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Original article

Effect of chemical preservatives on shelf life of mushroom (*Pleurotus ostreatus*) cultivated on cassava peels

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Summary Short shelf life is a major impediment to the processing and distribution of mushroom. The effect of chemical preservatives on some quality attributes of mushroom during storage was investigated. Mushroom were soaked in four preservatives at two concentrations for 10 mins, packaged, stored at 4 °C for 30 days and analysed at intervals for their microbial population, colour, firmness and weight loss. Sodium benzoate (0.05%, 0.1%) lost its preservative effect on all the micro-organisms enumerated after 3 days, and samples treated with 0.1% potassium sorbate had the lowest microbial load at the end of the storage period. Change in colour of the potassium sorbate (0.1%)-treated sample was lower than and significantly different from the citric acid (2%, 4%)-treated samples. The values of the firmness of the 4% citric acid preserved mushroom were significantly different ($P \leq 0.05$) from other samples throughout the storage period. There was a significant negative correlation ($r = -0.807$, $P < 0.01$) between the firmness and weight loss of the preserved mushroom. Potassium sorbate (0.1%) and citric acid (4%) extended the shelf life of mushroom for 24 days.

Keywords Firmness, mushroom, potassium sorbate, preservatives, shelf life.

Introduction

Mushrooms are macrofungi with visible structures that produce spores otherwise known as fruiting bodies. They offer tremendous applications as they can be used as food and medicines besides their key ecological roles (Wani *et al.*, 2010). Mushrooms are low in fat, high in complex carbohydrates and protein (Bano *et al.*, 1993), and they also lack cholesterol and are good sources of vitamins and minerals (Mattila *et al.*, 2001). *Pleurotus ostreatus* the oyster mushroom is an edible mushroom and is one of the more commonly sought mushrooms.

Substrate is one of the most important parameters in mushroom production because it supports the growth, development and fruiting of the mushroom (Chang & Miles, 2004), and these substrates are majorly agricultural wastes such as sawdust, rice bran and maize cobs (Chukwurah *et al.*, 2013). Large amounts usually 15% of solid wastes are generated during cassava processing as peels (Aro *et al.*, 2010). The utilisation of these peels can contribute to reducing the global problem of environmental pollution and can lead to the creation of other useful products such as mushroom.

Also, fresh mushroom is an ideal medium for microbial growth due to their high water activity, high respiration rate, neutral pH (Martínez-Carrera *et al.*, 1998) and continuous metabolism after harvest which results in changes in some of its quality parameters such as colour, weight and texture (Okhuoya, 2005). These characteristics limit the postharvest shelf life of fresh mushroom to few days (1–3 days) which is an impediment to their distribution and storage (Ares *et al.*, 2007), hence the need of a technology that can be used to preserve the postharvest quality of mushroom while ensuring its safety. Food-grade chemical preservatives such as potassium sorbate, calcium propionate and sorbic acid have been used within their maximum permissible levels to prolong the shelf life of foods. There is scarce information in literature on the use of readily available and cheap chemical preservatives which were

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used in this study, namely potassium sorbate, sorbic acid and sodium benzoate to extend the shelf life of *P. ostreatus*. The aim of this work was to cultivate *P. ostreatus* on cassava peels and identify chemical preservative(s) that are suitable for extending the shelf life of mushroom harvested from cassava peels.

Materials and methods

Materials

Cassava peels were obtained from Abeokuta, Nigeria. The peels were sundried, crushed and mixed with water and calcium carbonate in ratio 32 : 66 : 2 to make them moist. The moist peels were continuously turned for 3 days, and they served as substrate for mushroom cultivation. Two hundred grams of substrate was dispensed into polythene bags and autoclaved at 121 °C for 2 h. The substrate was inoculated with 2.5% spawn inside the polythene bags. The bags were hermetically sealed and punctured at the base for aeration and incubated at 25 °C for 5 weeks in a mushroom-growing house that had a relative humidity of 65–70%. They were monitored daily during the incubation period until the substrates were colonised with white mycelia. The colonised substrates were exposed to air by loosening the polythene bag to allow air circulation and light penetration till the pinheads appeared. The mushrooms were harvested after 3–4 days of fruiting. Mushroom from different flushes and phases was continuously harvested from the substrates. The harvested mushrooms were used for further analyses.

Chemical preservatives used

Different food-grade GRAS (generally regarded as safe) chemical preservatives, namely sodium benzoate, potassium sorbate, sorbic acid and citric acid (Ganesh Benzoplast Limited, Mumbai, India), were purchased. The maximum permissible level of each preservative was obtained from the Codex Alimentarius, 2011 food additive guide (CAC, 2011). The concentrations of the chemical preservatives were as follows: citric acid (2%, 4%), sodium benzoate (0.05%, 0.1%), potassium sorbate (0.05%, 0.1%) and sorbic acid (0.05%, 0.1%).

Sample preparation

Oyster mushrooms (*P. ostreatus*) were picked from the desired flush and phase. After picking, they were kept chilled (4 °C) in the refrigerator to keep them in a fresh state. The mushrooms were soaked in the treatment solutions at the above concentrations and in distilled water (control sample) for 10 min. They were afterwards placed on absorbent pads to remove excess surface water. The samples were wrapped with microperforated films and weighed and were finally stored at 4 °C. They were subjected to microbial, colour, texture and weight loss analyses at 3 days interval starting from day 0 (the first sampling was done after storing the samples at 4 °C for 3–4 h) and finishing on day 30.

Methods

Mushroom quality and safety are defined by a combination of parameters which including whiteness, texture and microbial counts. The following analyses were carried on fresh mushroom: microbiological analysis, colour analysis, texture analysis and the weight loss expressed in percentage.

Shelf life extension was measured by the reduction of microbial load and the ability of the treatments to keep the mushroom samples within acceptable limits according to the microbiological standards for foods of the European Commission Regulation (EC) No, 2073/2005.

The yield of the mushroom from cassava peels was measured using a digital weighing balance (Scaletech Instruments and Services Pvt. Limited, Surat, Gujarat, India).

Microbiological analysis

One gram of *P. ostreatus* was homogenised at 5000 rpm using a homogenizer (Ika-Werk, ultra-turrax T25 blade-type homogenizer, Staufen, Germany) in 9.0 mL sterile 0.1% peptone water for 30 s, and then, a 10-fold serial dilution in peptone water was carried out. Aliquot of 1 mL was plated on *Pseudomonas* agar (CM0559; Oxoid Ltd., Basingstoke, Hants, UK) with Cetrimide, Fucidin, Cephaloridine (CFC) selective supplement (SR0103, Oxoid Ltd), Mannitol salt agar (CM 0085; Oxoid) supplemented with 5% egg yolk, MacConkey agar (DM 143; Micro master, Maharajahs, India), Nutrient agar (CM003; Oxoid Ltd.) and *Salmonella Shigella* agar (CM 0099; Oxoid) being suitable media for the growth of *Pseudomonas* spp., *Staphylococcus* spp., coliforms, aerobic bacteria and *Salmonella* spp., respectively. Plated aliquots for coliform and *Salmonella* spp. were incubated at 37 °C for 48 h. Also, plated aliquots for *Staphylococcus* spp. and total aerobic were incubated at 37 °C for 24 h, while *Pseudomonas* spp.-plated aliquots were incubated at 25 °C for 24 h. Control plates were also prepared, a colony counter (LM-10; Analab, New Delhi, India) was used to enumerate the microbial population, and the counts of the microorganism were recorded in log cfu g^{-1}. The percentage increase/decrease in the microbial population of

P. ostreatus at the end of the storage period was also calculated.

Colour analysis

Colour analysis was done according to the method of Mariscal & Bouchon (2008). The *L*, *a*, *b* coordinates were obtained using Adobe Photoshop 6.0 software (Adobe Systems Inc., California, USA) which was thereafter normalised to *L** (lightness), *a** (redness), *b** (yellowness) according to Yam & Papadakis (2004). Also, the colour differences between the control and preserved mushroom samples were determined by taking the Euclidean distance between them according to the following equation:

$$\Delta E^* = [(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2]^{1/2} \quad (1)$$

where L_0* = Lightness value of control mushroom *L** = Lightness of preserved mushroom at ith day of storagea_0* = Redness value of control mushroom*a** = Redness of preserved mushroom at ith day of storageb_0* = Yellowness value of control mushroom*b** = Yellowness of preserved mushroom at ith day of storage.

Texture assessment

The firmness was determined using the method of Han *et al.* (2004) by measuring the compression force of the samples with the aid of texture analyser (TA.XT Plus; Stable Micro Systems, Limited, Surrey, UK).

Weight loss

Weight loss was measured by monitoring the weight changes during the storage period and was determined according to the method of Han *et al.* (2004).

Statistical analyses

All analyses were carried out in triplicates, and data collected from the study were subjected to analysis of variance. Differences among means were separated using Duncan's multiple range test, and significances were accepted at 5% confidence level (Duncan, 1955). Pearson's correlation coefficient between texture and weight loss of the preserved and stored mushroom was calculated using SAS 9.0 (2008) for windows (Statistical Analysis System Institute, Inc., Cary, NC, USA).

Results

At day 0, as shown in Tables S1 and S2, all the treatments reduced the population of all the micro-organisms enumerated when compared with the control. Treatment of samples with NA (0.05%, 0.1%) and refrigeration increased the microbial population of all the samples throughout the storage period starting from day 3 (Tables S1 and S2). At day 6, all the treatments significantly reduced ($P \leq 0.05$) TAC, but 0.05NA and 0.1NA significantly increased ($P \leq 0.05$) TAC which represent 62% and 64% increase over the control, respectively. Between day 0 and 9, STAP and PSEU were <0.30 log cfu g^{-1} in samples treated with CA and KSO (Table S2). At day 9, TAC (Table S1) for 2% CA, 0.05KSO, S0.05 and S0.1 were not significantly different ($P \leq 0.05$) from the control, but 0.05NA and 0.1NA were significantly higher ($P \leq 0.05$) than the control which represent 61% and 63% increase over the control, respectively. Also at day 9, COL was insignificantly lower ($P \geq 0.05$) than the control which represent 25% and 19% decrease over the control, respectively.

At day 12, using TAC, the effect of chemical preservatives (2% CA, 0.05KSO, S0.05 and S0.1) and refrigerator was not significantly different ($P \leq 0.05$) from that of the control. The 0.05NA and 0.1NA were significantly higher ($P \leq 0.05$) than the control, which represents 61% and 64% increase over the control, respectively, but 0.1KSO was significantly lower ($P \leq 0.05$) representing 13% decrease. At day 15, TAC for 2% CA, 4% CA, 0.1KSO were significantly different ($P \leq 0.05$) from the control and had 21%, 27% and 20% decrease over the control, respectively. At day 18, 0.05KSO reduced COL by 0.71 log cfu g^{-1} (36% decrease), while 0.1NA significantly increased ($P \leq 0.05$) COL by 1.30 log cfu g^{-1} (65% increase). At day 21, 0.1KSO and refrigeration significantly ($P \leq 0.05$) reduced TAC, PSEU, STAP and COL by 24%, 29%, 46% and 85%, respectively.

At day 24, groups treated with 0.05NA had the highest microbial population. During the 27 days refrigeration storage period, the TAC, COL, STAP and PSEU of the preserved mushroom within each treatment increased and reached 6.27, 4.98, 4.69 and 4.44 log cfu g^{-1}, respectively (Tables S1 and S2). At day 27, when compared with the control sample, COL, TAC, PSEU and STAP of the 4% CA decreased by 14%, 23%, 32% and 40%, respectively, while 0.1KSO also decreased them by 44%, 25%, 36% and 50%, respectively. All samples indicated physical spoilage at day 30, and they were thus excluded from further analysis. At the end of the storage period, samples treated with 0.1KSO had the lowest microbial population. *Salmonella* spp. was not detected in all the mushroom samples throughout the storage period.

All treatment increased the lightness of the mushroom on day 0, 3 and 6, but this increase was insignificant compared to the control as shown in Table S3. Insignificant effect of treatments on the redness of

preserved and 4 °C stored mushrooms was observed between day 0 and 30 (Table S4). All the treatments decreased the yellowness of the mushroom samples on day 27 and day 30, and these samples were significantly different from the control (Table S5). The change in colour gradually increased throughout the storage periods in all treatments (Table S6). On the final day of storage, the control sample had the highest colour change value (25.94) and 0.1% sodium benzoate-treated sample had the lowest value (11.88).

Based on the measurement of the mushroom weight, all samples maintained a constant weight of 16 g at day 0. There was no significant difference ($P > 0.05$) in the percentage weight loss of all the samples compared with the control until the ninth day. At day 9, the percentage weight loss of samples treated with 0.1% potassium sorbate was significantly different from the percentage weight loss of other samples. The percentage weight loss of all the samples increased throughout the storage period starting from day 3 as shown in Fig. S1. The 4% citric acid-treated sample had the highest weight loss percentage at the end of the storage period.

Throughout the storage period, the firmness of the citric acid-treated samples was significantly ($P \leq 0.05$) different from the control and other treatments. Also, in all the treatment groups, the firmness of the samples gradually decreased, and on the last day of storage (day 30), the firmness of the control sample had the highest value (3.15 N) as shown in Fig. S2. There was a negative correlation ($r = -0.807$, $P < 0.01$) between the firmness and weight loss of the entire preserved mushroom. Also, 200 g of substrate produced 29.84 g of mushroom.

Discussions

Effect of chemical preservatives on the microbial population *P. ostreatus* stored at 4 °C

Mushrooms deteriorate easily because they have high respiration rates, and this makes them to have short shelf life (Valerie & David, 2008). Some visible symptoms of mushroom deterioration include texture breakdown, tissue browning, moisture loss and shrivelling (Mau *et al*., 1993). It is important for a food product to conform with the microbiological criteria in order to certify that is of good quality and that it will not pose any risk to the health of consumers. Based on this, different classes have been established as a means of evaluating the microbiological safety of food products. Based on international food standards, class A foods are classified as satisfactory, class B foods are less than satisfactory but acceptable for consumption, and class C are categorised as unsatisfactory. Class D implies that the microbiological status of the food sample is unacceptable as it contains unacceptable levels of specific pathogens that are potentially hazardous to consumers according to European Commission Regulation (EC) No 2073/2005.

It has been stated that microbial populations play an important role in the postharvest quality of mushrooms (Soler-Rivas *et al*., 1999).With reference to the microorganisms enumerated in this study, the total aerobic bacteria, total coliform and *Staphylococcus* spp. count in the mushroom sample must be <3, <1.3 and <1.30 log cfu g^{-1} to be classed satisfactory. For it to be acceptable, it must be >3 ≤ 4, >1.30 < 2 and >1.30 < 2 log cfu g^{-1}, respectively, and counts >4, >2, >2 log cfu g^{-1}, respectively, are considered unsatisfactory (European Commission Regulation (EC) No 2073/2005). The microbial population of the preserved mushroom was evaluated against these standards. In a study by Carole *et al*. (2010), treatments that extended the shelf life of the mushrooms were those that reduced microbial load and mushroom deterioration. In an attempt to find a treatment that could improve mushroom shelf life, several antimicrobial substances were screened using the standard method for assessing treatments. This method compared the effect of the treatment to the effect of water which served as control. Under the conditions of this study, a 10-min immersion of whole mushroom in chemical preservatives and storage at 4 °C solutions produced comparable reductions in total aerobic bacteria, total coliform, *Pseudomonas* spp. and *Staphylococcus* spp. populations (Tables S1 and S2). These findings conform with the study of Derrickson-Tharrington *et al*. (2005) who found that the immersion of apple slices for 10 mins in citric acid resulted in reduction in bacterial populations.

Treatment of sample with sodium benzoate (0.05%, 0.1%) and refrigeration increased the microbial population of the entire sample throughout the storage period starting from the third day (Tables S1 and S2). This may be attributed to benzoic acid and its salt being effective only in acid foods (Brul & Coote, 1999), whereas mushroom is not considered an acidic food. Treatment of mushroom with 0.05% and 0.1% sorbic acid and refrigeration at 4 °C did not extend the shelf life of mushroom beyond 12 days because values for microbial populations were higher than acceptable limits. This may be due to the partial solubilisation of sorbic acid in distilled water. Surekha & Reddy (1999) had earlier reported limited water solubility of some long-chain organic acids which was attributed to their limited use as preservatives.

Potassium sorbate being the potassium salt of sorbic acid is much more soluble in water than the acid (Linda, 1999). In this study, 0.1% potassium sorbate and refrigeration extended the shelf life of the mushroom for 24 days (microbial populations were still

within acceptable limit). Sorbates have been observed to be effective against many bacteria, moulds and yeasts at different stages of their life cycle (Linda, 1999). This effectiveness has been shown against *Staphylococcus* spp., *Salmonella* spp., coliforms, psychotropic spoilage bacteria (especially the *Pseudomonas* spp.) e.t.c (Linda, 1999). The mechanism for the inhibition of microbial growth by sorbates has been linked to the alteration of cell membranes, inhibition of transport systems as well as the inhibition of some key enzymes among others (Sofos & Busta, 1986). These actions may be responsible for its effectiveness and shelf life extension (based on microbial load) of potassium sorbate at 0.1% compared to other preservatives.

Ligia *et al*. (2008) studied the effect of some chemical preservatives (sodium benzoate, sodium nitrite and potassium sorbate) on the growth of three foodborne pathogens (*Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus*) on smoked blue catfish steaks. Potassium sorbate was completely effective against all the pathogens.

Also, at day 24, microbial populations were beyond acceptable levels based on microbiological criteria except for 4% citric acid and 0.1% potassium sorbate-treated samples. Brennan and Gormley (1998) reported that treatment with citric acid (4%) was effective in extending the shelf life of refrigerated *Agaricus bisporus* for 12 days. However, only *Pseudomonas* spp. was enumerated for in the study, and the shelf life was based on the number of days it took for the lightness value (L^*) of the sample to reach 76 (Brennan and Gormley, 1998). Citric acid is an acidity regulator and has antimicrobial properties (Surekha & Reddy, 1999). This acid is also a metal chelator, which removes metallic ions some of which are necessary for the growth of bacterial and enzymatic browning reactions (Surekha & Reddy, 1999). The extension of the shelf life of the mushroom based on its microbial load by 4% citric acid might be attributed to these properties; however, at lower concentration (2%), it was less effective. The effectiveness of preservatives has been observed to be dependent on concentration (Beth *et al*., 2004).

Effect of chemical preservatives on the lightness, redness, yellowness and change in colour of *P. ostreatus*

In a study by Tianjia Xiaolin *et al*. (2011), shiitake mushroom (*Lentinus edodes*) had lightness, redness and yellowness value of 97, −2 and 0, respectively. The gradual decrease in lightness and also the observed colour change during days of storage could be associated with enzymatic browning (Khuram Wasim Aslam *et al*., 2014). Tyrosinase was reported as the major enzyme responsible for browning in mushroom (Kumar & Flurkey, 1991). Some of preservatives are reported to have different mechanism for browning inhibition (Brul & Coote, 1999). All the preservatives at different levels were observed to slow down the rate of browning reactions in the preserved mushroom for 30 days although 0.1% sodium benzoate was more effective than other preservatives during storage at 4 °C (Tables S3–S6).

Gormley (1972) reported that discolouration of button mushrooms could be prevented by soaking in various concentrations of citric acid (0–1%). Sodium benzoate, a salt of benzoic acid, has been reported as a browning inhibitor due to their structural similarities with phenolic substrates (Surekha & Reddy, 1999), and this might be the reason why samples treated with it had the lowest value for colour change. L^* (lightness) is the most important parameter for measuring mushroom colour; values higher than 93 are considered excellent with 100 being the theoretical maximum, 90–93: very good, 86–89: good, 80–85: fair: 79–69: poor and 69 below: very poor (Gormley, 1972). At the end of the 30-day storage period, based on Gormley (1972) classification, mushroom treated with 0.1% sodium benzoate was of good quality in terms of colour (lightness). In addition, mushroom samples treated with 0.1% potassium sorbate and 0.05% sodium benzoate were of fair quality, and mushroom treated with other chemical preservatives had poor qualities.

Effect of chemical preservatives on the weight of *P. ostreatus*

Plant foods lose their water content during storage, which causes shrinkage and weight loss (Mahajan *et al*., 2008). Observed weight loss as shown in Fig. S1 can be attributed to the action of microorganisms such as *Pseudomonas* that can degrade mushroom by breaking down their intracellular matrix (Tianjia *et al*., 2010). *Pseudomonas* spp. are psychrophilic in nature and have the tendency to degrade mushroom even at low temperature. These can lead to exudation of tissue fluids and apparent weight loss. Also, observed weight loss during the storage period might be because of the loss of carbon upon formation of CO_2 during mushroom respiration (Kim *et al*., 2006).

Effect of chemical preservatives on the firmness of *P. ostreatus*

The chemical compositions of foods including the processes they go through determine their texture. The structural integrity of the cell wall and middle lamella, as well as to the turgor pressure generated within the cells, makes up the texture of plant food (Tianjia

Xiaolin *et al*., 2011). In addition, it is one of the first of many quality attributes judged by the consumer hence its importance to the overall product acceptance (Tianjia Xiaolin *et al*., 2011). The application of force with a small-diameter probe has been indicated as a reliable method for measuring vegetable firmness (Howard & Buescher, 1990). In a study by Aboul-Anean *et al*., 2013; the firmness of citric acid-treated artichoke and mushroom decreased by 19.30% after storing under cold temperature for 12 days. Most chemical preservatives have been reported to cause breakdown of plant tissues, which lead to shrinkage, and reduce firmness (Conserve O Gram, 2007), and these might be the reason for the reduction in firmness that was observed among the treatments (Fig. S2).

The gradual decline of mushroom firmness during storage can also be attributed to the degradation of cell walls of the mushroom by enzymes released by micro-organisms that attacked the mushroom and increased activity of endogenous autolysins (Zivanovic *et al*., 2000). Loss of textural quality has been related to weight loss (Kidmose & Martens, 1999), and the negative correlation ($r = -0.807$, $P < 0.01$) between texture and weight loss (percentage) indicates that as the weight loss of the stored mushroom was increasing, its firmness was decreasing.

Conclusions

Treatments with 0.1% potassium sorbate, 4% citric acid and storage at 4 °C aided the control of microbial populations during storage and resulted in safe products for up to 24 days. Sodium benzoate (0.05%, 0.1%) made the mushroom more prone to spoilage. Mushroom soaked in 0.1% potassium sorbate for 10 min gave a mushroom of better quality in terms of colour, texture and reduced weight loss than 4% citric acid-treated sample at the end of the storage period. 0.1% sodium benzoate was a better enzyme inhibitor for mushroom stored at 4 °C compared to other treatments. These results demonstrate the need to combine chemical treatment with other preservation method as a form of hurdle technology in order to extend the shelf life of mushroom further.

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Conflict of interest

The authors have no conflict of interest to declare.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Percentage weight loss of cassava peel cultivated mushroom stored at 4 °C for 30 days.

Figure S2. Firmness of mushroom stored at 4 °C for 30 days in Newton (N).

Table S1. Determination of total aerobic and coliform counts (log cfu/g) of *P. ostreatus* stored at 4 °C during 27 days

Table S2. Determination of *Staphylococcus* and *Pseudomonas spp*. counts (log cfu/g) of *P. ostreatus* stored at 4 °C for 27 days

Table S3. Lightness of mushroom stored at 4 °C for 30 days

Table S4. Redness of *P. ostreatus* stored at 4 °C for 30 days

Table S5. Yellowness of *P. ostreatus* stored at 4 °C for 30 days

Table S6. Change in the colour of cassava peel cultivated mushroom stored at 4 °C for 30 days