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Optimization and Storage Studies of *A. bisporus* Vinegar-Oil Pickle to Utilize Stipe as a Value Added Product

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A B S T R A C T

Storage studies of vinegar-oil based pickle were carried out on strain U3 of *Agaricus bisporus* for providing value to mushrooms and to utilize the wasted stipe. Synthetic and natural (sugarcane) vinegars @vinegars @5% acidity and 14ml/200g mushrooms of volume were used as preservative means. Best results were obtained when natural vinegar was added to the pickle. The study showed the efficiency of natural vinegarin giving minimal storage changes for proteins and fats with no change in carbohydrates while better color and textural properties were seen for synthetic vinegar with minimal storage changes in pH, moisture and ash. The microbial count was in limits (<1000/10000 dilutions). This pickle method was applied to the wasted stipe for its cost effective utilization.

Introduction

Mushrooms represent microbial technology to recycle agricultural, industrial, forestry and household waste into food and manure. Edible mushrooms are of great value for being the least expensive source of protein (Chang and Quimio, 1997). They are rich in good quality proteins with lysine and tryptophan along with vitamin C and vitamin B complexes (pantothenic acid, niacin, folic acid, thaimine). They also supply a range of valuable minerals especially potassium and iron (Shrivastava, 1998). Edible mushrooms contain fibers and some nutrients having medicinal aspects; also they are poor source of carbohydrates due to which they can be included in the diet of people with diabetic disorder.

The button mushroom, *Agaricus bisporus*, is one of the most extensively cultivated mushrooms in the world. It belongs to phylum Basidiomycota, class Agaricomycetes, order Agaricales and family Agaricaceae with widest acceptability as a food item among the mushrooms, contributing about 40% of total world production of mushrooms (Vijay and Gupta, 1995). Button mushroom contains 91.5% moisture, 3.7% protein, 4.2% carbohydrate, 0.3% fat and 1.25% ash on wet

wt. basis (FAO, 1970). The protein of mushroom is in the range of 24 to 44% on dry basis that contains 9 essential amino acids (Chang and Miles, 2004). Presence of chitin and beta glucan makes this mushroom dietary fiber rich food (Sadiq *et al.,* 2008).

Morphologically, edible mushroom has a stipe that holds the wide cap named pileus (Kalac, 2009). According to the findings, the stipe of button mushroom contains protein, fiber, high chitin (Vetter, 2007) and more calcium and magnesium content as compared to the cap. On dry weight basis, the nutritional composition of stipe is reported to be 25% crude protein, 1% fat and 18% minerals (Maeda *et al.,* 1993). In spite of an inexpensive by-product for human nutrition, most of the stipe of button mushroom is either wasted or consumed as animal feed which could otherwise if utilized can meet the nutritional requirements.

Mushrooms, however, are highly perishable and tend to lose quality right after harvest as their shelf life is less than 3 days under usual shipping and marketing conditions. During postharvest storage, mushroom undergo changes like liquefaction, loss of moisture, texture and opening of caps thereby making them unacceptable from the consumer's view point (McGarry and Burton, 1994). Hence, the development of appropriate storage and processing technology in order to extend their marketability and availability to the consumers in fresh or processed form is of great significance (Bhupinder and Ibitwar, 2007).

Product value addition can be regarded as one of the post harvest processing practice for long term storage. An adoption of value added products is an appropriate processing methodology because it's the need of the hour for the mushroom growers not only to reduce the losses but also to enhance the income and boost the consumption of this important horticultural crop. One such popular product is pickle (Mehta *et al.,* 2011). Pickling is an ancient preservation process for fruits and vegetables which makes the food tastes better, acts as an appetizer, adds palatability to the meal and contributes mostly towards better assimilation of gastric juices. Addition of salt and /or vinegar makes the prime means of preservation approach in pickle. Vinegar is considered an important preservative because it reduces the thermal death time of microorganisms and either inhibits or kills microorganisms, depending on the concentrations used. The type of vinegar primarily used depends on the flavor of the pickled product. Two types of pickles of vinegar type (natural and synthetic) can be prepared. Vinegar may be added for taste and longer storage and the contents in the bottle should be topped up with oil. The preservative action of vinegar is based upon its acetic acid content. Pathogenic bacteria are rapidly destroyed in pickle solutions containing 3% acetic acid and 1.5% salt. Several recipes of pickle preparation in oil or vinegar are in practice in different parts of the country (Girdharilal *et al.,* 1967). The pickle may contain onion, garlic, ginger, sugar, jaggery, edible vegetable oil, green or red chillies, spices, spice extracts/oil, limejuice, vinegar/acetic acid, citric acid, dry fruits and nuts. Of these, oil-based pickles have the largest market share. Mushrooms for pickling are either blanched or fried in oil till brown depending upon taste; various condiments as per local preferences and practices are also ground or fried in oil separately and added to the mushroom (Saxena and Rai, 1990).

In the above context, the present study was undertaken with the objective to standardize *Agaricus bisporus* vinegar-oil based pickle using two different vinegar types and to assess the keeping quality of these pickles under ambient storage condition. Also, the best

opted method would be applied to the stipe of the button mushroom strains which otherwise go wasted.

Materials and Methods

Material sources

The mushroom [*Agaricus bisporus* Lange (Sing.)] pickle was prepared using strain U3 which was procured from Mushroom Research Complex, PAU, Ludhiana. All other standard ingredients such as spices, salt and oil were also purchased from local market of Ludhiana. Natural sugarcane vinegar and synthetic white vinegar were obtained from the department of microbiology, PAU Ludhiana. The concentration of 5% for both vinegar types was used at 70 ml per kg of pickled mushroom volume. For sampling, 200g of prepared product was filled in food grade pack plastic jars (each having capacity of 250 g). Storage was done under ambient conditions.

All the reagents used in the present investigation were of analytical grade.

Preparation of mushroom pickle

Standardization of methodology of mushroom pickle was prepared from strain U3 of button mushroom. Modifications in standard protocol were made (Ranganna, 1986). Two vinegar types (sugarcane and synthetic) at 5% acidity were used in varied volumes with added spices in fixed amount. Freshly harvested *Agaricus bisporus* strain U3 was used for this purpose. 2 kg of mushrooms were taken and the outer dirt, foreign particles, casing soil etc. were removed if any, from their outer skin. Mushrooms were sorted on the basis of their physical appearance having small size, uniform diameter of pileus. Washing with plain running water was given and rest of the water was drained out from them using sieve. The lower part of each mushroom fruiting body was trimmed from the base to remove the unwanted part. Pileus with attached 1 cm stipe was used as whole. It is done to inactivate enzyme activity. The mushrooms were blanched for five minutes in water with 2% salt keeping the temperature of water at 85°C-90°C. Mustard oil was used for frying the ingredients. The oil was heated for 2-3 minutes before putting any ingredient into it. Different spices were used for the preparation of mushroom pickle. All the spices were grinded well before use. The composition is given as below.

Standardized Mushroom Pickle Spices Composition (For 1 kg of mushrooms)

Spices	Amount in grams
Red chilli powder	10g
Haldi	10g
Zeera	10g
Cinnamon	10g
Cardamom	10g
Rye	10g
Black pepper	10g
Ginger paste	50g
Garlic paste	25g
Salt	50-100g
Vinegar	(white and sugarcane@5%) 60 ml, 100 ml and 140 ml
Mustard oil	250ml

Mustard oil was poured to shallow fry the mushrooms till they get golden brown. The mushrooms were separated and extra oil was drained out. Ginger and garlic paste were added followed by rest of the spices. In the end, salt was added and the mushrooms were mixed with the spices on low flame. The contents were mixed properly and allowed to cool on wide tray. 200 g of mushrooms were filled into 250 g of food grade plastic air tight jar. Different volumes of vinegar were poured into each jar. Spaces over the mushroom pieces were sufficiently covered with

remaining extra oil. Air tight lids were used for sealing the jars. Mushroom pickles were stored in ambient conditions and various physical, chemical and microbial analyses were carried out on them for a period of 6 months at 2 months interval (0, 2, 4 and 6 months).The steps discussed in section above were applied in making pickle so as to utilize the stipe of mushrooms. The stipes were cut into 1-2cm sized pieces. Vinegar volume of 14ml/200g of mushrooms with 5% strength is used for both synthetic and natural vinegar (Optimization done) (Singh, 2015).

Sensory evaluation

The organoleptic evaluation of both types of pickle samples were conducted by a panel of semi-trained judges (7 judges) for appearance, taste, mouthfeel and overall acceptability using the 9- point Hedonic Rating Scale (Amerine *et al.,* 1965).

Shelf life analysis on pickled button mushrooms

Morphological analysis

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.

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.,* (2007) with modification of the strain to 75% of sample height and probe (75-mm compression platen).The hardness was calculated as given by Bourne,(1982).

Physical analysis

Total solids and moisture content were determined as per AOAC, 1990 and AOAC, 1975procedures respectively. Digital pH meter (pHep pH Tester) was used for pH determination which was calibrated using buffers of different acidic and basic pH. 5 g sample was dispersed in 100 ml of distilled water, mixed properly and filtered through Whatmann filter paper. 10 ml aliquot was dispensed in small beaker to obtain the readings. Total ash and fibre were estimated by AOAC, 1965 and AOAC, 1970 protocols respectively.

Chemical analysis

The titrable acidity of pickled mushroom was obtained by using method of AOAC, 1999 taking acetic acid for percent acidity. Salt content as sodium chloride was determined by Mohr's method AOAC, 1975. Fat content was estimated by the Soxhlet Extraction method (AOAC, 1995). Micro Kjeldahl method was used to estimate protein content (AOAC, 1995). Total carbohydrates are calculated by difference. Under this approach, the other constituents in the food (protein, fat, water, alcohol, ash) are determined individually, summed and subtracted from the total weight of the food. This is referred to as total carbohydrate by difference and is calculated by the following formula: 100 - (weight in grams [protein + fat + water + ash + alcohol] in 100 g of food) (FAO, 2002). The peroxide value for checking the rancidity of pickle was estimated by using AOAC 1975 method. Vitamins were determined by AOAC 1996. PPO activity was determined by Fang *et al.,*

(1974). Antioxidant activity was obtained by DPPH method (S´anchez-Moreno *et al.*, 1999).

Microbiological analysis

Enumeration of Total plate count (TPC) was accomplished by the method of Adegoke *et al.*, (2004) to evaluate the stored pickles sample. Appropriate serial dilutions were prepared (10^{-3} to 10^{-5}) concentration and this method was used for the enumeration of total aerobic count, like *E. coli*, *Staphylococcal* and *Salmonella* counts on nutrient agar medium and the media were prepared according to the manufacturer's instruction and used for enumeration of total viable bacteria count. The medium was sterilized at 15 psi (121^0 C) for 20 minutes before use. Plating was done using pour plating technique. The plates were incubated at 37^0 C for 24-48 hrs and the total bacterial count (cfu/ g fresh mushrooms) was recorded. Yeast count plating was done using pour plating technique on Glucose yeast extract agar media. The plates were incubated at 25^0 C for 5-7 days and the total yeast count (cfu/ g fresh mushrooms) was recorded while mold count was done on Potato Dextrose Agar media. The plates were incubated at 25^0 C for 5-7 days and the total mold count (cfu/ g fresh mushrooms) was recorded.

Statistical analysis

The data obtained for all the parameters and effect of storage on them was statistically analyzed through ANOVA (CRD) to see the critical difference at 5% level of significance using CPCS1 Software.

Results and Discussion

Storage studies of mushroom pickle

The standardization was made by conducting various organoleptic, physiological, nutritional and microbiological tests on samples of mushroom pickle. The effect of storage on different parameters was also studied for a period of six months at two months interval. The results are discussed as below.

Organoleptic evaluation

Mushroom pickle samples were prepared and organoleptic analysis on hedonic scale for the same was conducted. All the samples showed a non-significant difference in color index among them. Aroma, taste and texture were pleasant in natural (sugarcane) (Table 1).

Quality characteristics and change in them during storage on pickled mushrooms

All *A. bisporus* pickle samples were subjected to quality analysis. The color and texture were affected by storage for six months. The color index of processed mushrooms was found to be lower than the unprocessed one because of blanching, cooking and ingredient mixing. The pickled samples had initial L value as 21.21 and 20.44 respectively. Using 14 ml/200g of synthetic vinegar in mushroom pickle showed maximum stability in L, a and b values by 1.79% with increasing storage time. Minimum change in texture (hardness) was found in sample with used 14 ml synthetic vinegar/200g pickle (Table 2).

Physical characteristics

Physical properties (moisture and pH) were studied during storage period of 6 months. *Agaricus bisporus*, strain U3, was used initially to standardize the method of pickling on the basis of type of vinegar used. Sample which is whole with added 14 ml synthetic vinegar) (75.76%) showed high moisture content. The moisture content was lower than control (92.85%) probably because the salt added may have oozed out some of the water from mushrooms. Precooking in oil on the

other hand has kept the moisture content to a limit in mushrooms. Increase in storage period lead to decrease in moisture content till 6th month for pickle samples. Pickle sample with added 14 ml synthetic vinegar showed low total solids (24.24%).

The total solid content for the samples was higher than control (7.15%). Pickle with added 14ml synthetic vinegar showed low pH (4.7). pH range for all pickles was lower than control (6.5). Most of the samples showed stable decrease in pH but sample dipped in synthetic viengar showed increase in pH till end upto 5.7. The overall samples were significantly different from each other (Table 2).

Nutritional changes during storage of pickled mushrooms

The protein content was almost similar for all the samples (14.47 and15.72%) and showed a decline with increase in shelf life. But the change was very low. The carbohydrate content for all the samples ranged in limit and in accordance with the fresh commodity with values similar to control ranging from as low as 30.2% (sample 6) to as high as 49.9% on dry weight basis (sample 3). Most pickle samples showed a slight decline in fat content with storage period.

The values for fat in pickle samples was very high than 3.1% of normal range on dry weight basis. The decrease in ash content was upto 30% with storage time for pickled samples. The ash content in pickle samples was 6.5% and 6.80%. With increase in storage period, the amount of ash decreased for all the pickles which is very low. The fibre content of mushroom pickles was lower than the control. This may be due to solubilization of some fibers. The fibre values for all the samples decreased from first month and later remained stable for rest of the months on storing them for 6 months (Table 2).

Chemical parameters during storage of mushroom pickle

Button mushroom pickles were prepared and standardized. All the samples had the medium range of acidity (0.36%). Storage studies revealed that samples with synthetic vinegar showed stable titrable acidity. Overall, all samples showed increase in titrable acidity by the end of the 6th month due to absorbance of vinegar into them. Pickle sample with synthetic vinegar showed decrease and again increase upto same level. It showed minimal percentage change. The volatile acidity increased as in accordance with the increased titrable acidity reported. After storage, all the samples showed slight drop by 4th month with recovery of the same value or slight increase than the initial value of salt (Table 2). The samples were non significantly different from each other. The peroxide value represents the rancidity in pickles. It was observed that the values increased to low levels with storage period for samples dipped with synthetic vinegar. The values were higher than control for all the samples and significantly different from each other. The PPO activity in mushroom pickle samples was reduced by processing the mushrooms into pickle. The values were lower than control (0.3145 ΔA/min/g). Maximum stability was maintained in sample with natural vinegar for PPO activity. The pickle samples showed decrease in the activity of enzyme at the end of the storage period with an increase in 4th month (Table 2). There was slight decrease in the amount of antioxidant due to cooking. The reduction was between 3-24% with increase in storage time. Minimum changes were seen in pickle with natural vinegar using DPPH methods of antioxidant detection. The ascorbic acid content of processed mushrooms decreased from the raw mushrooms. Effect of storage period showed an irregular trend in its stability with both increase and decrease with increasing storage time. Pickle showed

maximum stability for ascorbic acid content. Riboflavin (5.23mg/100g-9.87mg/100g) and niacin (26.09mg/100g-36.43mg/100g) contents showed only a small reduction by 5-8% with increasing storage period. Minimum percentage change was seen for sugarcane used vinegar in pickle. Similar trend was seen for thiamine content.

Microbiological changes during storage of mushroom vinegar pickle

The mushroom pickle samples were subjected to bacterial, yeast and mold count on every two months of interval for six months. Samples showed normally an increasing trend in bacterial count with maximum increase by the end of 6th month. Among pickles, all samples showed lower values of bacteria, yeast and mold with storage at 100 dilutions. All the samples showed no significant mold growth. The growth could be seen in last month. The samples confined to permissible limit of microbial count of 1000/10000 (Table 2).

Utilization of stipe of mushroom as a value added product

Sensory analysis

The unused stipes of button mushroom could be used to make pickle. The methodology standardized was utilized to the stipes of mushroom too. Sensory analysis suggested that when two different vinegar types (synthetic and natural at 5% acidity) were used, synthetic vinegar gave better color and texture while natural (sugarcane) vinegar gave better aroma and taste (Table 3).

Effect of storage on physico-chemical and microbial parameters of stipe of *A. bisporus*

Physico-chemical and microbial parameters were done on stipe mushroom pickle. Similar trend follows as color, texture, protein, vitamins, PPO activity and sodium content were better when natural vinegar (sugarcane) was used while moisture, dry matter, peroxide value was better for synthetic vinegar used stipe pickle (Table 4). Free amino acids, carbohydrates, vitamins, antioxidant activity, titrable acidity in stipe pickle samples showed non-significant changes for both vinegar types with storage of 6 months. Nasiri *et al.*, (2012) showed that the behavior of moisture and fat content in different flushes for cap and stipe was similar. Moisture and fat content of stipe, same as cap decreased significantly ($p<0.05$) from first flush to the third flush. The fiber content of stipe was significantly ($p<0.05$) higher than cap and the average of fiber content in three different flushes reported as 27.11 for cap and 38.51 for stipe. According to our results, both parts of mushroom (cap and stipe) are valuable sources of nutrition and using both parts in diet might be effective in human health. Also, the stipe part of button mushroom can be applied as a valuable food source for quantitative and qualitative provision of some human needs and help the economy of the country by applying agriculture.

Table.1 Optimization of vinegar mushroom pickles on the basis of sensory evaluation

Sample code	Color	Appearance	Aroma	texture	taste
Synthetic vinegar	8.16	8.83	8.16	8.16	8.80
Natural vinegar	8.00	8.66	8.73	8.73	8.76
CD (5%)	**NS**	**0.145**	**0.0798**	**0.0554**	**0.0714**

*Mean value of three replicates & 4 months. Like extremely 9, Like very much 8, Like moderately 7, Like slightly 6, Neither like/Dislike 5, Dislike slightly 4, Dislike moderately 3, Dislike very much 2, Dislike extremely 1

Table.2 Effect of storage on physico-chemical and microbial parameters of *A. bisporus* pickle

Parameters		Time duration								C (Raw mushroom)	CD (5%)
		0 month		2 month		4 month		6 month			
		Synthetic vinegar	Natural vinegar	Synthetic vinegar	Natural vinegar	Synthetic vinegar	Natural vinegar	Synthetic vinegar	Natural vinegar		
Color	**L**	21.21	20.44	17.38	17.95	25.83	26.21	21.59	29.29	85.4	0.09
	a	3.55	2.33	2.83	3.69	4.24	0.89	3.21	2.14	1	0.11
	b	11.03	10.42	7.91	8.67	13.63	8.06	13.17	22.74	12.65	0.45
Texture (g)		716	350	627	111	627	342	618	179	1417	20.43
Moisture	**(%)**	75.76	71.15	71.43	71.64	69.09	74.57	75	69.23	92.85	0.12
pH		4.7	5.7	5	5.3	4.6	5.3	4.7	5	6.5	0.74
Dry matter	**(%)**	24.24	28.84	28.57	28.36	30.91	25.42	25	30.77	7.15	0.83
Protein	**(%)**	14.47	15.72	12.97	13.62	11.09	15.89	10.21	15.87	27.4	0.31
Fat (%)		21.91	21.91	21.2	21.2	21.1	21.1	21.1	21.1	3.12	0.06
Carbohydrates		49.9	30.1	49.8	30	49.4	30	49	29	4.09	0.3
Ash (%)		6.5	6.8	6.3	6	6.1	5.8	10	4.8	8.96	0.12
Fibre (%)		13.2	13.32	10.17	6.77	10.02	6	10	6.89	19.05	0.04
Vitamins	**Ascorbic acid**	3.44	5.16	8.6	8.6	12.04	5.16	6.88	5.16	29.12	0.06
(mg/100g)	**Ribo-flavin**	8.68	9.87	8.43	9.87	8.32	9.88	8	9.54	5.94	0.1
	Niacin	32.98	26.09	30.99	26	30.76	25.8	30.65	25.98	14.76	0.19
	Thiamine	0.26	0.27	0.24	0.26	0.21	0.24	0.15	0.23	0.28	NS
Antioxidant activity	**DPPH (%)**	26.98	25.76	25.21	26.1	24.121	24.12	22.31	24.21	31.621	0.11
PPO	**(ΔA/Min/g)**	0.014	0.092	0.005	0.009	0.003	0.197	0.014	0.011	0.31	0.003
Titratable acidity	**(%)**	0.36	0.36	0.24	0.12	0.36	0.24	0.36	0.6	0.12	0.04
Na content	**(%)**	2.48	2.34	1.61	1.31	0.88	0.29	2.77	4.23	0.15	0.07
Peroxide value	**(meqv/min/1000 g fat)**	1200	11000	1300	13083	1542	16000	1590	17000	50	44.77
Microbial count (cfu/g)	**Bacteria**	0	0	0	0	2.1	2.7	16	0.5	8.035	0.002
	Yeast	0	0	8	4	5	3	3.6	3	1.03	10-May
	Mold	1	0	1	0	0	0	0	0	0.51	0.001

Permissible limit in pickle: less than 1000/10000 (FDA 1995)

Table.3 Sensory analysis

Sample code	Color	Appearance	Aroma	Texture	Taste
Synthetic Vinegar	**8.16**	**8.13**	**7.61**	**8.16**	**7.33**
Natural vinegar	**8.00**	**7.60**	**7.83**	**7.33**	**8.26**
CD (5%)	**0.11**				

*Mean value of three replicates & 4 months. Like extremely 9, Like very much 8, Like moderately 7, Like slightly 6, Neither like/Dislike 5, Dislike slightly 4, Dislike moderately 3, Dislike very much 2, Dislike extremely 1

Table.4 Effect of storage on below mentioned parameters of stipe of *A. bisporus* pickle

Parameters		Time duration								C (Raw mushroom)	CD (5%)
		0 month		**2 month**		**4 month**		**6 month**			
		Synthetic vinegar	**Natural vinegar**	**Synthetic vinegar**	**Natural vinegar**	**Synthetic vinegar**	**Natural vinegar**	**Synthetic vinegar**	**Natural vinegar**		
Color	**L**	**20.91**	**22.73**	**19.59**	**17.68**	**30.39**	**29.07**	**18.22**	**21.42**	**85.4**	**0.02**
	a	**4.83**	**6.31**	**4.17**	**3.76**	**5**	**4.35**	**5.31**	**4.09**	**1**	**0.01**
	b	**11.17**	**12.7**	**8.86**	**7.88**	**13.76**	**12.11**	**15.06**	**11.29**	**12.65**	**0.01**
Texture	**(g)**	**100**	**100**	**20**	**63**	**14.23**	**75**	**13**	**57**	**1417**	**17.3**
Moisture	(%)	**66.66**	**73.33**	**66.03**	**66.1**	**66.66**	**64.86**	**61.82**	**63.46**	**92.85**	**0.17**
pH		**5.5**	**5.7**	**5**	**4.9**	**4.5**	**4.1**	**4.5**	**4.6**	**6.5**	**0.05**
Dry matter	(%)	**33.33**	**26.66**	**33.96**	**33.9**	**33.33**	**35.13**	**38.18**	**36.53**	**7.15**	**0.5**
Protein	(%)	**15.02**	**15.12**	**13.67**	**14.32**	**12.12**	**12.12**	**9.32**	**10.32**	**27.4**	**0.6**
Fat	(%)	**11.33**	**11.33**	**13**	**13**	**12.9**	**12.1**	**12.9**	**12.3**	**3.12**	**0.6**
Carbo		**3.01**	**4.2**	**3**	**4.15**	**3.04**	**4.1**	**3**	**4.07**	**4.09**	**NS**
Ash	(%)	**7.5**	**8**	**7**	**6**	**6.5**	**6.02**	**6**	**5.89**	**8.96**	**0.6**
Fibre	(%)	**13.32**	**13.21**	**6.77**	**7.44**	**6**	**7**	**6.89**	**6.87**	**19.05**	**0.6**
Vitamins	**Ascorbic Acid**	**5.16**	**6.88**	**12.04**	**6.88**	**8.6**	**5.16**	**6.88**	**5.16**	**29.12**	**1.3**
(mg/100g)	**Ribo-flavin**	**7.65**	**9.65**	**7.61**	**9.34**	**7.09**	**9.12**	**5.87**	**9.09**	**5.94**	**NS**
	Niacin	**28.67**	**26.77**	**28.56**	**26.71**	**28.43**	**26**	**28.08**	**25.45**	**14.76**	**NS**
	Thiamine	**0.24**	**0.26**	**0.23**	**0.21**	**0.21**	**0.2**	**0.24**	**0.26**	**0.28**	**NS**
Antioxidant activity	**DPPH (%)**	**31.345**	**27.234**	**31.021**	**26.211**	**30.211**	**25.111**	**31.345**	**27.234**	**31.621**	**1**
PPO	(ΔA/Min/g)	**0.05**	**0.02**	**0.01**	**0.01**	**0.18**	**0.37**	**0.05**	**0.02**	**0.31**	**0.001**
Titratable acidity	(%)	**0.36**	**0.36**	**0.12**	**0.24**	**0.36**	**0.36**	**0.36**	**0.36**	**0.12**	**NS**
Na content	(%)	**0.88**	**1.75**	**2.04**	**1.02**	**0.58**	**0.29**	**2.77**	**3.21**	**0.15**	**0.34**
Peroxide value	(meqv/min/1000g fat)	**6042**	**87302**	**6100**	**96092**	**6590**	**11500**	**7800**	**3833**	**50**	**64.9**
Microbial count (cfu/g)	**Bacteria**	**2**	**0**	**4**	**0**	**2.4**	**12.5**	**1**	**7.5**	**8.035**	**0.8**
	Yeast	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**2**	**1.03**	**5.00E+04**
	Mold	**0**	**0**	**0**	**1**	**0**	**2**	**5**	**5**	**0.51**	**5.00E+04**

Permissible limit in pickle: less than 1000/10000 (FDA 1995)

The color index for pickles found to show no significant differences (p>0.05) among samples in terms of lightness and greenness of roselle pickle (Nasution, 2013). Blanching suppressed browning in fruits, resulting in constant or only slight increase in b* value (Krokida *et al.,* 2000). Texture gets affected by storage conditions as firmness of samples was seen to decrease after 10 days of pickling process (Nasution, 2013). Blanched samples had lower values of firmness compared to other samples. Some fruits and vegetables are very heat sensitive and quality indicators such as color and texture are usually degraded to a large extent during thermal treatment (Lau *et al.,* 2000). Similar findings were reported by Joshi (2010), where on storage of white button mushroom preserve, hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience, decreased significantly ($P \leq 0.05$) up to 60 days of storage. Studying the physiological factors gave different outcomes of results when pickle is stored for six months. Mishra *et al.,* (2010) investigated that the moisture content of mushroom pickles decreased during storage due to the evaporation of moisture. The moisture value for pickle was 63.40% which is similar with the readings obtained ranging from 60.52% to 78.72%. Khaskheli *et al.,* (2015) reported that in the beginning pH were recorded as 5.70; pH value dropped gradually most of the time during processing of pickle. The pH continued to decrease from 5.50 to 5.04 and 5.86 to 5.56 for (MOVS) (SWVS) respectively until day 15 and remained steady at the end of the storage period. The low pH for a period of storage may be due to the activity of certain types of the bacteria, which is producing acid (Gopal *et al.,* 1985). Decrease in pH was observed in both pickles. Similar decreasing trend in pH throughout storage of pickles was reported by several authors (Behanan, 1992). However, Kumar and Ray (2007) showed an increase in pH for mushroom pickles. The range of carbohydrates is similar to the one reported as 47.5 % and 59.11% on dry basis by Stamets (2005) and Singh *et al.,* (2011) respectively. The readings showed a slight decrease in 6th month of storage. When storage effect was studies for nutritional quality, it was observed by Kumar and Ray (2007) that change in protein content was non-significant for mushroom samples. Kumar and Ray (2007) found no significant change in fat content with storage. According to Nasiri *et al.,* (2013), the ash content ranges from 9.5% to 10.17% on dry weight basis. Readings fall in alignment with Kumar *et al.,* (2014) (9.4%-14.5%). They showed a similar trend with the readings obtained from Kumar and Ray (2007) which showed no significant change in ash with storage period for two types of pickles using different oils. The fibre values for pickle samples showed a decrease and later get stable after first month. This is similar to the findings reported by Joshi (2010). The value for control was in agreement with the readings obtained by Parashare *et al.,* (2013) (20.93%) and Stamets (2005) (19.90%-20.90%). Mishra *et al.,* (2010) showed that the initial acidity of mushroom, vegetable and mixed pickles were 0.55%, 0.89% and 0.80% as acetic acid, respectively. The increase in acidity was significantly increased during of these pickles for 60 days at 28±5°C. The variation in increase in acidity may be attributed to the absorption of acidulants from the pickling media and action of microorganism present in the pickle. According to Joshi (2010), the acidity increased significantly. However, Kumar and Ray (2007) showed the decreasing trend in titrable acidity for mustard and sesame oil based white button pickles. According to Mishra *et al.,* (2010), during storage of pickles, the salt concentration increased significantly ($P<0.01$) with increase in storage period. A significantly higher salt concentration was found for vegetable pickle

followed by mixed and mushroom pickles. This increase could be due to diffusion of salt from the pickle solution into the vegetable components. He also reported that the final value of free fatty acid content of mustard oil used for the preparation of mushroom, vegetable and mixed pickles during storage for 60 days at room temperature were 0.69%, 0.96%, 0.81% as oleic acid, respectively. The free fatty acid content of mustard oil increased significantly ($P<0.01$) with increase in storage period. The increase in free fatty acid content of mustard oil of vegetable pickle was significantly higher than mushroom and mixed pickles. Puttarajappa *et al.,* (1996) also observed an increase in the free fatty acid content of pickling media for meat pickle. The increase in fatty acid content of pickling media has been observed due to hydrolysis of fatty acids from fat by organic acids present as acidulants. Such an increasing trend was reported by mushroom added fish patty on storage for 12 days (Nayak *et al.,* 2015). Joshi,(2010) found the initial total plate count of mushroom preserve was 3.79 log of colony forming units whereas yeast and mold count was 3.06 log of colony forming units. All these counts decreased significantly ($P \leq 0.05$) with the advancement of storage (up to 60 days) in both the samples. However, in the later stages of storage, no counts were detected. Kumar and Ray,(2007) reported that microbial examination showed significant increase in total viable count during storage for one year but it remained in the range of 2 log cycles throughout the storage period. The decrease in total phenol content during storage indicated that phenols were utilized by the enzyme PPO concentrated in the skin of the mushroom (Burton, 1986). The activity of PPO is reported to increase with increase in storage temperature when mushrooms were not processed (Rai and Saxena, 1989). Similar results were seen by Sun *et al.,* (2011). Tak (2006) also reported that blanching and cooking decreased the antioxidant value with storage. The values for vitamins were in range and in agreement with the order of different coworkers. Baston *et al.,* (2014) showed a slight decrease in the frozed stored mushroom. However, Caglarkmak, (2001) showed non-significant change in vitamins. Nasiri *et al.,* (2013) used agricultural waste to reduce the costs and environmental pollution. Button mushroom´s stipe is a cheap by-product that is rich in nutrients for human nutrition. The stipes were separated from caps after harvesting and parameters such as moisture, protein, carbohydrates, fat, fiber, ash, minerals (potassium phosphorus, calcium, iron and selenium) and the fatty acid compositions were determined. The result indicated that the caps on average contained 90.76% moisture and total solid content consisted of 33.65% protein, 20.59% carbohydrate, 2.48% fat, 33.11% fiber and 10.17% ash while the stipes contained 90.01% moisture where the solid content consisted of 19.01% protein, 31.41% carbohydrate, 2.00% fat, 38.08% fiber and 9.5% ash. The analysis of the extracted lipid revealed that the major unsaturated fatty acid was linoleic acid, while the predominant saturated fatty acid was Palmitic acid in both cap and stipe. According to statistical analysis, significant differences ($p<0.05$) were obtained between the chemical composition of caps and stipes. The results also indicated that the amount of calcium content in the stipe is twice higher than cap (2.08 g/kg) and the amount of iron and linoleic acid in the stipe is significantly higher than cap ($p<0.05$). The cap of the mushroom has significantly higher concentration of protein ($p<0.05$) than the stipes. From statistical point of view, button mushrooms stipe, contains higher quantities of fiber and carbohydrate in comparison with cap ($p<0.05$), that could have useful health promotion effects. Therefore, this valuable product that is often considered as an agricultural waste might be employed as a rich source of nutrients in food industries.

In conclusion, it was inferred that the shelf life of oil vinegar based mushroom pickle is of six months at room temperature. Synthetic vinegar added pickle gave better color and textural properties along with other physical parameters (pH, moisture, dry matter, ash). Nutritionally, natural vinegar types pickle was more profound than synthetic vinegar showing more protein, fat content. Carbohydrate levels remained same in both. The shape of the mushroom did influence the properties. Microbiologically, all samples showed acceptable range of microbial count. The unused stipes of button mushrooms were utilized to make pickles using the method of pickling standardized. Sensory analysis suggested that synthetic vinegar gave better color and texture while natural (sugarcane) vinegar gave better aroma, texture and taste. Physico-chemical and microbial parameters were done on stipe mushroom pickle. It was found that the color, texture, protein and sodium content were better when natural vinegar was used while moisture, dry matter, peroxide value were better for stipe pickle made from synthetic vinegar.

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