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EFFECT OF STEEPING SOLUTION ON THE QUALITY OF BUTTON MUSHROOMS (*A. BISPORUS*) PRESERVED UNDER AMBIENT CONDITIONS

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ABSTRACT
Steeping of the mushrooms was done with a purpose to enhance the shelf-life and various quality changes viz., pH, colour, microbial flora and antioxidant potential were studied for up to a period of 80 days. The pH of the solutions was maintained below 4.5 that helped in the inactivation of polyphenoloxidase enzyme and in lowering microbial growth. A gradual decrease in pH, antioxidant potential, colour and all the sensory parameters was observed whereas an increase in the growth of microbes was observed during storage. Sensory evaluation of the mushrooms was done using hedonic scale and of all the treatments, T_3 was observed to be the best treatment on the basis of sensory and physico- chemical analysis.

INTRODUCTION

Food consumption patterns are rapidly changing all over the world. Consumer food choices have been attributed, in part, to the rise in health problems. Therefore, the positive health impact of nutrients in fruits and vegetables has become one of the consumer's chief concerns. Consumption of fresh fruits and vegetables has been revealed to be beneficial for the alleviation of many degenerative diseases (Suttirak and Manurakchinakorn, 2010). In most countries, there is a well-established consumer's acceptance of cultivated mushrooms, probably not only due to their unique flavor and texture but also for their physico-chemical properties and nutritional characteristics (Sharma *et al.*, 2013). Mushrooms are also considered as low-energy functional foods, which could notably contribute to the design of healthy patterns (Kalogeropoulos *et al.*, 2013). Mushrooms may contribute significantly in overcoming protein deficiency in the developing countries. Low cost labour, varied agro-climatic conditions and abundant cheap raw materials for production of various mushrooms may make India a future mushroom exporter both in form of quality and quantity of diverse food (Sarma *et al.*, 2010). The fat content of the mushrooms is as low as 0.3% (but is rich in linoleic acid, an essential fatty acid) and are called as diabetics delight to prevent hyperglycemia and is an ideal food for obese persons. They also exert antioxidant properties which are mainly attributed to their phenolic content (Ferreira *et al.*, 2009). The mushroom market is growing continuously mainly due to increasing interest in their culinary, nutritional and health benefits and of the different mushrooms grown, button mushroom continues to occupy a prominent place and contributes about 85% of the total mushroom production of the country (Murmu *et al.*, 2014). However, button mushrooms only have a short shelf life of 3-4 days. Their commercial value is lost within a few days, due to browning, water loss, senescence and microbial attack (Gao *et al.*, 2014). Browning after harvest is a common phenomenon in mushroom crops, which decreases the commercial value of the products. Loss of whiteness upon storage is particularly important in the mushroom industry (Burton, 1988). So in order to enhance the shelf life and to preserve those, steeping of mushrooms can be done. In white button mushroom water is continuously being lost as a result of transpiration and respiration. In this direction it is necessary to keep and store the mushroom in such a way that it can be stored for longer period maintaining its quality. The steeping preservation method is simple and economical and mushrooms can be preserved for short periods by steeping them in solution of salts or acids (Ratnoo and Doshi, 2013). Steeping preservation of fruits and vegetables involving permissible chemical preservatives is one of the methods to enhance their storability without much deterioration in the quality (Sethi and Maini, 2000). Blanching, along with combination of different chemicals helped not only to prevent browning by chelating with metal ions but also keeps a check

on microbial growth (Brennan and Gormley, 1998).

The present study is an attempt to preserve mushrooms in order to enhance their shelf life without much deterioration in their quality.

MATERIALS AND METHODS

Freshly harvested button mushrooms were made available by the department of Plant Pathology, SKUAST-J. The research was conducted in the department of Food Science and Technology, SKUAST-J. Mushrooms of uniform size are washed and cut in to thick slices. The slices were blanched for 2-3 minutes, cooled to room temperature and dipped in glass jars containing steeping solution of different concentration. Each treatment has been replicated thrice. Concentration of different steeping solution used is as follows

Treatments	Steeping Solutions
T_1	2% NaCl + 0.1% KMS + 0.1% citric acid + 0.3% tartaric acid
T_2	2.5%NaCl + 0.1% ascorbic acid + 0.2% citric acid + 0.1% $NaHCO_3$ + 0.1% KMS
T_3	2% NaCl + 2%sugar + 0.3% citric acid 0.1% KMS + 1% ascorbic acid
T_4	2.5% NaCl + 0.1% acetic acid +0.2% citric acid + 0.1% $Na2CO_3$ + 0.1% KMS
T_5	Control

pH measurement

The pH of the solution was measured with the help of pH meter for up to a period of 80 days (Ministry of Health and Family Welfare, 2005).

Microbial analysis

Microbial analysis was done according to total plate count method using nutrient agar. Nutrient agar was prepared and sterilized in an autoclave at 121ºC for 20 minutes. Serial dilution of mushrooms was carried out and 0.1mL of the aliquot was added on the petri plate containing nutrient agar as medium (using micropipette). The plates were then covered and incubated at 37ºC for 48 hours. The total microbial count was recorded as $cfuml^{-1}$ (Nwachukwo and Ezeigbo, 2013).

Colour

The surface colour of mushrooms was measured with the help Hunter Lab Mini Scan XE Colorimeter with an 8-mm-diameter diaphragm calibrated with a white tile (X = 81.1, Y = 86.0 and Z = 91.8) (Simon and Gonzalez- Fandos, 2009) where, L* indicates (whiteness/ darkness), a* (greenness/ redness) and b* (yellowness/ blueness). Each treatment was replicated thrice for colour measurement.

Browning Index

The browning index was calculated using the following expression (Bozkurt and Bayram, 2006):

$$BI = 100 \times \frac{[x - 0.31]}{0.71}$$

Where,

$$X = \frac{(a^* + 1.75L^*)}{(5.645L^* + a^* - 3.012b^*)}$$

Sensory analysis

Mushrooms were sensory evaluated on the basis of appearance, flavor, texture, taste and overall acceptability using 9 -point hedonic scale. The data obtained was analyzed statistically (Rangana, 2005).

Antioxidant potential

Antioxidant potential was determined by ferric-reducing antioxidant power assay (FRAP) according to Benzie and Strain (1996) and modified by Alvarez-Parrilla *et al*. (2007) with some modifications. FRAP reagent was prepared daily by mixing 0.3 mM acetate buffer (pH 3.6) with 10 mM 2,4,6-tripyridyl-s triazine (TPTZ) solution in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H2O$ (10:1:1 ratio). 1.8mL of the freshly prepared FRAP reagent was taken in a test tube and incubated at 30ºC in water bath for 10 minutes. Then absorbance was taken at 0 minute (t_o). Immediately 100μL of sample extract and 100μL of methanol was added to the test tube, mixed and incubated at 30ºC for 30 minutes. The absorbance was taken at 593 nm. Ferrous sulphate was used as a standard curve and calculated using the following equation:

FRAP value = Absorbance (sample + FRAP reagent) – Absorbance (FRAP reagent)

Statistical analysis

The results obtained were statistically analyzed using completely randomized design (CRD) and CRD factorial for interpretation of results through analysis of variance.

RESULTS AND DISCUSSION

Physico-chemical analysis of mushrooms with control on 20th day of storage

Blanched mushrooms preserved in various steeping solutions had a shelf life upto 80 days. But mushrooms steeped in water got spoiled after 20th day. Analysis of various treatments on 20th day of storage showed that the highest pH (7) was observed in control and lowest (3.72) in T_1 (2% NaCl + 0.1% KMS + 0.1% citric acid + 0.3% tartaric acid) as maintained by the addition of different chemicals (Table.1). The highest total plate count of 7.2 x 10^2 was observed in control and the lowest (1.2 x 10^2) in T_3 (2% NaCl + 2% sugar + 0.3% citric acid + 0.1% KMS + 1% ascorbic acid) after 20th day of storage. Increase in total plate count also lead to a decrease in antioxidant potential (1.74μmol FRAP/g) in control as compared to other steeping treatments. With storage the decrease in L* value and an increase in a* and b* value was observed (Table 2). The lowest L* value (80.29) was observed in control and the highest (85.73) in T_3 and the lowest a* (0.29) and b* (3.25) was observed in T_3 whereas the highest a* (1.88) and b* (6.32) value was observed in control. The browning index of mushrooms which is related to decrease in L* and increase in a* and b* value also increased with storage and the highest browning index (2.32) was observed in control and lowest (0.96) in T_3 on 20th day. Since the control was not acceptable after 20 days as observed by microbial count, it was discarded and not analyzed further.

It could be seen that the score for sensory parameters was lesser in control (Table 3) as compared to other steeping treatments on 20th day of storage. On the basis of overall

Table 1: Effect of different steeping solution on pH, total plate count and antioxidant potential of mushrooms on 20th day of storage

Treatment	pH	Total plate count (cfu/g)	Antioxidant Potential (Umol FRAP/g)
T_1 2% NaCl + 0.1% KMS +0.1% citric acid + 0.3% tartaric acid	3.72	2.1×10^2	1.99
T_2 2.5%NaCl + 0.1% ascorbic acid + 0.2% citric acid + 0.1% $NaHCO_3$ + 0.1% KMS	4.20	1.6×10^2	2.10
T_3 2% NaCl + 2%sugar + 0.3% citric acid 0.1% KMS + 1% ascorbic acid	3.98	1.2×10^2	2.15
T_4 2.5% NaCl + 0.1% acetic acid +0.2% citric acid + 0.1% Na_2CO_3 + 0.1% KMS	4.41	2.4×10^2	1.95
Control	7	7.2×10^2	1.74
C.D. (p=0.05)	0.08	0.21	0.18

All values are mean significant values

Table 2: Effect of different steeping solution on the colour and browning index of mushrooms on 20th day of storage

Treatment	Colour	Browning index
T_1 2% NaCl + 0.1% KMS +0.1% citric acid + 0.3% tartaric acid	L* 84.75 a* 0.73 b* 4.12	1.3
T_2 2.5%NaCl + 0.1% ascorbic acid + 0.2% citric acid + 0.1% $NaHCO_3$ + 0.1% KMS	L* 85.48 a* 0.41 b* 3.59	1.08
T_3 2% NaCl + 2%sugar + 0.3% citric acid 0.1% KMS + 1% ascorbic acid	L* 85.73 a* 0.29 b* 3.25	0.96
T_4 2.5% NaCl + 0.1% acetic acid +0.2% citric acid + 0.1% Na_2CO_3 + 0.1% KMS	L* 84.43 a* 1.32 b* 4.25	1.47
Control	L* 80.29 a* 1.88 b* 6.32	2.32
C.D. (p=0.05)	L* 0.18 a* 0.18 b* 0.19	0.18

All values are mean significant values, L* = whiteness/darkness, a* = redness/greenness and b* = yellowness/blueness.

Table 3: Effect of different steeping solution on the sensory evaluation of mushrooms up to 20 days of storage

Treatments	Appearance	Flavor	Texture	Taste	Overall acceptability
T_1 2% NaCl + 0.1% KMS +0.1% citric acid + 0.3% tartaric acid	8.32	8.26	8.16	8.21	8.21
T_2 2.5%NaCl + 0.1% ascorbic acid + 0.2% citric acid + 0.1% $NaHCO_3$ + 0.1% KMS	8.47	8.32	8.31	8.59	8.40
T_3 2% NaCl + 2%sugar + 0.3% citric acid 0.1% KMS + 1% ascorbic acid	8.40	8.48	8.27	8.48	8.44
T_4 2.5% NaCl + 0.1% acetic acid +0.2% citric acid + 0.1% Na_2CO_3 + 0.1% KMS	8.55	8.15	8.28	8.12	8.26
Control	7.42	7.96	7.25	7.82	7.61
C.D. (p=0.05)	0.20	0.18	N.S	0.17	0.18

All values are mean significant values

acceptability treatment T_3 has got the highest score as compared to other treatments.

Comparison of various steeping treatments during storage

There was significant difference in pH of various steeping solutions, however there was little variation in pH during storage (Table 4). Presence of citric acid in various steeping solution helped in maintaining the pH below 4.5 which prevented microbial growth, browning and enhanced the shelf life of mushrooms. A gradual change in the mean value of pH was recorded in all the treatments for a period of 80 days and this might be due to increased acidity during storage in steeping solution. These results are in conformity with those of Hussain *et al.* (2005) and Sinha *et al.* (2013). Significant difference among the various steeping treatments and storage period was observed with the lowest mean value in T_3 and highest in T_4. Steeping preservation helped in reducing the microbial growth below the acceptable limit (1000 c.f.u/ml, Food Safety and Standard Regulation, 2010) (Table 4). Similar findings were reported by Iqbal (1996) who stated that addition of acids had discernible lethal effect on microorganism as well as on enzyme inhibition during one month storage of mushrooms in brine solution and Kaur *et al.* (2009) that preserved blanched baby corn with 15% salt concentration that remained without any fungal growth up till 45 days.

Table 4: Effect of different solutions on pH and total plate count of steeped mushrooms

Treatment	pH	Total plate count (cfu/g)
T_1 2% NaCl + 0.1% KMS +0.1% citric acid + 0.3% tartaric acid	3.63	5.87×10^2
T_2 2.5%NaCl + 0.1% ascorbic acid + 0.2% citric acid + 0.1% $NaHCO_3$ + 0.1% KMS	4.07	4.87×10^2
T_3 2% NaCl + 2%sugar + 0.3% citric acid 0.1% KMS + 1% ascorbic acid	3.90	3.87×10^2
T_4 2.5% NaCl + 0.1% acetic acid +0.2% citric acid + 0.1% Na_2CO_3 + 0.1% KMS	4.29	6.72×10^2
C.D. (p=0.05)	Treatment = 0.18 Storage = N.S T x S = N.S	Treatment = 0.20×10^2 Storage = 0.18×10^2 T x S = 0.40×10^2

All values are mean significant values, taken from three replications, Statistical analysis with means separations using OPSTAT (F CRD) with 95% confidence interval.

Table 5: Effect of steeping solution on colour and browning index of mushrooms

Treatment	Colour		Browning index
T_1	L*	81.69	2.33
2% NaCl + 0.1% KMS +0.1% citric acid + 0.3% tartaric acid	a*	1.55	
	b*	6.75	
T_2	L*	82.71	1.94
2.5%NaCl + 0.1% ascorbic acid + 0.2% citric acid +	a*	1.22	
0.1% $NaHCO_3$ + 0.1% KMS	b*	5.68	
T_3	L*	82.67	1.82
2% NaCl + 2%sugar + 0.3% citric acid 0.1% KMS +	a*	0.95	
1% ascorbic acid	b*	5.45	
T_4	L*	81.09	2.74
2.5% NaCl + 0.1% acetic acid +0.2% citric acid	a*	1.96	
+ 0.1% Na_2CO_3 + 0.1% KMS	b*	7.51	
C.D. (p=0.05)		L* a* b* Treatment = 0.10 0.10 0.16 Storage = 0.10 0.10 0.16 T x S = 0.20 N.S 0.32	Treatment = 0.08 Storage = 0.08 T x S = 0.17

All values are mean significant values, taken from three replications, Statistical analysis with means separations using OPSTAT (F CRD) with 95% confidence interval.

Table 6: Effect of different steeping solution on the sensory quality of mushrooms

Treatments	Appearance	Flavor	Texture	Taste	Overall acceptability
T_1 2.5% NaCl + 0.1% acetic acid 2% NaCl + 0.1% KMS +0.1% citric acid + 0.3% tartaric acid	7.47	7.48	7.32	7.64	7.43
T_2 2.5%NaCl + 0.1% ascorbic acid + 0.2% citric acid + 0.1% $NaHCO_3$ + 0.1% KMS	7.88	7.85	7.65	7.95	7.86
T_3 2% NaCl + 2%sugar + 0.3% citric acid 0.1% KMS + 1% ascorbic acid	7.82	8.08	7.53	8.09	7.89
T_4 2.5% NaCl + 0.1% acetic acid +0.2% citric acid + 0.1% Na_2CO_3 + 0.1% KMS	7.43	7.18	7.16	7.31	7.34
C.D. (p=0.05)	Treatment =0.11 Storage = 0.11 T x S = 0.23	Treatment =0.13 Storage = 0.13 T x S =0.27	Treatment =0.10 Storage = 0.08 T x S = 0.16	Treatment=0.8 Storage= 0.08 T x S = 0.16	Treatment = 0.08 Storage = 0.08 T x S = 0.17

All values are mean significant values, taken from three replications, Statistical analysis with means separations using OPSTAT (F CRD) with 95% confidence interval.

Steeping of mushrooms helped to retain the colour of mushrooms. With storage a slight decrease in whiteness has been observed and it might be due to non-enzymatic browning which occur at a faster rate at high temperature. This is in agreement with Vivar-Quintana *et al.* (1999) who observed that storing of mushrooms with brine solution containing citric acid and citric + ascorbic acid at low temperature maintained colour and kept a fresh appearance. Though the whiteness of the mushrooms was retained a slight change in the colour of the steeping solution to yellow was observed during storage. The mean score showed that the highest L* value was of T_3 and lowest of T_4. These values may be considered acceptable (Lopez-Briones *et al.*, 1993) who considered whole mushrooms with L* values above 80 to be acceptable.

The antioxidant potential of steeped mushrooms decreased with storage in all the treatments (Fig.1). It was observed that blanching caused a decrease in the antioxidant potential of steeped mushrooms. This has also been observed by Nayak *et al.* (2012) who reported that polyphenols content of segments as well as syrup in anola decreased as the steeping preservation in fruits prolonged. They also reported that the decrease in antioxidant activity might be linked to a decrease in total phenol content. The main reason for loss was leaching as they are water- soluble and may leach easily into water due to osmosis (Bhattacherjee *et al.*, 2013). Steeping solutions helped in maintaining the sensory attributes for up to 80 days as compared to control and the difference among the solutions might be due to the combination of chemicals used. The

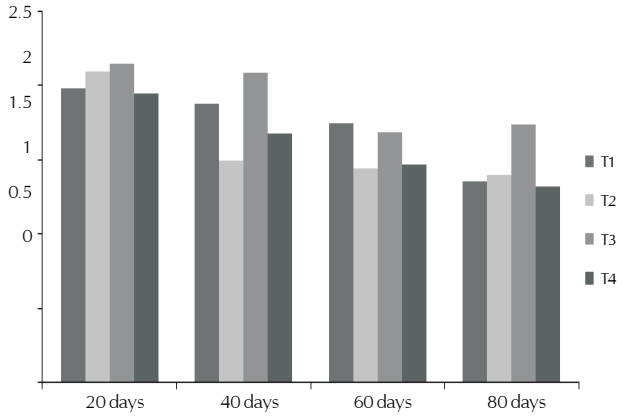

Figure 1: Change in the antioxidant potential of mushrooms preserved under steeping solution

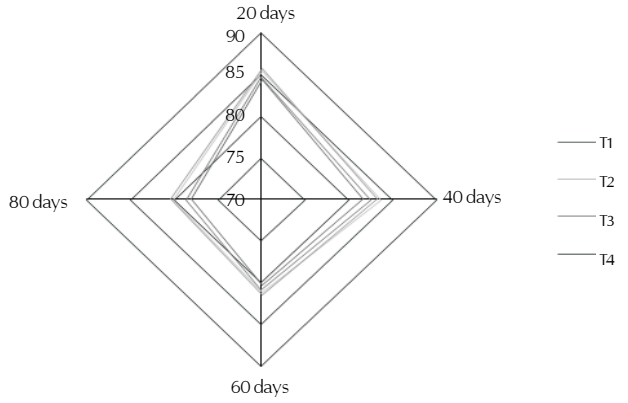

Figure II: Web chart showing change in L* value of mushrooms during steeping preservation

sensory score for all the parameters decreased with storage.This decrease might be due to the degradation of flavor and taste of stored samples due to increased acidity. Our results are in agreement with Barwal *et al*. (2005) who preserved cauliflower by steeping in different concentrations (10-15%) of salt and potassium meta bisulphite (0.2%), and citric acid (1.0%) after blanching which was accepted up to 90 days of storage and Pruthi (1963) who observed that different vegetables like potatoes, carrot, cabbage, mushrooms, peas and animal food (meat, fish and poultry) can be preserved in an acidified sulphited brine solutions through steeping and can be used for pickling or home cooking after leaching out the salt and acid.

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