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Nutritional Studies and Antioxidant Profile of Pickled Oyster Mushrooms of North East India

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ABSTRACT

Mushroom is a very popular food that is consumed around the globe yet it finds very limited acceptance in India, that too mainly among the elite. Non-availability of mushrooms around the year and its highly perishable nature makes it an expensive commodity for the common people to afford. Hence pickling may be viewed as a method to increase the shelf life of oyster mushrooms from 4-7 days under refrigerated condition to at least up to 12 months at room temperature. Hence a recipe for pickling of mushrooms using Indian spices was formulated so as to suit the Indian palate. The proximate analysis, calorific value, mineral and fatty acid profile along with antioxidant profile of the mushroom pickle was elucidated. Sensory parameters and shelf life and stability determining data like pH, titratable acidity and microbiological profile of the pickle was also performed. Finally it was observed that the mushroom pickle formulation has a shelf life of at least 12 months at room temperature with an overall acceptability (OAA) score above 'very good' mark.

Keywords: Oyster mushroom; Pickle; Composition; Micronutrient; Antioxidants; *Agaricus bisporus*

1. INTRODUCTION

Mushroom is a general term used mainly for the fruiting body of macrofungi (kingdom Mycetae) representing only a short reproductive stage in their life cycle. Mushroom can be epigeous or hypogeous, large enough to be seen with the naked eyes and can be picked by hand. It is estimated that although more than 2000 species of mushrooms exist in nature, yet roughly around less than 25 species only are widely accepted as food and consequently cultivated commercially and have attained the importance of being a cash crop[3]. Mushrooms rank between meat and legumes, in terms of crude protein content and have excellent usage in low caloric diets for their low contents of fat and energy. Consumption of wild edible mushrooms is gaining momentum due to a good content of proteins and trace minerals and because of disease outbreaks related to animal meat consumption like bovine spongiform encephalitis (BSE) from beef, Trichinosis from pork, Salmonella mediated infection from poultry and Scrapie from lamb and mutton and upcoming surge of vegetarianism in countries like India where percentage of vegetarian people has risen by 4 per cent from 2004 to 2014. Hence the nutritional potential of mushroom as a gradual replacement of meat needs careful examination involving detailed chemical and biological studies. Mushrooms are low in calorie, yet having a high concentration of polyunsaturated fatty acids. In recent years, there is a trend to cultivate value added mushrooms like selenium bio-accumulated mushrooms. Edible mushrooms are now termed as culinary-medical mushrooms as it is observed that in addition to nutritional values they possess considerable therapeutic effects against many pathologic conditions. Mushrooms are known to be used medically as antioxidant, anticancer, antidiabetic, antiallergic, immunomodulating, cardiovascular protector, antitumor, anti-inflammatory, anticholesterolemic, antiviral, antibacterial, antiparasitic, antifungal, detoxificant and hepatoprotectant. Many bioactive compounds like polysaccharides (beta-glucan), phenolics, flavonoids, folates, lectins, etc. are found either in fruiting bodies, cultured mycelium or cultured broth. Mushrooms are particularly known for antioxidant compounds, particularly polyphenols and flavonoids. For evaluation of antioxidant properties, the reducing power of mushrooms extracts and their scavenging effects on 1,1-diphenyl-2-picrylhidrazyl (DPPH) radicals were studied. Other studies reveal the neutralisation of linoleate radical by mushroom extract and report the presence of phenolic compounds and organic acids in them. The most cultivated mushroom worldwide is *Agaricus bisporus*, followed by *Lentinus edodes*, *Pleurotus* spp. etc. Oyster mushrooms generally fall under the genus *Pleurotus*, and are easily cultivable having high nutritional values and medicinal importance.

The respiration rate of fresh mushroom is extremely high (greater than 60 mg CO_2 $kg^{-1}h^{-1}$) and contains around 85-95 per cent moisture content when harvested. Hence fresh mushrooms are highly perishable items and have to be processed by various methods for enhancing the shelf life. Apart from the high water activity, enzymatic browning by activation of tyrosinase and

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polyphenoloxidase and also by the presence of microorganisms also affects the degree of ripeness and damage of the fruiting body. Among the different methods adopted for mushroom preservation range from age-old method of drying to modified atmosphere packaging (MAP) of fresh mushrooms. Apart from traditional blast drying, vacuum drying using microwave heating is also. Moreover sealing in air-tight container and freezing are also popular methods of preservation of mushrooms. Salting and pickling are also very popular procedures adopted to preserve mushrooms. Although salting has negative effects on nutritive values of mushrooms due to less suitable Na:K ratio and lower content of water soluble components, yet it is popular in Europe. Pickling of *Pleurotus ostreatus* has been reported in conjunction with freshly shredded cabbage having a shelf life of six months. Pickling is preferred over other methods as pasteurisation of mushrooms before storage is not required for pickling and presence of lactic acid bacteria imparts a nice aroma and taste to the food. Pickling is a very popular technique of food preservation in India, where the items to be pickled range from common vegetables like carrot, yam to not-so-common vegetables like bamboo shoots and also red meat like pork that are commonly consumed mostly in north east India. The aim of this paper is to enumerate the nutritive parameters, antioxidant profile and shelf life based on sensory evaluation data of a mushroom pickle recipe prepared from freshly harvested oyster mushroom (*Pleurotus* spp., namely *P. ostreatus* and *P. sajor-caju* etc.) from north east India.

2. MATERIALS AND METHODS

2.1 Preparation of Pickle

Freshly harvested trimmed mushrooms are chopped into bite size square or rectangular pieces (1.5 cm x 1.5-3.0 cm), taken in a piece of cloth and subjected to hot water treatment for 2 min followed by rapid cooling to prevent surface contamination. Thereafter hand touching of mushroom pieces were to be avoided and a clear spoon or ladle used for this purpose. 50 per cent of freshly cut mushroom pieces were boiled in single distil water for around 25 minutes, containing 16.66 per cent sugar, 2.66 per cent salt, 1.67 per cent chilli powder, 0.35 per cent turmeric powder, 0.67 per cent cumin powder and fenugreek powder. A thick gravy was formed as a result of boiling. Then the gravy was reduced to dryness under lowered temperature conditions (around 50^0C) and 5 per cent vinegar was added and continuously stirred for another 10-15 min for further evaporation. Thereafter 0.66 per cent fresh curry leaves, 0.33 per cent cinnamon and clove powder was added to the pan and mixed thoroughly (Preparation 1). In another pan, 73.25 per cent freshly sliced onion, 7.24 per cent grated garlic and ginger and 3.62 per cent sliced green chilli were sautéed in mustard oil until browning of onion occurs (Preparation 2). Some mustard oil was heated suitably and cooled to room temperature to be added as a topping on the pickle surface. Preparation 1 and 2 were thoroughly mixed and filled into previously steam sterilised and dried glass containers. Then the surface of the content is levelled with a dry clean spoon and topped with earlier heated mustard oil. All the heating processes were carried out in heating mantle to ensure proper maintenance of temperature. All the percentages mentioned are on a weight/weight basis. All the spices, vinegar and vegetables (other than mushroom) are procured from local market. Mushroom used for the process is cultivated in DRL campus in Tezpur.

To determine the shelf life of the pickle water activity and sensory evaluation of the samples were done at 0 month, 6 months and 12 months interval. Nutrition (proximate analysis, fatty acid composition, mineral composition, antioxidant profile, salt content and titratable acidity) and microbiological parameters of the pickle sample were done after storing the of the sample at room temperature for 12 months.

2.2 Chemical Composition and Energy Value

Pickle sample was analysed for chemical composition (moisture, protein, fat, carbohydrates crude fibre and total ash) using the AOAC procedures[1].The crude protein content (NX4.38) of the sample was estimated by the micro-Kjeldahl method ; the crude fat was determined by extracting a known weight of pickle sample with petroleum ether, using a Soxhlet apparatus and the ash content was determined by gravimetry after incineration at 600 ± 15 ^{O}C. Total carbohydrates were calculated by titration method using Fehling's solution. Crude fibre was estimated gravimetrically from sample obtained after moisture and fat analysis, using a Gooch crucible and after sequential acid and alkali digestion. The calorific value of pickle sample was estimated in an auto bomb calorimeter (Make: Changsha Kaiyuan Instrument Co. Ltd, China; model no. 5E-1AC/ML) as per the manufacturer's instructions.

2.3 Fatty Acid Composition

Fatty acids in the pickle sample were determined by GC-MS (Agilent Technologies, USA; Model: GC-7890A) with capillary column (19091J-413; HP-5; 5 per cent Phenyl Methyl Siloxane capillary column having dimensions 30 m x 320 µm x 0.25 µm) and MS-240 ion trap detector based on the ISO 5509 (2000)[2] trans-esterification method. The temperatures of the injector and detector were 250 ^{O}C with helium was used as carrier gas at an internal pressure of 120 kPa. Separation was achieved in an oven condition of 50 ^{O}C temperature for 1 min hold and then programmed to increase up to 250 ^{O}C at a rate of 7 ^{O}C/min and coming to a hold for 5 min at 250 ^{O}C. The MS data was analysed in a split less injection mode (injection volume 1µl) with a delay time of 3 min and atotal run time of 34.5 min covering a m/z range of 50-500 under a full scan mode. The MS analysis was done by the protocol followed by Barros[3], *et al.* The results are expressed in relative percentage of each fatty acid, calculated by internal normalisation of the chromatographic peak area. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with matching with library standards.

2.4 Mineral Composition

Mineral composition analysis of pickle sample was achieved using an inductively coupled plasma optical emission spectrometer (ICP-OES; Make: Perkin Elmer, Model no. Optima 2100 DV) as per the method followed by Gebrelibanos[4], *et al.* Wet digestion of the 0.5 g pf pickle sample, 0.5 g each, was performed in a oxi-acid mixture of 2:1:1 (v/v/v)

(HNO_3:H_2SO_4:H_2O_2) having a total volume of 12 ml for 3 h at 150 °C. Then, the clear solution was made up to the final volume of 50 ml with deionised water. Digestion of the blank control, in the absence of analytes, was also carried out in a similar manner. The instrument was calibrated using a series of working standards. Standard solutions were prepared, at five points, from 10 mg/L onwards for respective metals. Hence the concentrations of the various metals in the digest were determined. Metal standards and calibrators were procured from Merck-Millipore, USA. Acids and H_2O_2 were procured from SRL, India and were of analytical grade.

2.5 Antioxidant Profile

The antioxidant profile of mushroom pickle was assessed in three different tests; 2, 2-diphenyl-1-picrylhydrazyl (DPPH) is a stable (in powder form) free radical with red colour which turns yellow when scavenged) radical scavenging activity[5], total polyphenols estimation by reaction of polyphenols with phosphomolybdic acid of Folin-Ciocalteau's reagent in alkaline pH and flavonoid estimation[6]. DPPH radical scavenging activity is expressed as IC_{50} (Inhibitory Concentration; mg/ml) which was defined as the concentration of antioxidants in pickle sample that caused 50 per cent radical inhibition in a 0.1mM DPPH soln. Total polyphenols concentration is expressed as mg of gallic acid equivalents (GAEs)/g of sample and total flavonoids concentration is expressed as mg of rutin equivalent/g of sample. Polyphenols react with phosphomolybdic acid of Folin-Ciocalteau's reagent in alkaline solution to produce a coloured complex measured at 760 nm, the intensity of which is directly proportional to the concentration of polyphenols. All chemicals and standards were procured from Sigma St Louis, Missourie, USA.

2.6 Microbiological Profile

For microbial profiling of pickle sample, total plate count (TPC; cfu/gm) using plate count agar, total yeast and mould count (cfu/gm) using potato dextrose agar and *E. coli* count (cfu/gm) using Eosin Methylene Blue (EMB) Agar were done from pickle sample. All the media were procured from Himedia, India. For TPC and total yeast and mould count plates were incubated at 27 ^{O}C (room temperature) for 72 h and for *E. coli* count plates were incubated at 37 ^{O}C for 24 h.

2.7 Titratable Acidity

The titratable acidity of the pickle sample as per IS 13844: 2003 (Re: 2008)[7] and value expressed as gram of citric acid/100 gram sample.

2.8 Water Activity

The water activity (a_w) of the pickle sample was estimated by AquaLab Dew Point water activity meter (Model: 4TE, WA, USA) as per instruction manual from manufacturers. Water activity is defined as P/P_0 where P is the water vapour pressure over a food andP_0 is that over pure water; hence it is a dimensionless number, being a ratio of two similar quantities. The water activity is measured at zero month of pickle preparation, after 06 months and 12 months of storage of pickle at room temperature after preparation as compared to fresh raw oyster mushrooms and dry oyster mushroom powder.

2.9 Salt Content

The salt content of the pickle sample was estimated by manual titration method called Mohr's method using silver nitrate as the titrant to precipitate the chloride ions in the sample as insoluble silver chloride. When silver nitrate added to the sample is in excess, it binds with a chromate ion indicator to produce a red colour in solution, marking the endpoint.

2.10 Sensory Evaluation

Sensory evaluation for the flavour, taste, consistency, colour and overall acceptability (OAA) were done in order to determine consumer acceptability of mushroom pickle vis–a-vis standard commercially available mixed vegetable pickle from M/s MTR Foods, Bengaluru, India by a panel of 10 semi trained judges from employees and research scholars of DFRL, Mysuru. A numerical 09 point hedonic scale (4.5 and below: Rejected as unacceptable and 8: Exceptional) was used for sensory evaluation[8] . Sensory evaluation of samples at zero month, six months and twelve months are performed. The mean values of the sensory parameters are used to assess the acceptability and shelf life of pickle.

3. RESULTS AND DISCUSSIONS

The results of the chemical composition and calorific value of the pickle sample is given in Table 1. The moisture value is 80.21 per cent which is in accordance with per cent moisture levels of fresh weight of *P. ostreatus, P. sajor-caju* which are in the range of 73.7 - 90.9 per cent[9]. Protein in the pickle was found to be 20.13 per cent which is in concurrence with accordance with per cent protein levels of fresh weight of *P. ostreatus, P. sajor-caju* which are in the range of 20.82 - 21.22 per cent[10]. However, the protein contents of mushrooms are affected by a variety of factors, namely the species of mushrooms, substrate used for cultivation, the stage of development at which it is harvested and the part sampled[12]. Although the fat per cent in oyster mushrooms is very low in the range of 0.62-2.2 per cent (Cuptapun[10], *et al*.) yet the pickle contains around 17.84 per cent fat due to its mustard oil base. Total ash from the pickle sample is about 5.04 per cent which is also in range with different *Pleurotus*spp (3.66-6.5 per cent; Chang[5], *et al*. and Cuptapun[10], *et al.*). Crude fibre in the pickle is 15 per cent which is too in agreement with previously recorded values of *P. ostreatus* and *P. sajor-caju* (13.3-18.52%)[13]. Total

Table 1. Proximate composition and calorific value of mushroom pickle sample (mean ± SD; n = 3)

Test parameters	g/100g
Moisture	80.21 ± 0.71
Protein	0.20 ± 0.055
Fat	17.84 ± 0.02
Total Ash	0.04 ± 0.001
Crude Fiber	0.15 ± 0.011
Carbohydrates	1.56± 0.03
Calorific value	167.60 ±0.68 (kcal/100g)

carbohydrates were estimated to be 34.6 per cent which is in range with literature values (32.5-48%)[14]. The calorific value estimated by bomb calorimetric method came out to be 631 kcal/100 g of pickle sample which is 1.5-1.8 times the value of that of *P. ostreatus* (350-420 kcal/100 g). This increase in calorific value may be attributed to the addition of sugar (calorific value 400 kcal/100 g) and mustard oil (879 kcal/100 g) calorific value to the preparation.

The fatty acid composition, in mushroom pickle is shown in Table 2. Pentadecanoic acid, palmitic acid, margaric acid, stearic acid are the major saturated fatty acids found in the pickle. Among the unsaturated fatty acids, cis-linoleic acid is a polyunsaturated fatty acid while paullinic acid (4.474 %) and erucic acid (17.493 %) are the ω7 and ω9 monounsaturated fatty acid present respectively. Dimou et al. (2002)[14] estimated the concentrations of palmitic, stearic and linoleic acids in two different strains of *P. ostreatus* and *P. sajor-caju.*Concentration of palmitic acid (22.5 %) in pickle sample is in agreement with *P. ostreatus* LGM40 and LGM861008 strain (22.78 % and 15.01%) and also with *P. sajor-caju* MUCL 29148 and LGM 851003 strain (22.83 and 25.10 %). Concentration of stearic acid (1.639 %) in pickle sample is found to be much lower than that in *P. ostreatus* LGM 40 and LGM 861008 strain (8.99 and 4.25%) and also with *P. sajor-caju* MUCL 29148 and LGM 851003 strain (14.46 % and 11.58 %). The concentration of linoleic acid (12.35 %) in pickle sample is also lower as compared to *P. ostreatus* LGM 40 and LGM 861008 strain (42.76 % and 65.48 %) and also to *P. sajor-caju* MUCL 29148 and LGM 851003 strain (45.42 % and 46.38 %), but the value is in range with the published data. Maftoun[15], *et al*. reported that concentrations of pentadecanoic acid (1.35 %) and margaric acid (0.21 %) in *P. sajor-caju.* The concentrations of pentadecanoic acid (5.83 %) and margaric acid (7.08 %) in pickle sampleare much higher as that reported by Maftoun et al. (2015).The disparity between reported values of fatty acids and experimental values from pickle sample may be due to the presence of mustard oil in the pickle. Mustard oil having about 12 per cent saturated fatsmay contribute to the increased values of pentadecanoic and margaric acids. The erucic acid may be contributed solely by mustard oil into the pickle sample as mustard oil is reported to have 42 per cent erucic acid[16]. One interesting finding is the presence of nervonic acid (0.451 %) in pickle sample. Since this pickle is prepared entirely from mushrooms of *Pleurotus spp* yet the presence of nervonic acid is an interesting finding as nervonic acid is generally found in *Flammulina velutipes* (Enokitake) mushrooms[17] and not in mushrooms from *Pleurotus* spp. The presence of nervonic acid may be explained from the fact that erucic acid is the immediate precursor of nervonic acid[18] and erucic acid is present in mustard oil. The trans isomers of unsaturated fatty acids were not detected in the pickle making it more healthier as consumption of trans fatty acids are positively correlated with increased incidence of cardiovascular disease.

Table 2. Fatty acid profile (%) of pickled mushroom (mean ± SD; n = 3)

IUPAC Fatty acid name (trivial name; lipid no.)	Composition %	Retention time (min)
Pentadecanoic acid (15:0)	5.830± 0.36	22.548
Hexadecanoic acid (Palmitic acid, 16:0)	22.5± 0.26	23.229
cis-9, cis-12-Octadecanoic acid (cis-Linoleic acid, 18:2)	12.3± 0.65	24.842
Heptadecanoic acid (Margaric acid, 17:0)	7.088± 0.15	25.257
Octadecanoic acid (Stearic acid, 18:0)	1.639± 0.21	25.877
cis- 13-Eicosenoic acid (Paullinic acid, 20:1, ω7)	4.474 ± 0.54	27.422
13-Docosenoic acid (Erucic acid, 22.1, ω9)	17.493±0.26	29.792
cis-15-tetracosenoic acid (Nervonic acid, 24:1, ω9)	0.451±0.013	32.578

Mineral composition in mushroom pickle is shown in Table 3. Heavy metals like cadmium and lead are in the "not detected" (ND) range and 1.14 mg/kg respectively, the later being well within the limit specified by FSSAI Regulations[19]; the limit set by FSSAI for lead being 2.5 mg/kg for any nonspecific kind of food. The probable source of lead in the pickle might be different spice powders that are used for pickling. The pickle contains high levels of calcium (51.44 mg/kg), potassium (25.48 mg/kg), sodium (37.08 mg/kg) and selenium (37.24 mg/kg) and substantial levels of iron (5.24%) and magnesium (10.14 %). Traces of copper (0.28 %), manganese (0.3 %) and zinc (1.24 %) are also present. High levels of sodium as compared to dry oyster mushroom may be attributed to the added salt (2.66 %) in the preparation.The calcium levels in pickle are higher but are in the same range as reported by Mattila[20], *et al.* in *P. ostreatus* dry mushroom samples (10 mg/kg). The variability in mineral composition in different mushroom species is evident from the current study case, as no cadmium is detected in our pickle sample yet cadmium is reported to be present in trace quantities in oyster mushrooms[21]. The potassium in pickle sample is particularly low as reported by Khan[22], *et al.* in *P. ostreatus* dry mushroom samples (1400 mg/kg) and by Falandysz and Borovička[23] in *Pleurotus* spp. (22-40 g/kg dry mass). The

Table 3. Mineral composition of mushroom pickle sample (mean ± SD; n = 3)

Name of element	mg/kg
Calcium	51.44±0.68
Cadmium	ND
Copper	0.28±0.002
Iron	5.24±0.25
Potassium	25.48±0.67
Magnesium	10.14±0.43
Manganese	0.3±0.003
Sodium	37.08±0.12
Lead	1.14±0.001
Selenium	37.24±0.96
Zinc	1.24±0.005

ND = Not detected

apparent low concentration of potassium in the pickle sample may be due to the fact that freshly harvested mushrooms were used for pickling as opposed to dry mushrooms and drying (especially blast drying) of mushrooms lead to elevation in potassium concentration[24]. Magnesium level in pickle sampleis in accordance with previous publications about magnesium composition in dry *Pleurotus* spp. powder. Iron is present in low concentrations in all the mushrooms, but the previous data concerning iron vary very widely ie. from 2-1280 mg/kg dw. Zinc, copper and manganese are trace elements present in mushrooms with the level of Zn being higher as compared to both Cu and Mn in *P. ostreatus*. Reported value range of selenium in *P. ostreatus* is about 0.35-1.05 mg/kg dw[25]. The Se levels obtained in pickle sample is however higher than the reported data. Se acts as a cofactor of glutathione peroxidise enzyme system thereby enhancing alpha-tocopherol activity and boosting up DNA repair mechanism.

Antioxidant profile of the pickle sample is given in Table 4. Total phenolic contents (TPC) in pickle sample is 23.4 mg of GAEs/g of sample. The TPC value of pickle sample corroborates with that for P. ostreatus and P. sajor-caju ranging from 29.30 – 42.47 mg of GAEs/g dw[26]. Antioxidant activity of pickle is expressed in terms of IC_{50} of DPPH radical scavenging activity which reads 87 mg/ml. The IC_{50} values of *P. ostreatus* and *P. sajor-caju* extracts range from 11.56-58.44 mg/ml[27]. This increase in DPPH radical scavenging activity in the pickle sample may be due to the addition of different spices, curry leaves (containing antioxidants like pyrrolidine; and onion slices which is known to have quercetin, a flavonoid. Total flavonoids content in pickle is 1.4 mg of rutin equivalent/g of sample which matches with that of *P. ostreatus* flavonoid data taking quercetin as standard (3.39 mg quercetin equivalent QE/g dw)[28].

Table 4. Antioxidant profile of mushroom pickle sample (mean ± SD; n = 3)

Total polyphenols (mg of GAEs/g of sample)	23.4±0.65
Total flavonoids (mg of rutin equivalent/g of sample)	1.4±0.002
DPPH radical scavanging activity (IC_{50}; mg/ml)	87.0±0.26

The microbiological profile, titratable acidity, salt content and pH (all estimated after one year of storage of the pickle sample at room temperature) of pickle sample are given in Table 5. Total plate count, total yeast and mould count and *E. coli* count has been found to be nil. Titratable acidity, salt content and pH are found to be 1.24 g/100 g, 1.88 g/100 g and 4.19 respectively. The salt content is well under the final recommended concentration of 2.0-2.5 %[29]. The principle function of salt is to withdraw juice from the vegetables and make a favourable environment for fermentation. Salt analysis and adjustment are essential for the manufacturing of pickled food products. Titratable acidity is routinely measured to assess the direct acidification process of vegetables and the amount of free acid is actually estimated using a known amount of sample in distilled water. According to FSSAI Regulations[30] for pickles in vinegar the titratable acidity (expressed in terms of acetic acid) should not be less than 2.0 %, but for pickles in oil no such specification exists. The pH of the pickle sample is acidic as expected. Although growth

Table 5. Microbiological profile, titratable acidity, salt content and pH (mean ± SD; n = 3) of mushroom pickle on storage at room temperature

Test parameters	Results
Total plate count (TPC; cfu/gm)	ND
Total yeast and mould count (cfu/gm)	ND
E. coli count (cfu/gm)	ND
Titratable acidity (g of citric acid/100g)	1.24±0.04
Salt content (g/100g)	1.88±0.03
pH	4.19±0.01

ND= Not Detected

of pathogenic bacteria *E. coli* O157:H7 have been reported in commercially fermented vegetable products and apple cider, having pH between 3.5 and 4.0 yet this pickle sample shows no *E. coli* count in EMB medium.

The water activity data of pickle sample (Table 6.) is as follows; 0.966 at zero month post preparation, 0.866 after 06 months and 0.865 after 12 months post preparation. The a_w for fresh oyster mushrooms is 0.982 and that of dry oyster powder is 0.332 respectively. 0.85 is considered the safe cut off level for pathogen growth like *Streptococcus aureus*. Foods having a water activity above 0.85 are considered as moist foods that require refrigeration or another barrier to control the growth of pathogens. So the pickle under study may be considered as moist food and may be preserved under refrigeration if required and it also has an oil barrier to control pathogen growth which is apparent from the microbiological profile obtained after one year of storage at room temperature. The stability in water activity data over a period of six months (a_w for 06 months vs a_w for 12 months) proves the stability of the pickle even under room temperature.

Table 6. Water activity of mushroom pickle sample.

Water activity (a_w)	
Dry oyster mushroom	0.332
Fresh oyster mushroom	0.982
Pickle sample (0 month post preparation)	0.966
Pickle sample (six months post preparation)	0.866
Pickle sample (twelve months post preparation)	0.865

The sensory assessment of mushroom pickle as compared to standard mixed vegetable pickle is elucidated in Fig. 1. According to Dictionary of Food and Nutrition the term hedonic scale is used in tasting panels where the judges indicate the extent of their like or dislike for the food. Hedonic scale is a semi-quantitative scale where the sensory parameters of food is evaluated by a human being; hence it is not an absolute measure of sensory parameters but definitely has bearing with individual perception to sensory variables, psychological state at the time of test etc. The successive scale points were selected in such a way that the psychological distance between these scale points is approximately equal. This equal-interval of property of scale points helps in analyzing the responses to testing differences in average acceptability using parametric statistics. The flavour of the pickle is 7.02 at zero month and

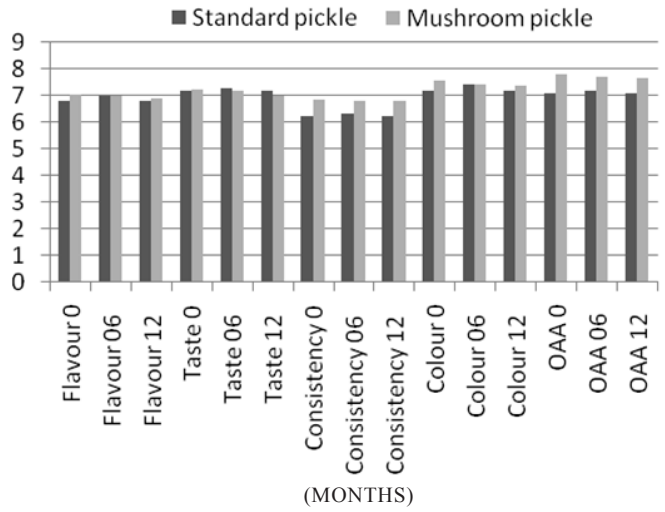

Figure 1. Sensory evaluation of mushroom pickle on a 09 point hedonic scale at zero (0) month of preparation and after six months and twelve months of storage at room temperature, respectively.

goes down slowly to 6.88 after 12 months. Taste scores at 7.24 at zero month with decrease of score to 6.97 at 12 months. Consistency scores at 6.85 at zero time with gradual decrease to 6.79 after 12 months. Colour of the pickle scores at 7.58 at zero time point while the score goes down to 7.37 after 12 months. OAA is a gross summative index (but not an actual sum) of the individual sensory parameters of taste, flavour, colour and consistency and determines the preference of any food as a whole. OAA scores at 7.8 at zero month while decreasing to 7.66 after 12 months. Hence we can comment from the sensory data that according to the interpretative value of the 09 point hedonic scale the quality of mushroom pickle drops maximum w.r.to consistency (6.79 at 12 months) although having an OAA of 7.66 after 12 months. Although 6.79 accounts for something between run-of the-mill (6.50) and good (7.00), OAA score remains above very good mark (7.50) even after 12 months of storage at room temperature. Hence it may be concluded that as per as sensory evaluation is concerned the mushroom pickle has a shelf life of at least 12 months if stored at room temperature.

4. CONCLUSIONS

The pickle may be seen as a rich source of antioxidant minerals like selenium along with calcium and potassium. Moreover it is rich in polyphenols and antioxidants (as evident from high DPPH scavenging activity) and free from any trans fatty acids. Most of the fatty acids reported are cis monounsaturated fatty acids. Since this pickle has a shelf life of at least 12 months hence it may be viewed as a ready to eat source of mushroom based food in India.

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