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Effects of anti-browning agents on biochemical composition of summer white button mushroom

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Abstract
In the present study, the effects of anti-browning agents on the biochemical composition of summer white button mushroom *Agaricus bitorquis* (Quel.) Sacc was taken up. To evaluate the quality of *Agaricus bitorquis*, various pre-treatments were given such as blanching (hot water treatment at 96℃ for 10 minutes) and control taken as unblanched, and then blanched and unblanched samples were dipped in anti-browning agents of different concentration for 15 minutes. The anti-browning agents used were NaCl at concentration of 0.1, 0.2 and 0.5%, ascorbic acid at 0.5, 0.75 and 1.0% and potassium meta bisulphite at 0.75, 1.0 and 1.5%. Moisture content in treated mushroom was recorded in the range of 87.26 to 89.66 per cent and was significantly different from each other. Total solids content in treated mushroom was recorded in the range of 12.73 to 10.33 per cent and was significantly different from each other. Protein content in treated mushroom was recorded in the range of 28.33 to 28.90 per cent. Fat content in treated mushroom was recorded in the range of 3.51 to 4.02 percent. Ash content in treated mushroom was recorded in the range of 5.93 to 6.26 percent. Alcohol insoluble solids content in treated mushroom was recorded in the range of 3.03 to 3.27 percent. There is non-significant difference between the anti-browning agents, and protein, fat, ash and alcohol insoluble solids content of *Agaricus bitorquis*.

Keywords: *Agaricus bitorquis,* anti-browning agents, Ascorbic acid, Potassium meta bisulphite and Sodium chloride

Introduction
The use of mushrooms as food is probably as old as civilisation and mushrooms currently have greater importance in the diet of mankind. Mushrooms have been evaluated for their nutritional status on the basis of their chemical composition. Cultivated and wild mushrooms contain reasonable amounts of proteins, carbohydrates, minerals, fibres and vitamins. Furthermore, mushroom share low in calories, sodium, fats and cholesterol. (Barros *et al.*, 2007) [2]. Due to their high content of vitamin, protein and mineral, mushrooms are considered as "poor man's protein''. Mushrooms can be used for the food to solve the malnutrition problem. Mushrooms have good nutritional value particularly as a source of protein that can enrich human diets. Mushrooms generally possess most of the attributes of nutritious food as they contain many essential nutrients in good quanity (Kalac 2009) [5].

Blanching food is a heat treatment. Blanching treatments are presented according to the heat medium used: blanching in boiling water or in steam. The blanching time varies depending on the technique used, the type of product, size or maturity status. This process inactivates the enzymatic systems responsible for sensory alterations and thus limits the oxidation (Ioannou and Ghoul 2013) [4]. Anti-browning agents also inhibited the enzymatic browning of the mushroom and helped to maintaining the colour of mushroom during storage. Mushrooms contain about 85-95 per cent water, 3 per cent protein, 4 per cent carbohydrates, 0.1 per cent fats, 1 per cent minerals and vitamins (Tewari, 1986; Thakur, 1998) [7, 8]. It contains 19-35 per cent protein on dry weight basis as compared to 7.3 per cent in rice, 13.2 per cent in wheat and 25.2 per cent in milk. Mushroom contains 20-35 per cent superior quality protein (dry weight) which is higher than those of vegetables and fruits. Mushrooms are now getting significant importance due to their nutritional and medicinal value and presently their commercial cultivation is being done in more than 100 countries of the world. Mushroom cultivation is the most economical and relatively short biological process for the biotransformation of organic materials into protein rich food (Martinez *et al.*, 2000; Chiu and Moore, 2001) [6, 3]. Therefore the objective of the present study was to evaluate the effects of anti-browning agents on biochemical composition of *Agaricus bitorqui*s.

Materials and Methods
The present investigations were conducted at Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar during the year 2014-2015.

Freshly harvested fruit bodies of uniform size of *Agaricus bitorquis* were taken, trimmed, washed with running cold water to remove dirt and dust particles and given various pre-treatments such as: Blanching (hot water treatment at 96℃ for 10 minutes) and control taken as unblanched. Blanched and unblanched samples were kept in anti-browning agents for 15 minutes to minimum enzymatic discolouration such as NaCl (0.1, 0.2, & 0.5%) ascorbic acid (0.5, 0.75, and 1.0%) and potassium meta bisulphite (0.75, 1.0 and 1.5%). All the treatments were replicated thrice and then these samples were dried and analysed for chemical composition with respect to moisture content, total solids, protein, fat, ash and alcohol insoluble solids of *Agaricus bitorquis.*

Moisture content (%)

Moisture content in each treatment, replicate was determined by hot air oven method (AOAC, 1995) [1] 10 gm of fresh mushroom sample was weighed accurately and dried at 70±2°C for 12 hours. The loss in weight was determined to calculate the moisture content by using the following formula:

$$\text{Moisture content (\%)} = \frac{\text{Weight of original sample (g)} - \text{Weight of dried sample (g)}}{\text{weight of original sample (g)}} \times 100$$

Total solids (%)

Total solids (%) in each treatment replicate was determined by hot air oven method (AOAC, 1995) [1]. 10 g of sample in triplicate was weighed accurately and dried at 70±2°C for 12 hours. The left over residue was determined to calculate the total solids (%) by using the equation 100 - moisture content.

Protein (%)

Protein estimation was determined by using Kjeldhals method. Half gram of dried mushroom in powdered form per replicate was placed in Kjeldhals tubes and 5gm of digestion mixture (Potassium sulphate + Iron sulphate +copper sulphate in ratio of 5:0.5: 0.25) was added. After adding 10 ml of concentrated H_2SO_4, the mixture was heated till colour changed to green. Then the tubes were cooled and 10 ml of distilled water was added to each sample. Then tubes were fitted in assembly. Mixture from tubes was dipped in flask fitted in assembly containing 25 ml of boric acid (4%) and 5 ml of mixed indicator. Then 40-50 ml of NaOH (40.0%) was added to it till colour changed to brown. Flask containing boric acid and indicator was titrated with 0.1 N HCl till colour changed to brown.

Protein estimation was calculated from nitrogen content (N%) in accordance with the following formula:

$$\text{N (\%)} = \frac{\text{Quantity of sample - blank (ml) x Normality of HCl} \times 14}{1000 - \text{weight of sample}} \times 100$$

Then per cent protein was calculated by multipling nitrogen (%) by 6.25 i.e.

Protein (%) = N% × 6.25

Alcohol insoluble solids (%)

Alcohol insoluble solids (%) in the sample were determined by the procedure based on (AOAC, 1995) [1]. The sample was blended to a smooth homogenous paste. 10 g of grounded mushroom were taken in 200 ml beaker and 100 ml of 80 per cent ethanol was added to it. The solution was boiled for 30 minutes and cooled, filtered through Whatman filter paper. The filter paper was dried in oven at 100 °C for 2 hours. Alcohol insoluble solids (%) was calculated by using the following formula:

$$\text{Alcohol insoluble solids (\%)} = \frac{\text{Weight of residues}}{\text{Weight of sample taken for estimation}} \times 100$$

Ash (%)

Ash content in mushroom samples was determined according to the procedure of AOAC (1995) [1]. 5 g of sample was placed in pre-weighed silica dish, charred over a flame and kept in muffle furnace at a temperature of 550°C for about 4-5 hours. The dish was cooled in a desicator and weighed accurately. Percent ash was determined by using the following equation:

$$\text{Ash (\%)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$$

Crude fats (%)

The procedure given by AOAC (1995) [1] was adopted for the estimation of crude fats content in mushroom samples. 3 g of each dried sample was taken in a thimble and put in an extraction tube of soxhlet apparatus. The temperature of the heater was adjusted till continuous drops of ether fell on the sample in the extraction tube. The process of extraction was carried out with petroleum ether (B.P 50±10°C) for 16 hours. The sample was then removed and the solvent was allowed to evaporate under the fume hood at ambient room temperature. There after extract was completely dried in hot air oven for 30 minutes at 105°C temperature. After cooling in a desicator, the weight of the extract was recorded and crude fats was calculated by employing the following formula:

$$\text{Crude fats (\%)} = \frac{\text{Weight of fat (extract) in sample (g)}}{\text{weight of sample (g)}} \times 100$$

Results and Discussion

The data (Table-1,2,3,4,5 and 6) reveals that moisture content, total solids, protein content, fats, ash and alcohol insoluble solids of *Agaricus bitorquis* after pre-treatments like blanching at 96°C for 10 minutes, or unblanched, dipped in anti-browning agents like NaCl, Ascorbic acid and KMS for 15 minutes. Maximum moisture content was observed due to these treatments of KMS and ascorbic acid which are at par. Minimum moisture content was observed in NaCl (87.76, 87.73 and 87.23%) at 0.1, 0.2 and 0.5 per cent which are at par with each other. Statistically significant difference was exhibited between anti-browning agents on moisture content of *Agaricus bitorquis.* Maximum total solids was observed in NaCl (12.73%) at 0.5 per cent. Minimum total solids were observed in KMS (10.33%) at 0.75 and 1.5 per cent which are at par. Statistically significant difference was exhibited between anti-browning agents on total solids of *Agaricus bitorquis.* There is non-significant difference between the anti-browning agents, and protein, fat, ash and alcohol insoluble solids content of *Agaricus bitorquis*. Protein content in treated mushroom was recorded in the range of 28.33 to 28.90 per cent and was non significantly different from each other. Fat content in treated mushroom was recorded in the range of 3.51 to 4.02 per cent and was non- significantly different from each other. Ash content in treated mushroom was recorded in the range of 5.93 to 6.26 percent and was non- significantly different from each other. Alcohol insoluble

solids content in treated mushroom was recorded in the range of 3.03 to 3.27 per cent and was non-significantly different from each other.

Moisture content in treated mushroom was recorded in the range of 87.26 to 89.66 per cent and was significantly different from each other. The present findings pertaining to moisture content are in agreement to findings of Zhenqiang Xia (2013) [10]. Total solids content in treated mushroom was recorded in the range of 12.73 to 10.33 per cent and was significantly different from each other. Moisture content and total solids are inversely proportional to each other. Protein content in treated mushroom was recorded in the range of 28.33 to 28.90 per cent and was non- significantly different from each other. The present findings pertaining to quality parameters viz. protein content are in agreement to finding of Singh *et al.* (2014) [9]. Fat content in treated mushroom was recorded in the range of 3.51 to 4.02 percent and was non-significantly different from each other. Ash content in treated mushroom was recorded in the range of 5.93 to 6.26 percent and was non- significantly different from each other. Alcohol insoluble solids content in treated mushroom was recorded in the range of 3.03 to 3.27 percent and was non-significantly different from each other.

Table 1: Effect of anti-browning agents on moisture content (%) of *Agaricus bitorquis*

Anti-browning agents	Blanched			Mean	Unblanched			Mean	Mean			Overall Mean
	C_1	C_2	C_3		C_1	C_2	C_3		C_1	C_2	C_3	
NaCl	87.76 (9.39)	87.73 (9.42)	87.26 (9.42)	87.58 (9.42)	88.83 (9.45)	88.33 (9.42)	87.86 (9.45)	88.17 (9.44)	88.05 (9.41)	88.03 (9.43)	87.56 (9.43)	87.88 (9.42)
Ascorbic acid	89.06 (9.49)	89.36 (9.50)	89.20 (9.49)	89.21 (9.49)	89.53 (9.52)	89.46 (9.51)	89.33 (9.50)	89.44 (9.51)	89.30 (9.50)	89.41 (9.50)	89.26 (9.50)	89.32 (9.50)
KMS	89.20 (9.50)	89.33 (9.49)	89.16 (9.49)	89.23 (9.49)	89.66 (9.52)	89.46 (9.51)	89.66 (9.52)	89.60 (9.51)	89.43 (9.50)	89.40 (9.50)	89.41 (9.51)	89.41 (9.50)
Overall Mean	88.67 (9.46)	88.80 (9.47)	88.54 (9.46)	88.67 (9.47)	89.34 (9.50)	89.25 (9.48)	88.95 (9.48)	89.07 (9.49)	88.93 (9.47)	88.95 (9.48)	88.74 (9.48)	

C.D. ($p \leq 0.05$)
Blanched and unblanched : 0.005
Anti-browning agents : 0.006
Concentration : 0.006
*Values in parenthesis are square root transformed values

Table 2: Effect of anti-browning agents on total solids (%) of *Agaricus bitorquis*

Anti-browning agents	Blanched			Mean	Unblanched			Mean	Mean			Overall Mean
	C_1	C_2	C_3		C_1	C_2	C_3		C_1	C_2	C_3	
NaCl	12.23 (3.63)	12.26 (3.64)	12.73 (3.70)	12.41 (3.66)	11.73 (3.56)	11.66 (3.55)	12.06 (3.61)	11.82 (3.58)	11.98 (3.60)	11.96 (3.60)	12.40 (3.66)	12.11 (3.62)
Ascorbic acid	10.93 (3.45)	10.63 (3.41)	10.80 (3.43)	10.78 (3.43)	10.46 (3.38)	10.53 (3.39)	10.73 (3.42)	10.57 (3.40)	10.70 (3.42)	10.58 (3.40)	10.76 (3.43)	10.68 (3.41)
KMS	10.73 (3.42)	10.66 (3.41)	10.83 (3.44)	10.74 (3.42)	10.13 (3.33)	10.53 (3.39)	10.33 (3.36)	10.33 (3.36)	10.43 (3.38)	10.60 (3.40)	10.58 (3.40)	10.53 (3.39)
Overall Mean	11.29 (3.50)	11.18 (3.49)	11.45 (3.52)	11.31 (3.50)	10.77 (3.42)	10.90 (3.44)	11.04 (3.46)	10.91 (3.45)	11.03 (3.47)	11.04 (3.47)	11.24 (3.49)	

C.D. ($p \leq 0.05$)
Blanched and unblanched : 0.012
Anti-browning agents : 0.015
Concentration : 0.015
*Values in parenthesis are square root transformed values

Table 3: Effect of anti-browning agents on protein content (%) of *Agaricus bitorquis*

Anti-browning agents	Blanched			Mean	Unblanched			Mean	Mean			Overall Mean
	C_1	C_2	C_3		C_1	C_2	C_3		C_1	C_2	C_3	
NaCl	28.90 (5.46)	28.70 (5.45)	28.80 (5.45)	28.80 (5.45)	28.66 (5.44)	28.76 (5.45)	28.70 (5.45)	28.71 (5.45)	28.78 (5.45)	28.73 (5.45)	28.75 (5.45)	28.75 (5.45)
Ascorbic acid	28.76 (5.45)	28.66 (5.44)	28.76 (5.45)	28.73 (5.45)	28.76 (5.45)	28.76 (5.45)	28.60 (5.44)	28.71 (5.45)	28.76 (5.45)	28.76 (5.45)	28.68 (5.44)	28.72 (5.45)
KMS	28.60 (5.44)	28.63 (5.44)	28.73 (5.45)	28.65 (5.44)	28.33 (5.41)	28.60 (5.44)	28.76 (5.45)	28.56 (5.44)	28.46 (5.42)	28.67 (5.44)	28.75 (5.45)	28.61 (5.44)
Overall Mean	28.75 (5.45)	28.66 (5.44)	28.76 (5.45)	28.72 (5.45)	28.58 (5.43)	28.70 (5.45)	28.68 (5.45)	28.66 (5.44)	28.66 (5.44)	28.72 (5.45)	28.72 (5.45)	

C.D. ($p \leq 0.05$)
Blanched and unblanched : NS
Anti-browning agents : NS
Concentration : NS
*Values in parenthesis are square root transformed values

Table 4: Effect of anti-browning agents on fat content (%) of *Agaricus bitorquis*

Anti-browning agents	Blanched			Mean	Unblanched			Mean	Mean			Overall Mean
	C_1	C_2	C_3		C_1	C_2	C_3		C_1	C_2	C_3	
NaCl	3.78 (2.18)	3.78 (2.18)	4.02 (2.24)	3.86 (2.19)	3.51 (2.12)	3.83 (2.19)	4.02 (2.24)	3.78 (2.18)	3.64 (2.15)	3.80 (2.19)	4.02 (2.24)	3.82 (2.19)
Ascorbic acid	3.73 (2.17)	3.82 (2.19)	3.77 (2.18)	3.77 (2.18)	3.80 (2.19)	3.79 (2.19)	3.69 (2.16)	3.76 (2.18)	3.76 (2.18)	3.81 (2.19)	3.73 (2.17)	3.76 (2.18)
KMS	3.77 (2.18)	3.79 (2.19)	3.83 (2.19)	3.79 (2.19)	3.80 (2.19)	3.70 (2.16)	3.80 (2.19)	3.76 (2.18)	3.79 (2.18)	3.75 (2.18)	3.82 (2.19)	3.78 (2.18)
Overall Mean	3.76 (2.17)	3.79 (2.19)	3.87 (2.20)	3.80 (2.19)	3.70 (2.15)	3.77 (2.18)	3.83 (2.19)	3.76 (2.18)	3.73 (2.17)	3.78 (2.19)	3.85 (2.20)	

C.D. ($p \leq 0.05$)
Blanched and unblanched : NS
Anti-browning agents : NS
Concentration : NS
*Values in parenthesis are square root transformed values

Table 5: Effect of anti-browning agents on ash content (%) of *Agaricus bitorquis*

Anti-browning agents	Blanched			Mean	Unblanched			Mean	Mean			Overall Mean
	C_1	C_2	C_3		C_1	C_2	C_3		C_1	C_2	C_3	
NaCl	5.93 (2.63)	6.06 (2.65)	6.00 (2.64)	5.99 (2.64)	6.03 (2.65)	6.10 (2.66)	6.13 (2.67)	6.08 (2.66)	5.98 (2.64)	6.08 (2.66)	6.06 (2.65)	6.04 (2.65)
Ascorbic acid	6.20 (2.65)	6.03 (2.65)	6.23 (2.68)	6.15 (2.67)	6.06 (2.65)	6.06 (2.65)	6.26 (2.69)	6.12 (2.67)	6.13 (2.67)	6.05 (2.65)	6.25 (2.69)	6.14 (2.67)
KMS	6.16 (2.67)	6.13 (2.67)	6.13 (2.67)	6.20 (2.68)	6.16 (2.67)	6.13 (2.67)	6.00 (2.64)	6.09 (2.66)	6.16 (2.67)	6.13 (2.67)	6.16 (2.67)	6.15 (2.67)
Overall Mean	6.09 (2.65)	6.07 (2.65)	6.12 (2.66)	6.11 (2.66)	6.08 (2.65)	6.09 (2.66)	6.13 (2.66)	6.09 (2.66)	6.09 (2.66)	6.09 (2.66)	6.16 (2.67)	

C.D. ($p \leq 0.05$)
Blanched and unblanched : NS
Anti-browning agents : NS
Concentration : NS
*Values in parenthesis are square root transformed values

Table 6: Effect of anti-browning agents on alcohol insoluble solids (%) of *Agaricus bitorquis*

Anti-browning agents	Blanched			Mean	Unblanched			Mean	Mean			Overall Mean
	C_1	C_2	C_3		C_1	C_2	C_3		C_1	C_2	C_3	
NaCl	3.20 (2.05)	3.27 (2.07)	3.20 (2.05)	3.22 (2.05)	3.20 (2.05)	3.21 (2.05)	3.15 (2.03)	3.19 (2.04)	3.20 (2.05)	3.25 (2.06)	3.18 (2.04)	3.21 (2.05)
Ascorbic acid	3.16 (2.04)	3.24 (2.05)	3.21 (2.05)	3.20 (2.05)	3.03 (2.00)	3.14 (2.03)	3.14 (2.03)	3.07 (2.02)	3.10 (2.02)	3.15 (2.03)	3.18 (2.04)	3.14 (2.03)
KMS	3.21 (2.05)	3.08 (2.02)	3.10 (2.02)	3.13 (2.03)	3.03 (2.00)	3.13 (2.03)	3.20 (2.05)	3.14 (2.03)	3.12 (2.03)	3.14 (2.03)	3.15 (2.03)	3.13 (2.03)
Overall Mean	3.19 (2.04)	3.19 (2.04)	3.17 (2.04)	3.18 (2.04)	3.08 (2.01)	3.16 (2.03)	3.16 (2.03)	3.13 (2.03)	3.14 (2.03)	3.18 (2.04)	3.16 (2.04)	

C.D. ($p \leq 0.05$)
Blanched and unblanched : NS
Anti-browning agents : NS
Concentration : NS
*Values in parenthesis are square root transformed values

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