The 'a' value determines greenness ( $a < 0$ ) or redness ( $a > 0$ ) and the 'b' value determines blueness ( $b < 0$ ) or yellowness ( $b > 0$ ). The 'L' value varies between 0 and 100, representing transition from black to white. Total color difference after storage was measured by following formula:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

Where  $\Delta L$ ,  $\Delta a$  and  $\Delta b$  are deviations from  $\Delta L$ ,  $\Delta a$  and  $\Delta b$  values of the fresh sample.

The hue (H), chroma (C) and browning index (BI), which represented the purity of brown color<sup>10</sup>, were also calculated according to the given equation:

$$\text{Hue} = \tan^{-1} (b/a), \text{Chroma} = (a^2 + b^2)^{1/2}$$

$$\text{BI} = 100(x - 0.31)/0.172,$$

$$\text{Where } x = (a + 1.75L) / (5.645 + a - 3.012b)$$

#### Texture analysis

Texture profile analysis (TPA) was done using a texture analyzer (model TA-XT2i; Stable Micro Systems, United Kingdom) with instrument parameters described by Kotwaliwale et al<sup>11</sup> with modification of the strain to 75% of sample height and probe (75-mm compression platen). The parameters of brittleness, hardness, cohesiveness, adhesiveness, chewiness, springiness and gumminess were calculated as given by Bourne<sup>12</sup>.

#### Moisture content

The moisture content of samples was expressed in percent and calculated by the formula.

$$\text{Moisture content (\%)} = \frac{\text{weight of fresh sample} - \text{weight of dry sample}}{\text{weight of fresh sample}} \times 100$$

The average fresh weight was determined to compare the moisture content at the end of each time interval. The weight loss was recorded by subtracting weight after 1,2,3,5 and 7<sup>th</sup> from the fresh weight of mushroom.

### Microbiological

#### Total bacterial count

Total bacterial count was determined transferring 1 gram of mushroom tissue aseptically into 10 ml water blank and dilutions were prepared upto 10<sup>-5</sup>. The pour plating was done on nutrient agar medium. The plates were incubated at 37p C for 24-48 hrs and the total bacterial count (cfu/ g) was recorded.

### Biochemical

#### Extraction and estimation of total sugars

Carbohydrates from *A. bisporus* (dried samples) following washing treatment were extracted. Dried sample (0.5g) was crushed and extracted with hot 80% ethanol twice followed by hot 70% ethanol thrice on boiling water bath for 20 minutes. The supernatant aliquots from each extraction were pooled and evaporated under reduced pressure at 50p C. The volume was made by distilled water. Saturated lead acetate solution (0.25 ml) was added to precipitate the proteins and contents were filtered through whatmann no.40 filter paper and centrifuged at 3000 rpm for 15 minutes. A clear, protein free, extract so obtained, was collected for estimation of total sugars by Dubois et al.<sup>13</sup> method.

#### Extraction and estimation of proteins

0.5 g dried mushroom powder was extracted twice by continuous stirring with 25ml 0.1 N NaOH for 30 minutes followed by centrifugation (14000 x g for 15 minutes). The supernatants were pooled and diluted to 50ml volume. To the 2 ml aliquot of the supernatant, added 2 ml chilled 20% trichloro acetic acid (TCA) and kept for 1 hour at 4p C, the contents were centrifuged (14000 rpm x g for 15 minutes) and the precipitates were dissolved in 2 ml 0.1 N NaOH. From this extract, total true protein was estimated according to the method of Lowry et al<sup>14</sup>.

#### Extraction and estimation of total lipids

Dried sample (1g) was crushed in 1-2 ml of isopropanol and 3wheated for 1 minute to remove the sugar. To this, 10 ml of the solution of chloroform:methanol was added and kept overnight at 4p C. Rest of the procedure was followed as given by Folsch et al.<sup>15</sup>.

#### Statistical analysis

The data obtained from different treatments were analyzed using factorial two-way

ANOVA. The critical difference was measured at 5% level of significance.

## RESULTS AND DISCUSSION

Two strains of *Agaricus bisporus* S11 and U3 were grown on four set of compost formulations consisting of wheat straw (WS), WS + paddy straw (PS) (25:75), WS + PS (50:50), WS + PS (10:90). Spawn run period was minimum (20 days) in WS + PS (50:50) while WS compost showed complete spawn run in 26 days.

Number of fruit bodies and their average weight was higher in U3 in all the substrate combinations as compared to S11. Number of fruit bodies showed huge variation from 496 to 2468 while average weight of fruit bodies varied from 7.5 to 14g. The yield data [Table 1] indicated the highest yield on WS based compost for both the strains as compared to other combinations. Among the strains, U3 strain revealed higher yield potential as compared to S11 in all the combinations. Maximum yield was reported in U3 (26.49 kg/qlts compost) with WS based compost which was statistically at par with PS (50:50) and WS + PS (25:75) combination at 5% level of significance. However, no other substrate combination proved better than WS compost for S11 strain. *Coprinus* and *Tricoderma* were seen on few bags of S11 grown on WS + PS (10:90) compost.

Rice straw has enough nutrients and regarded as the best material for mushroom growing in all countries which produce rice, e.g. China, Philippines and Indonesia<sup>16</sup>. Main components of rice straw are cellulose and hemicellulose coated by lignin and small amount of protein<sup>17</sup>. Baysal et al<sup>18</sup> reported highest mushroom yield (1707.2 g per 5.78 qlts of compost) using wheat straw mixed with pigeon manure with the peat of Caykara and perlite mixture as casing material. Rice straw compost (320. 6 g of mushrooms per bag and 6.91 flushes) showed better results than wheat straw compost (70.0 g of mushrooms per bag and 3.72 flushes) for number of flushes although there was no significant difference in mushroom weight still it was better for rice straw compost. Using horse manure in compost gave inadequate results due to its high sawdust content<sup>19</sup>. The use of cotton straw and manure in 1:1 proportion for *A. bisporus* cultivation gave much higher biological efficiency and faster mycelial growth rate<sup>20</sup>.

### Preservation of button mushroom (*Agaricus bisporus*) in steeping solutions

The fresh fruit bodies of *Agaricus bisporus* strains, S11 and U3 were washed with 0.1% KMS solution and blanched in boiling water for 3 minutes. Six steeping solutions were used to preserve blanched mushrooms. The mushrooms from four solutions in S11 strains and only one

**Table 1.** Cultivation of *Agaricus bisporus*

Substrate	Strain	Spawn run(d)	Case run(d)	NFB(No./q compost)	Av.wt of a fb(g)	Yield(kg/q compost)	Disease (+/-)
WS	S11	26	13	1272	12.3	15.65	-
	U3			1892	14.0	26.49	-
WS:PS2) (50:50)	S11	20	12	1281	9.1	11.70	-
	U3			2468	9.2	22.83	-
WS:PS3) (25:75)	S11	20	12	1480	7.5	11.10	-
	U3			2301	10.3	23.70	-
WS:PS5) (10:90)	S11	20	12	496	12.0	05.95	+
	U3			726	13.6	09.87	-
CD (5%)						S11= 3.48; U3= 4.26	

WS, wheat straw; PS, paddy straw; NFB, number of fruit bodies; cd, critical difference

Bag size: 20"x24" (polythene, 150 gauge)

No. of replicates: 15 each for one strain (10kg compost /bag)

Rate of spawn: 0.7% wet compost (70g/bag of 10kg compost)

Relative Humidity in growing rooms from the month of October to march: 80-90% throughout

Temperature in growing room from the month of October to march: 13-23°C

**Table 2.** Physiological indices (weight loss and color parameters ) of *A. bisporus* strains in different steeping solutions

Strain	Steeping Solution	Mushroom weight Before steeping (g)	After steeping (one month) (g)	L	A	b	Color $\Delta E$	HUE	Chroma	BI
S11	I	50			Unfit for human consumption					
	II									
	III	50	32.0	60.71 $\pm$ 0.69	3.14 $\pm$ 0.19	18.80 $\pm$ 0.15	40.88	80.52	19.06	39.65
	IV	50			Unfit for human consumption					
	V	50	35.2	58.82 $\pm$ 2.22	3.16 $\pm$ 0.10	18.79 $\pm$ 0.69	42.56	80.41	18.96	40.95
	VI	50	38.9	60.71 $\pm$ 0.69	3.14 $\pm$ 0.19	18.80 $\pm$ 0.15	40.88	80.52	19.06	39.65
CONTROL	—		50	75.61	4.35	15.88	26.74	74.68	16.46	27.05
U3	I	50			Unfit for human consumption					
	II	50								
	III	50	36.5	59.85 $\pm$ 2.47	4.31 $\pm$ 0.11	20.59 $\pm$ 0.71	42.53	78.18	21.04	46.08
	IV	50			Unfit human consumption					
	V	50								
	VI	50								
CONTROL	-		50	81.92	2.59	12.5	19.59	78.29	12.76	18.31

 $\Delta E$ = Total color difference

BI= Browning Index

DIFFERENT COMBINATIONS OF STEEPING SOLUTIONS:-

I0.05% KMS+0.2% Citric acid+6% Sucrose+3% NaCl

II0.1% KMS+0.2% Citric acid+6% Sucrose+3% NaCl

III0.2% KMS+0.2% Citric acid+6% Sucrose+3% NaCl

IV0.1% KMS+0.1% Citric acid+6% Sucrose+3% NaCl

V0.1% KMS+0.2% Citric acid+6% Sucrose+3% NaCl

VI0.1% KMS+0.3% Citric acid+6% Sucrose+3% NaCl

**Table 3.** Biochemical analysis of steeped *A. bisporus* (button mushroom) strains

Strain	Steeping Solution	Carbohydrate (g/100g of mushroom)	Protein(g/100g of mushroom)	Fats(g/100g of mushroom)	Bacterial count (cfu/gram)
S11	I	3.20g	3.90g	Unfit for consumption	
	II				
	III			0.70g	2.5x10 <sup>2</sup>
	IV	3.20g	3.70g	Unfit for consumption	
	V			0.60g	1.5x10 <sup>2</sup>
	VI			0.80g	0.3x10 <sup>2</sup>
CONTROL	-	3.21g	3.90g	0.81g	0.3 x10 <sup>3</sup>
U3	I	3.20g	3.60g	Unfit for consumption	
	II				
	III			0.80g	5x10 <sup>2</sup>
	IV	3.25g	3.75g	Unfit for consumption	
	V				
	VI				
CONTROL	-	3.25g	3.75g	0.83g	1.68 x10 <sup>4</sup>

DIFFERENT COMBINATIONS OF STEEPING SOLUTIONS:-

I0.05% KMS+0.2% Citric acid+6% Sucrose+3% NaCl

II0.1% KMS+0.2% Citric acid+6% Sucrose+3% NaCl

III0.2% KMS+0.2% Citric acid+6% Sucrose+3% NaCl

IV0.1% KMS+0.1% Citric acid+6% Sucrose+3% NaCl

V0.1% KMS+0.2% Citric acid+6% Sucrose+3% NaCl

VI0.1% KMS+0.3% Citric acid+6% Sucrose+3% NaCl

**Table 4.** Texture analysis of steeped *A. bisporus* (button mushroom) strains

Strain	Steeping Solution	Adhesiveness (g x mm)	Hardness (g)	Springiness (mm)	Resilience	Cohesiveness	Chewiness (g x mm)	Gumminess (g)
S11	I	-0.33	599	0.48	0.29	0.73	211	439
	II							
	III							
	IV	-5.87	1876	0.55	0.31	0.66	685	1243
	V							
	VI							
CONTROL	-	-0.053	3333	0.86	0.58	0.48	137	1930
U3	I	-4.65	3147	0.56	0.30	0.60	1064	1880
	II							
	III							
	IV	-9.418	3240	0.36	0.38	0.61	689	1876
	V							
	VI							
CONTROL	-	-9.418	3240	0.36	0.38	0.61	689	1876

DIFFERENT COMBINATIONS OF STEEPING SOLUTIONS:-

I0.05% KMS+0.2% Citric acid+6% Sucrose+3% NaCl

II0.1% KMS+0.2% Citric acid+6% Sucrose+3% NaCl

III0.2% KMS+0.2% Citric acid+6% Sucrose+3% NaCl

IV0.1% KMS+0.1% Citric acid+6% Sucrose+3% NaCl

V0.1% KMS+0.2% Citric acid+6% Sucrose+3% NaCl

VI0.1% KMS+0.3% Citric acid+6% Sucrose+3% NaCl

solution in U3 strain were fit for human consumption. After steeping, a decline in mushroom weight 18 to 36 per cent was observed. In case of mushroom, higher L value and lower BI which means more whiteness and less browning are the important parameter in determining market price. The color analysis revealed maximum L value for S11 with solution VI (60.71) with minimum the chroma and browning index which was at par with steeping solution III. However, Steeping solution V gave negligible variation with respect to steeping solution III and VI. The strain U3 had lower L value (59.85) as compared to control with solution III (81.92) and chroma and browning index values (21.04, 46.08) were also higher but better than rest of the steeping solutions [Table 2].

Mushrooms after storage in steeping solution for one month were subjected to texture analysis. The strain S11 showed increase in cohesiveness, chewiness and gumminess while decrease in adhesiveness, hardness, springiness and resilience with all the steeping solution as compared to control. The texture parameters for S11 strain preserved in steeping solution V were more close to control indicating better textural properties comparing to other treatments. Steeping solution III showed better textural properties among the other treatments for U3 strain and was more close to control [Table 4].

Preliminary processing such as blanching and soaking or vacuum moistening in solutions of compounds (table salt, citric acid, L-ascorbic acid) prevents the darkening of pilei<sup>21</sup>. Blanching or 'blanching and freezing' is reported to bring a decrease in the hardness<sup>22</sup> and stiffness of mushrooms<sup>23</sup> in *Agaricus bisporus*.

White button mushroom (*A. bisporus*) simultaneously softened and toughened during post harvest storage. Force of puncturing, represent softening declined while gumminess, represent toughening increased during the first 6d and decreased thereafter<sup>24</sup>. Okere and Beelman<sup>25</sup> also showed that blanching in a solution of citric acid and use of brine made from table salt and sodium calcium salt of versenic acid had a positive effect on the color and texture of the sterilized mushroom with increase in the microbiological stability of the product.

The carbohydrate content protein and fat content of the preserved mushroom was almost

same with control (fresh mushrooms) indicating effectiveness of steeping in maintaining nutritional quality. This may be due to prevention of microbial deterioration with steeping treatment. Therefore bacterial count of steeped mushrooms was found to be less than 500 cfu per gram of mushrooms (Table 3). Hussain *et al*<sup>26</sup> reported that mushroom preserved in 0.08% KMS and 0.08% sodium benzoate had less damaged protein due to inhibition of enzyme activity. The preserved mushroom gave better nutritive status to make up the protein deficiency in people. These mushrooms are likely to be less expensive than those preserved by other techniques like canning and refrigeration.

Similar results were obtained by Singh<sup>27</sup>, who used solution consisting of 2% sodium chloride, 0.3% citric acid, 2% sugar, 0.1% KMS and 1% ascorbic acid as steeping preservation of the blanched button mushroom for 8-10 days at 21-28°C. In another report, Ratnoo and Doshi<sup>28</sup> steeped blanched button mushroom in chemical solutions of (T<sub>1</sub>: NaCl 2%, KMS 0.1%, citric acid 0.1% and tartaric acid 0.3%, T<sub>2</sub>: NaCl 2.5%, ascorbic acid 0.1%, citric acid 0.2%, NaHC03 0.1% and KMS 0.1% for 8-10 days and T<sub>3</sub>: NaCl 2%, sugar 2%, citric acid 0.3%, KMS 0.1% and ascorbic acid 1.0%) for 3 months that maintained its toughness, whiteness and edibility during storage at ambient temperature.

According to Arora *et al*<sup>29</sup>, blanching of button mushrooms in boiling water for one month and treating in solution containing 0.1% citric acid and 0.25% KMS for 15 minutes at room temperature resulted in lowest browning index and the activation energy values of button mushrooms.

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