POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES

www.pan.olsztyn.pl/journal/
e-mail: joan@pan.olsztyn.pl

Pol. J. Food Nutr. Sci.
2008, Vol. 58, No. 1, pp. 95-105

EFFECT OF CHITOSAN COATING ON SHELF-LIFE AND QUALITY OF FRESH-CUT MUSHROOM

Hesham A. A. Eissa

Food Technology Department, National Research Centre, Cairo, Egypt

Key words: mushroom *(Agaricus bisporus*), chitosan, coating, polyphenoloxidase, peroxidase, catalase, phenylalanine ammonia lyase, laccase, cellulase, total amylase, α and β amylase, phenols, microbiology, colour, sliced process, shelf-life

The effect of chitosan coating in fresh-cut mushroom preservation, including microbiological, enzyme activities, colour characteristics and chemical quality attributes, was examined. However, application of chitosan coating to enzyme activity control and quality maintenance of fresh-cut mushroom was investigated. Fresh-cut mushroom were treated with aqueous solution containing 5, 10 and 20 g of chitosan/1 L, placed in polyethylene bags, and then stored at 4°C. Changes in total phenolic content, and cellulase (CEL), total amylase, α and β amylase, laccase (LAC), phenylalanine ammonia lyase (PAL), peroxidase (POD), catalase (CAT) and polyphenoloxidase (PPO) enzymes activities were measured.

Applications of chitosan coating delayed discoloration associated with reduced enzyme activities of LAC, PAL, POD, CAT and PPO as well as lowered total phenolic content. Also, it slowed down texture changes associated with reduced enzyme activities of CEL, total amylase and α-amylase. Results showed that increasing the concentration of chitosan coating resulted in higher contents of total soluble solids (TSS), total acidity and TSS/T acid ratio of fresh-cut mushroom. In mushroom, during storage at 4°C for 15 days, 20 g/kg chitosan coating inhibited the growth of total bacteria, yeasts and moulds counts. Chitosan also had a good effect on the evolution of the colour characteristics and parameters (C* and BI) of fresh-cut mushroom during storage at 4°C. The results showed that increasing the concentration of chitosan coating enhanced the beneficial effects of chitosan on extended shelf-life and maintained quality of fresh-cut mushroom.

INTRODUCTION

Edible mushrooms are considered as protein food products of the future [Biljana *et al*., 2002]. The shelf-life of minimally processed mushrooms, such as the commercial button mushroom *Agaricus bisporus*, is limited to a few days, due to enzymatic browning during storage. Inactivation of polyphenoloxidase (PPO) in mushroom (the principal enzyme responsible for the browning reactions) by heat or the application of antioxidants or enzyme inhibitors is essential to prevent enzymatic browning [Devece *et al*., 1999; Zhang & Flurkey, 1997]. Fresh mushrooms are extremely perishable and can be preserved only if properly processed. They contain water, mineral salts, vitamins, typical phenol compounds and various enzymes, including polyphenoloxidase (PPO), peroxidase (POD), catalase (CAT), laccase (LAC), phenylalanine ammonia lyase (PAL), cellulase (CEL) and amylase. These enzymes are causing a loss of sensory and nutritional qualities of mushroom. PAL, LAC, POD, CAT and PPO are phenolic oxidative enzymes, which cause browning in many fruits, vegetables and other foods. Each of them has been reported to occur in mushrooms [Mayer & Harel, 1991; Perry *et al*., 1993]. Mushroom tissue is a good source of both oxidative enzymes and phenolics. Current conventional techniques used to avoid browning include autoclaved and blanching methods. These conventional processes are inherently linked to important weight and nutritional quality losses in the product [Lopez *et al*., 1999], pointing to the need for new procedures being an alternative to industrial blanching techniques.

The fresh-cut produce can meet the ever-increasing demands of consumers for high quality, fresh, natural, nutritive, and conveniently prepared fruits and vegetables [Luo & Barbosa-Canovas, 1996; Saltveit, 1997]. However, the fresh-cut mushroom is very perishable and has a short shelf-life compared to intact mushroom, similar to other minimally processed goods [Saltveit, 1997]. It is, thereby, necessary to develop a suitable technology to extend their shelf-life for commercial use. Cut-surface browning is a major concern with regard to quality deterioration and short shelf-life of fresh-cut fruits and vegetables [Loaiza-Velarde & Saltveit, 2001; Luo & Barbosa-Canovas, 1996]. Various approaches are now applied to prevent browning of fresh-cut fruits, vegetables and mushroom, one of which is the use of different modified atmosphere conditions during low temperature storage [Annese *et al*., 1997]. Application of low O_2 atmosphere below 1 kPa is effective to inhibit browning of many fresh-cut fruits and vegetables mediated by polyphenoloxidase (PPO) [Gorny, 1997], but off-flavors often occur due to anaerobic respiration under low O_2 atmosphere conditions. Other approaches are to use chemical inhibitors to control browning [Food & Drug Administration, 1989; Friedman, 1996; Buta *et al*., 1999; Son *et al*., 2001].

Application of semi-permeable edible coating is promising to improve the shelf-life of perishable fruits, vegetables and

Author's address for correspondence: Hesham A. Eissa, National Research Center, Food Technology Department, Cairo, Egypt; tel. (00 202) 202 6881; faks: (00 202) 337 0931; e-mail: hamin_essa@yahoo.com

© Copyright by Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences

mushroom as well as other low-processed products [Baldwin *et al*., 1995; Li & Barth, 1998]. Polymeric coatings such as Pro-Long chitin (β (1-4)–N-acetyl–D- glucosamine) and chitosan (deacetylated chitin) are currently available in large quantities as by-products of the shellfish industry. Experimental evidence regarding chitosan has shown that it is non-toxic, safe and increases IgM production in human-human hybridoma cells [Darmadji & Izumimoto, 1994]. Chitosan already has a number of applications in the food industry. In the preservation of fruits, it has been used as a coating and antifungal agent, resulting in increased quality and storability [El Ghaouth *et al*., 1991]. Imeri & Knorr [1988] also reported the use of chitosan for the reduction of total acidity and colour index of carrot and apple juice. Chitosan coatings are generally good gas barriers and adhere well to cut surfaces of fruit and vegetables, but their hydrophobic nature makes them poor barriers to moisture and inhibits the growth of several fungi [Baldwin *et al*., 1995]. Chitosan also inhibited the growth of spoilage bacteria, resulting in better sensory attributes and it had a good effect on the development of the red colour of meat during storage [Darmadji & Izumimoto, 1994]. Also, the application of chitosan coating has been reported to form an ideal coating on fruits surface, and, thus, maintained the quality of harvested fruits and vegetables [Li & Yu, 2000; Su *et al*., 2001], and delayed browning of litchi and longan fruits [Jiang & Li, 2001]. Furthermore, chitosan, a high molecular weight cationic polysaccharide produced by a deacetylation of chitin, has been proved to be a dietary fiber analogue with lots of benefits for human health and, thus, to be safe, compared with sulphites [Van Der Lubben *et al*., 2001]. However, little information of chitosan effects on browning control and shelf-life extension of fresh-cut fruits and vegetables is available. There is no documentation on the use of chitosan as a preservative agent in mushroom. The objective of this research was to assess the potential of chitosan coating for browning control by inhibits enzyme activities, extend shelf-life and quality maintenance of fresh-cut mushroom during storage at low temperature (4°C).

MATERIAL AND METHODS

Materials and sample treatment

Common cultivated mushrooms (*Agaricus bisporus*) were obtained from a commercial market in Cairo (Egypt) selected for uniformity and size, and any bruised or diseased mushroom was discarded. The average size of the cap of the mushrooms used in this study was 5-10 mm. Mushrooms were washed and sliced to a thickness of 4 mm (6-slices/mushroom) with a sanitized sharp stainless knife. The prepared slices were surface-treated by immersion in a 0.1 g $NaClO_2$/100 mL for 1 min, air-dried at room temperature for 30 min, and then dipped for 1 min in the solutions containing 5, 10 or 20 g/kg chitosan, which were prepared as described by Jiang & Li [2001] with a pH value of 6.0. "Chitosan" solutions were prepared from high viscosity "chitosan" flakes (37% deacetylation) and practical grade "chitosan" from crab shells (Sigma Chemicals Co. USA) by slurrying the flakes in water (20 g/kg). The slurry was combined with an equal volume of 20 g/kg malic acid solution, and the "Chitosan" dispersed with a Franz MORAT KG – R270 homogenizer (GmbH & Co., Germany). The dispersion was heated to 60°C with stirring, and filtered through filter paper (Whatman No. 541) under suction to remove a small amount of the insoluble materials. The slices treated without chitosan were used as control. After air-drying at room temperature for another 30 min, the slices were placed into polyethylene bags and then stored at 4°C for analysis. The control and chitosan-treated mushroom samples stored for 0, 3, 5, 7 and 15 days at 4°C were subjected to enzymatic, microbiological and colour characteristic evaluation. For each treatment, three replicates were used.

Extractions and assay of oxidative enzymes activity

To measure oxidative enzyme activities (PPO, POD and CAT), mushroom tissue (10 g) from six slices was homogenized in 20 mL of 0.1 molphosphate buffer (pH 7.0) and the homogenate was filtered through two layers of cotton cloth and filter paper (Whatman No.1) to remove cell debris. The clear supernatant after centrifugation at 5000 ×*g* (HERMLE z323k, Germany) for 20 min at 4°C was collected [Sun & Song 2003]. The supernatant collected was used to determine polyphenoloxidase, peroxidase and catalase enzyme activities as follows.

Polyphenoloxidase (PPO) activity was assayed according to the method of Sun & Song, [2003], by measuring the oxidation of 0.1 mol/L catechol. The increase in absorbance at 420 nm was automatically recorded for 3 min, using a 4054 UV/ Visible spectrophotometer, LKB-Biochrom (Sweden). One unit of enzyme activity was defined as the amount of enzyme that caused a change of 0.001 in absorbance per minute.

Peroxidase (POD) activity was assayed by the procedure of Olmos *et al*. [1997]. The assay mixture consisted of 50 mmol/L potassium phosphate (pH 6.8), 10 mmol/L hydrogen peroxide, 9 mmol/L guaiacol and enzyme extract in a total volume of 3 mL. The increase in absorbance at 470 nm was recorded for 3 min using a 4054 UV/ Visible spectrophotometer, LKB-Biochrom (Sweden). One unit of enzyme activity was defined as the amount of enzyme that caused a change of 0.001 in absorbance per minute.

Catalase (CAT) activity was measured by "titrimetric method" as described by the procedure of Aebi [1983]. Enzyme activity can be expressed in arbitrarily defined "perborate units/gram" (The number of millimoles of perborate decomposed under the standard experimental conditions related to mg wet weight of tissue "perborate Units") [Aebi, 1983].

Extractions and assays of phenylalanine ammonia lyase (PAL)

PAL was extracted with the method of Lister *et al*. [1996]. Tissue (10 g) from six slices was homogenized in 30 mL of 50 mmol/L phosphate buffer (pH 7.0) containing 5 g polyvinylpyrolidone/100 mL (Mr 44 000), 0.05 mol/L sodium ascorbate, 18 mmol/L mercaptoethanol, 0.1 g Triton X-100/100 mL. The homogenate was filtered through four layers of cotton cloth and then centrifuged at 5000 ×*g* (HERMLE z323k, Germany) for 20 min at 4°C. The supernatant was collected as enzyme extract. PAL activity was assayed with a slightly modified method of Nita-Lazar *et al*. [2002] using a reaction mixture of 2 mL sodium borate buffer (60 mmol/L, pH 8.8)

containing 11 mmol/L of l-phenylalanine and 0.4 mL of crude enzyme, with a final volume of 2.4 mL. Tubes were incubated at 30°C for 2 h, and the reaction was stopped by adding 35 g trichloro acetic acid (0.6 mL)/100 mL. After the tubes were centrifuged for 5 min at 5000 ×*g* to pellet the denatured proteins, the absorbance was measured at 290 nm with a Shimadzu spectrophotometer UV-2401PC, UV-VIS recording spectrophotometer, Japan. One unit of the enzyme activity was defined as the amount of enzyme that caused a change of 0.001 in absorbance per hour.

Extractions and assays of laccase enzyme activity (LAC)

Extractions and assays of laccase enzyme activity (LAC) were carried out according to the method of Betty *et al*. [1994]. Tissue (10 g) from six slices was homogenized in 30 mL of 50 mmol/L sodium phosphate buffer (pH 6.0) containing 10 mmol/L ascorbic acid and homogenized in a Warring Blender for 1 min. The homogenate was filtered through four layers of cotton cloth and then centrifuged at 5000 ×*g* (HERMLE z323k, Germany) for 20 min at 4°C. The supernatant was collected as enzyme extract. Laccase activities were determined at 25°C in 100 mmol/L sodium acetate buffer (pH 5) using 5 mmol/L solution of p-phenylenediamine as a substrate. The increase in absorbance was monitored at 400 nm for p-phenylenediamine. The enzymatic reactions were carried out at room temperature (22–25°C) and one unit of laccase enzyme activity was defined as the amount of enzyme oxidizing 1 μmol of substrate per minute [Yaropolov *et al*., 1994; Benny *et al*., 1998].

Extractions and assays of cellulase enzyme activity (CEL)

The activity of cellulase was determined according to the method of Malik & Singh [1980] as follows: a known weight of each mushroom sample was treated with 50 mL of acetate buffer 100 mmol/L (pH 5.5). The mixture was centrifuged at 5000 ×*g* for 15 min. The extract or supernatant was filtered through Whatman No.1 filter paper and the filtrates were used as a source of enzyme extract. To 0.5 mL acetate buffer (100 mmol/L, pH 5.5) 1 mL of enzyme extract and 1.5 mL, 10 g/kg of carboxymethylecellulose was added and mixed. The mixture was incubated for two hours at 30°C and then 3 mL of dinatrosalicylic acid (DNSA) reagent (3.5 dinatrosalicylic acid, Rochelle salts, sodium hydroxide, phenol and sodium metabisulfite) were added. The tubes were boiled for 10 min. Then the contents were cooled and the absorbance was read at 560 nm. A blank experiment was carried out. A standard curve was plotted at different concentrations of glucose with dinatrosalicylic acid reagent. The total activity was expressed in unit per mL. One unit of enzyme activity is defined as nmols of cellulase equivalent released in one hour under the assay conditions.

Extractions and assay of amylase enzymes (α- and β-amylase) activity

One gram of mushroom samples was ground in a cooled mortar (4°C) with 20 mL of 100 mmol/L sodium acetate buffer (pH 5) and the obtained suspension was centrifuged at 5000 ×*g* for 30 min at 4°C. One milliliter of the supernatant was incubated with 1 mL of 10 g/kg soluble starch dissolved in 100 mmol/L sodium acetate buffer (pH 5.0) in a water-bath (27°C) for 1 h. The enzymatic action was terminated with DNSA reagent and the quantity of reducing sugars formed determined by taking the optical density at 540 nm against a blank that contained 1 mL of boiled enzyme extract that was similarly treated [Fasidi & Kadiri, 1991].

Extractions and assays of α-amylase enzyme activity

This was determined by heating 5 mL of the supernatant obtained after centrifuging the total amylase extract at 70°C for 15 min to inactivate β-amylase [Wilson, 1971]. One milliliter of the heated extract was incubated with 1 mL of 10 g/kg soluble starch in 100 mmol/L sodium acetate buffer (pH 5.0) in a water bath (27°C) for 1 h. The resultant solution was treated with DNSA reagent (3 mL) and the quantity of reducing sugars was determined as above [Fasidi & Kadiri, 1991].

All enzyme activities were measured 3 times and all enzymes activity was reported in arbitrary units (Unit/g).

Determination of microbial growth

The total bacteria, yeast and moulds counts were determined with the method of Gonulalan *et al*. [2003]. Untreated and chitosan treated mushroom samples were mixed with 1 g/kg peptone (DIFCO Labs., Detroit, MI) and pour-plated in duplicate. Total bacteria counts (TBC): one mL aliquot of each dilution was plated using a plate count agar medium (Merck KGaA, Darmstadt, Germany), and incubated at 35-37°C for 48 h prior to counting. Also, yeast and mould counts (Y & M) were obtained using malt extract agar (Merck KGaA, Darmstadt, Germany) and incubated at 25°C for 3 days prior to counting. The number of colonies (TBC or Y and M) that appeared on the plates was counted and expressed as log Colony Forming Unit per gram or log (CFU/g).

Quality evaluation

Chemical properties

Tissue (20 g) from 10 slices was homogenized in a grinder and then centrifuged for 20 min at 5000 ×*g* (HERMLE z323k, Germany). The supernatant phase was collected for determinations of: pH – using a digital pH-meter (HANNA, HI 902 meter, Germany); the percent Total Soluble Solids (TSS), expressed as °Brix (0-32) – using a Hand refractometer (ATAGO, Japan) and the total acidity or total acidity expressed as citric acid mg/kg – with 0.1 mol NaOH according to the method reported by Tung-Sung *et al*. [1995].

Colour

Hunter a*, b* and L* values of the investigated samples were measured using a spectro-colourimeter (Tristimulus Colour Machine) with the CIE lab colour scale (Hunter, Lab Scan XE – Reston VA, USA) in the reflection mode [CIE, 1978]. The instrument was standardized each time with white tile of Hunter Lab Colour Standard (LX No.16379): X=72.26, Y=81.94 and Z=88.14 (L*=92.46; a*=-0.86; b*=-0.16) [Sapers & Douglas 1987].

The Hue (H)*, Chroma (C)* and Browning Index (BI) were calculated according to the method of Palou *et al*. [1999].

Determination of total phenol contents

According to the method of Lavid *et al*. [2001], mushroom tissue (5 g) from six slices was extracted in 20 mL methanol (95%) containing 100 mmol/L HCl at 25°C three times, changing the solution every 5 h. The three solutions were combined, filtered and collected for total phenolic determination. Phenolic content of the extract was measured by the method of Amerine & Ough [1980], the absorbance was measured at 765 nm on a spectrophotometer (4054 UV/ Visible spectrophotometer, LKB-Biochrom, Sweden) and results were expressed as milligram of gallic acid as standard equivalent per gram.

Statistical analysis

Statistical analysis was performed using SPSS statistical package (Version 9.05) according to [Rattanathanalerk *et al*., 2005], analysis of variance (ANOVA) and Standard Deviation (Standard deviation), whereas, treatment samples were repeated three times (n=3).

RESULTS

Enzyme activities in fresh-cut mushroom

For fresh mushroom that was stored at refrigerator temperature (4°C) Figures 1-10 showed that quality characteristics were changed and began deterioration after 3 days. This could be attributed to the recorded activity of these enzymes that could affect colour (PPO, PAL & LAC), odour and taste (POD, CAT and protease) and softening (cellulase, amylase and protease). Also, enzymes that cause a loss of sensory properties and nutritional value in mushroom include peroxidase, lipoxygenase, polyphenoloxidase, cellulase, polygalacturonase and chlorophyllase as described by Baardseth [1979] and Fasidi & Kadiri, [1991].

However, mushrooms are extremely perishable and have a short shelf-life. Different methods of preservation, using chitosan coating could be applied to increase their shelf-life. The enzyme activities, *i.e.* polyphenoloxidase (PPO), peroxidase (POD), catalase (CAT), phenylalanine ammonia lyase (PAL), laccase (LAC), cellulase (CEL) and amylase, were determined in fresh mushroom and after coating with chitosan as presented in Table 1. Results in Figures 1-10 showed that fresh mushroom had different activities of enzymes such as PPO, POD, CAT, PAL, LAC, CEL and amylase being 0.571, 0.515, 0.00092, 0.437, 0.0704, 82.737 and 38.756 unit/g, respectively. These enzymes are responsible for different changes in chemical constituents that would affect the quality characteristics and deterioration of mushroom either fresh or processed. However, for cellulase, total amaylase, peroxidase, polyphenoloxidase and phenylalanine ammonia lyase the fresh mushroom showed greater activity than for the other enzymes (Figures 1-10).

Effect of chitosan coating on oxidative enzyme activities and total phenolics content of fresh-cut mushroom

As mentioned above, the major concern with regard to quality deterioration of fresh-cut mushroom is the surface discoloration, which is associated with phenol-related metabolic enzymes, such as POD, PPO, CAT, LAC and PAL [Jiang, 1999; Loaiza-Velarde & Saltveit, 2001; Lopez-Serrano & Barcelo, 1999; Betty *et al*., 1994].

The effect of chitosan coating on PPO activity was similar to that of POD (Figures 1 and 2). The degree of inhibition of PPO activity was dependent on the concentrations of chitosan used. After 15 days of storage, the PPO activity of the fresh-cut mushroom treated with 5, 10 and 20 g/kg chitosan was 22.22%, 12.70% and 2.54% of the control, respectively, of which the enzyme activity reached a peak. For POD activity there was the same distinct trend of PPO activity (Figures 1 and 2). Compared to those of PPO and POD, the activity of CAT of fresh-cut mushroom treated with 5, 10 and 20 g/kg chitosan decreased markedly when stored at low temperature

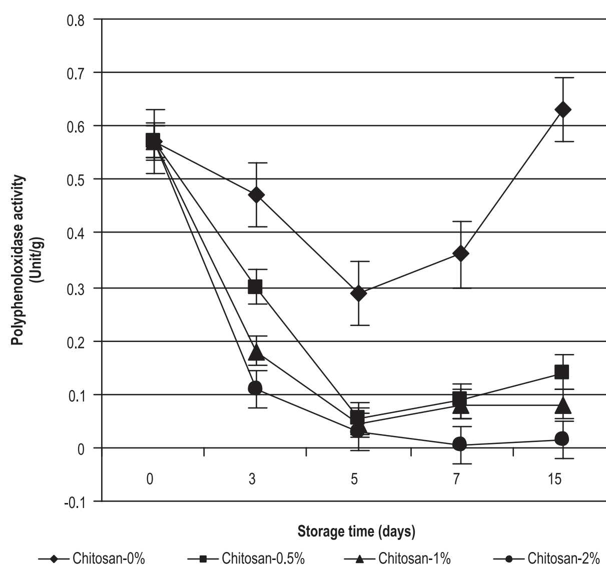

FIGURE 1. Effect of chitosan coating on polyphenoloxidase activity in fresh-cut mushroom during 15 days of storage at 4°C.

TABLE 1. Effect of chitosan coating on chemical properties of fresh-cut mushroom after storage for 15 days.

Treatments	SD	TSS (°Brix)	SD	pH	SD	Total acidity*	TSS / T. acidity
Fresh mushroom	0.08	6	0.06	7.00	0.02	10.70	0.561
Control 0.0%	0.06	5	0.04	7.53	0.03	9.30	0.538
Chitosan 0.5%	0.08	5.5	0.02	5.56	0.02	9.60	0.573
Chitosan 1.0%	0.15	6	0.14	5.69	0.006	9.80	0.612
Chitosan 2.0%	0.13	7	0.01	5.73	0.001	10.56	0.663

*Total acidity expressed as citric acid (mg/kg); **SD = Standard deviation (n=3)

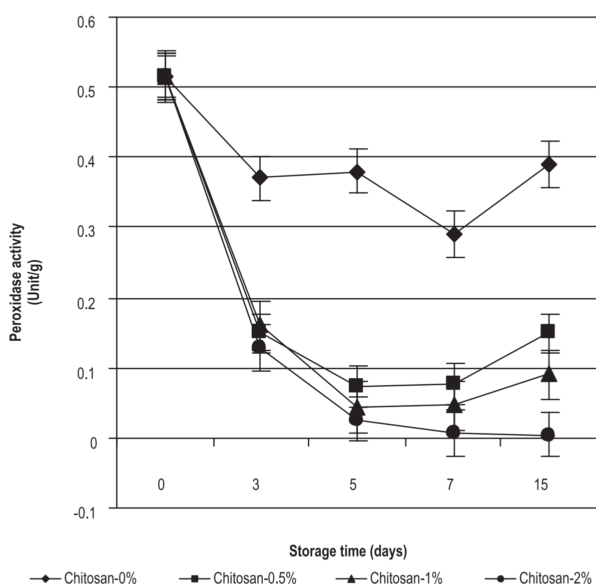

FIGURE 2. Effect of chitosan coating on peroxidase activity in fresh-cut mushroom during 15 days of storage at 4°C.

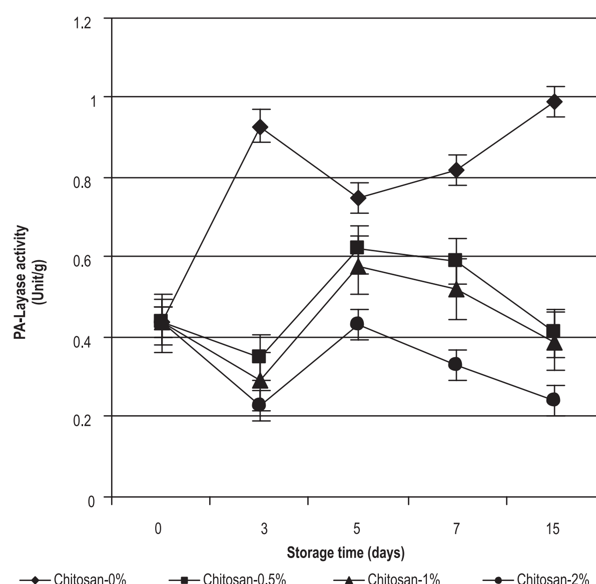

FIGURE 4. Effect of chitosan coating on layase activity in fresh-cut mushroom during 15 days of storage at 4°C.

within 15 days, whereas application of chitosan coating reduced the enzyme activity (Figure 3).

As shown in Figure 4, PAL activity increased substantially in control slices in the entire 15-day period of storage at 4°C. Treatment with chitosan coating inhibited the increase in PAL activity, and the inhibition enhanced as the treatment concentrations increased; however, lower PAL activity of the fresh--cut mushroom treated with 20 g/kg chitosan was observed at the end of the storage.

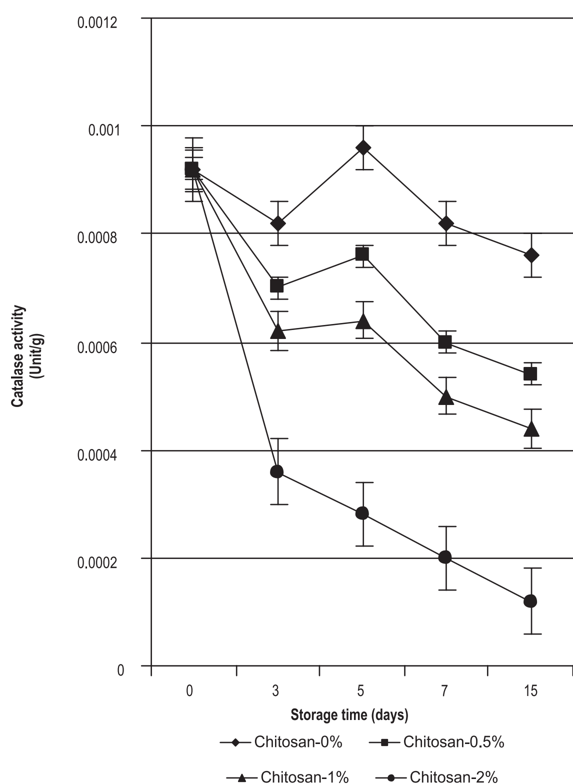

FIGURE 3. Effect of chitosan coating on catalase activity in fresh-cut mushroom during 15 days of storage at 4°C.

For laccase activity, the converse was obtained. However, the activity of LAC of fresh-cut mushroom (untreated sample) sharply increased when stored at low temperature (4°C) within 15 days, whereas application of chitosan coating delayed the increase in the enzyme activity compared with untreated mushroom sample (Figure 5). Results showed that the LAC activity was lower than PPO and POD activity in fresh-cut mushroom, whereas, the same observation was made by Betty *et al*., [1994] who reported that the laccase activity in fresh-cut mushroom seemed to be much lower than tyrosinase activity.

As seen in Figures 1-5, the refrigerator storage at 4°C did not inactivate the PPO, POD, LAC and PAL enzymes. Chitosan treatment and refrigerator storage at 4°C reduced the enzyme activity of PPO, POD, LAC and PAL to about 10% of their original activity after 5 days of refrigerator storage

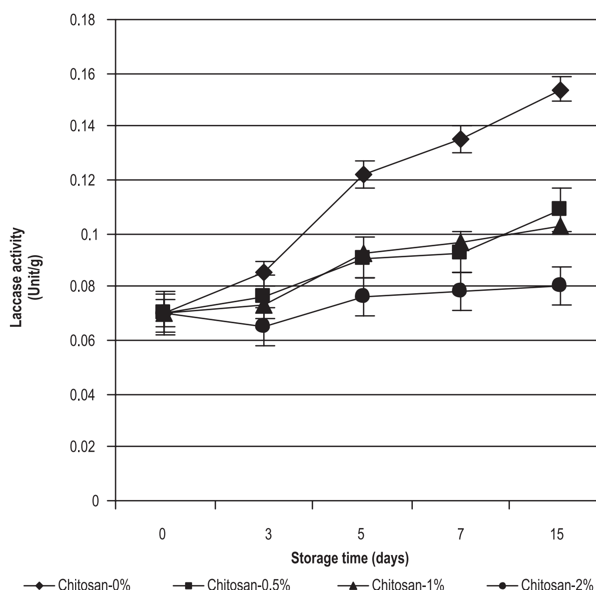

FIGURE 5. Effect of chitosan coating on laccase activity in fresh-cut mushroom during 15 days of storage at 4°C.

at 4°C followed by a rapid increase during the second week so that it achieved activity levels exceeding the original activity of the native enzyme. However, refrigerator storage at 4°C alone without chitosan treatment was not sufficient to inactivate the tested enzymes. Whereas, Scott [1975] mentioned that inactivation at low temperature was assumed to be due to the formation of intra molecular hydrogen bonds preventing sufficient unfolding of the molecule.

Phenolic content increased, reached a peak after about 3 days of storage, and then deceased in untreated mushroom (Figure 6). Treatment with chitosan coating inhibited the changes in the content of total phenols, in a concentration-dependent manner and decreased the content of total phenols during 15 days of storage. As PAL regulates the phenolic pathway by catalysing conversion of phenylalaine to trans-cinnamate [Ke & Saltveit, 1989]. However, a lower level of phenolics of the chitosan-treated slices may be due to the inhibition of PAL activity.

It is generally accepted that edible coatings form a protective barrier on the surface of fresh produce, reduce supply of oxygen, retard ripening and senescence, and, consequently, delay browning of fruits and vegetables [Jiang & Li, 2001; Li & Barth, 1998; Li & Yu, 2000; Su *et al*., 2001]. In this study, application of chitosan coating partially inhibited PAL activity, delayed the increase in the LAC activity, reduced or delayed the increase in phenolic content, PPO, POD and CAT activities of fresh-cut mushroom. Thus, it seems that the effect of chitosan coating on anti-browning is not only due to the reduction in oxygen supply, but also to the inhibitory effects of PAL, PPO, POD and CAT activities.

Effect of chitosan coating on cellulase and amylase enzyme activities of fresh-cut mushroom

Cellulase, total amylase and α-amylase activities of coated mushroom decreased with increasing chitosan concentration, but for β-amylase activity there was no distinct trend (Figures 7-10). Furthermore, the activities of the various enzymes were usually greater in the uncoated mushroom than in the coated one, but for β-amylase activity opposite results were obtained, whereas, no clear differences were observed between uncoated and coated mushroom (Figures 7-10). However, refrigeration, or lowering the storage temperature, can serve to slow metabolic reactions including respiration and transpiration, as well as to slow enzymatic activity which may deteriorate the produce. The temperature at which a commodity is stored is usually very spe-

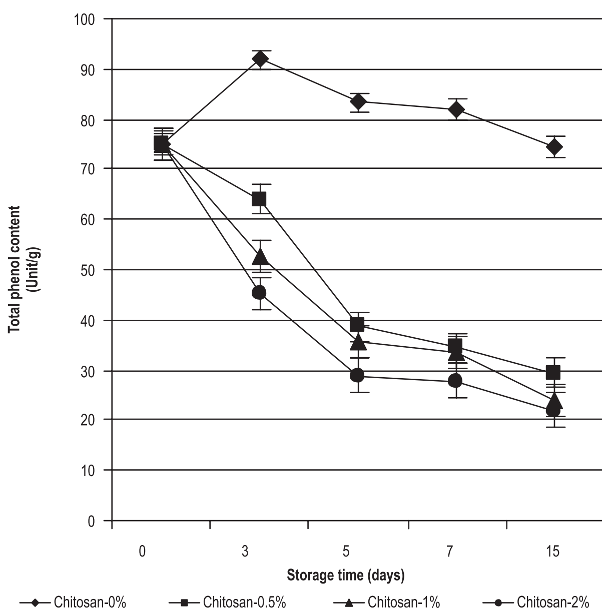

FIGURE 6. Effect of chitosan coating on total phenol content in fresh--cut mushroom during 15 days of storage at 4°C.

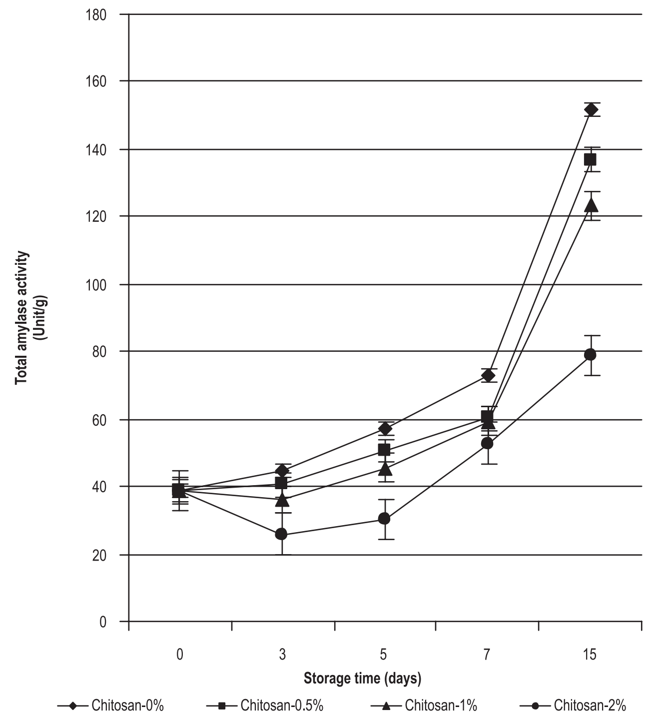

FIGURE 7. Effect of chitosan coating on total amylase activity in fresh--cut mushroom during 15 days of storage at 4°C.

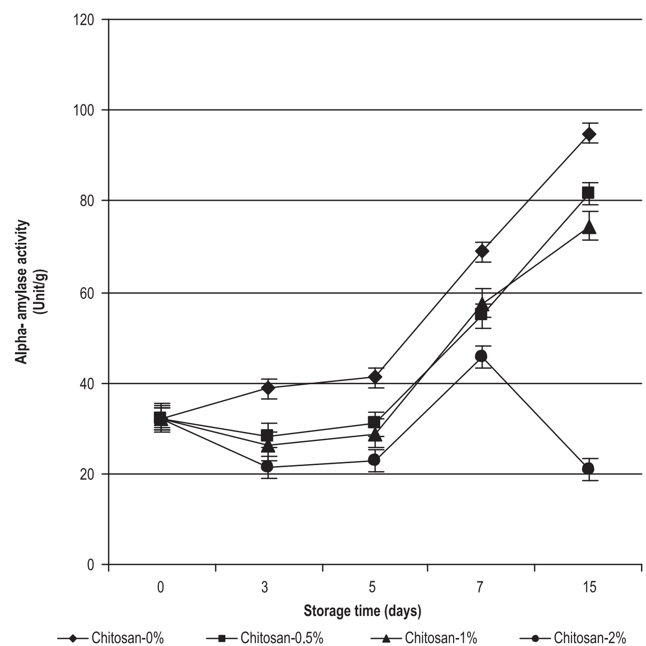

FIGURE 8. Effect of chitosan coating on alpha–amylase activity in fresh--cut mushroom during 15 days of storage at 4°C.

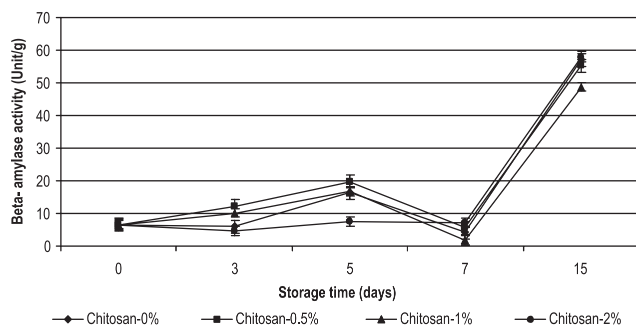

FIGURE 9. Effect of chitosan coating on beta-amylase activity in fresh--cut mushroom during 15 days of storage at 4°C.

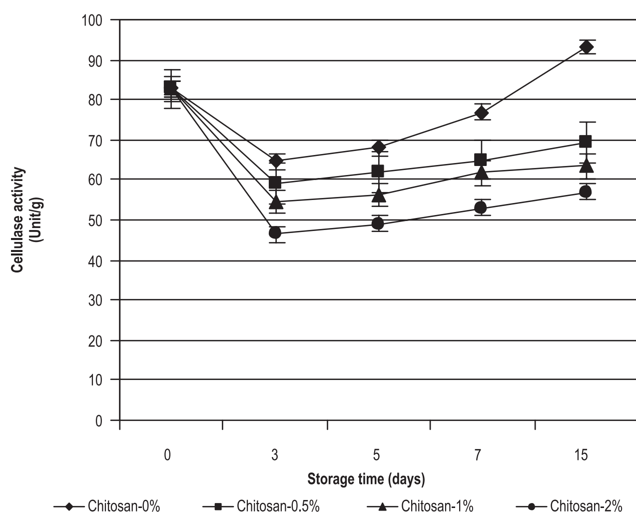

FIGURE 10. Effect of chitosan coating on cellulase activity in fresh-cut mushroom during 15 days of storage at 4°C.

cific to that particular product. If storage temperatures are too low, chilling injury may result. However, if temperatures are too high, metabolic processes can accelerate. In addition, a wide range of storage temperatures is also not advisable, because such conditions lead to rapid weight loss of the produce [Salunkhe *et al*., 1991]. Whereas, low temperature storage (4°C) and pre-treatments may be effective in controlling enzymatic activity in fresh-cut products. Within 24 h of cutting, iceberg lettuce cut into 2 x 2 cm pieces and stored at 4°C showed a 6 to 12 fold increase in PAL activity [Saltveit, 2000]. Fluctuations in the air temperature should be kept to a minimum (not more than 4°C); if they are not, product quality will suffer and there may be a significant build-up of free ice in the product due to the sublimation of water from the product and its deposition on the internal wall of the packaging. Temperature-related quality deterioration is more severe the warmer the storage temperature, and the quality losses that occur even during short periods of temperature abuse are cumulative and irreversible [Anonymous, 1999].

For cellulase and amylase in coated mushroom showed consistently higher activity during storage at 4°C for 15 days, as seen in Figures 7-10. This result is consistent with findings of Fasidi & Kadiri [1991] who observed that the stipes of mushroom exhibited higher cellulose activities than their corresponding pilei, this may be due to the greater preponderance of crude fibre in the stipes of mushroom.

The coated mushroom exhibited higher cellulase and amylase activities than of the other enzymes during storage at 4°C for 15 days. This may be due to the greater preponderance of crude fiber in the coated mushroom [Fasidi & Kadiri, 1991]. Since chitosan forms the upright support for the entire fruit body as a coating of mushroom, it is not surprising that it contains a greater amount of crude fiber than in uncoated mushroom. Similarly, the observed higher cellulase activity in coated mushroom also confirms the previous result that treated samples maintained a higher content of fibre.

The effect of chitosan coating on total amylase activity was similar to that of α-amylase (Figures 7 and 8). The degree of inhibition of amylase activity was dependent on the concentration of chitosan used and storage time. After 15 days of storage, the total amylase and α-amylase activities of the coated mushroom with chitosan were 51.96% and 22% of the control, respectively, of which the enzyme activity reached a peak. Compared to those of total amylase, α-amylase and β-amylase, the activity of cellulase of chitosan coated mushroom decreased markedly when stored at low temperature within 3 days and then closely constant within the period of 3-15 days, whereas application of chitosan coating delayed the increase in the cellulase enzyme activity compared with the untreated sample (Figure 10). This means that chitosan coating caused more inhibition of cellulase enzyme activity than total, Alpha and Beta amylase enzyme activities within 15 days storage (Figures 7-10).

The significance of the finding in the present study is that the uncoated mushroom would be expected to undergo a faster decline in flavour and food nutrients and to show more browning and texture changes than the chitosan coated mushroom during storage at 4°C for 15 days. This is due to there having greater activity of cellulase, total amylase, α and β amylase, laccase, phenylalanine ammonia lyase, peroxidase, catalase and polyphenoloxidase. However, our results indicate that the coating of mushroom by chitosan before processing caused an inhibition of all these enzymes in the range of 52-97.5% during cold storage at 4°C for 15 days (Figures 1-10) in which the deterioration might have begun after 15 days.

Effect of chitosan coating on microbiological analysis of fresh-cut mushroom

Mushroom could be exposed to microbial contamination during cultivation, harvesting, transportation, processing and storage. Data given in Figures 11 and 12 show that the initial total yeast and mould (TYMC) and total bacteria counts (TBC) of mushroom were reduced with increasing chitosan concentration. Untreated mushroom was contaminated with bacteria, yeasts and moulds. TYMC and TBC of untreated mushroom after 15-day storage at 4°C were 1.5 and 1.7 log CFU/g, whereas the TYMC and TBC were reduced by 1 log values at mushroom coated with 20 g/kg chitosan, respectively. This level is generally accepted as a maximal count of decontaminated fruit desired by the fruit trade [Lee *et al*., 2004]. Moreover, no growth of bacteria, yeasts nor moulds was detected after 5 days of storage at 4°C of mushroom coated with 20 g/kg chitosan, but the growth increased lightly after 15 days, as seen in Figure 3. This result indicated that 20 g/kg chitosan coating was effective in the reduction of harmful microorganisms (TYMC and TBC) in mushroom.

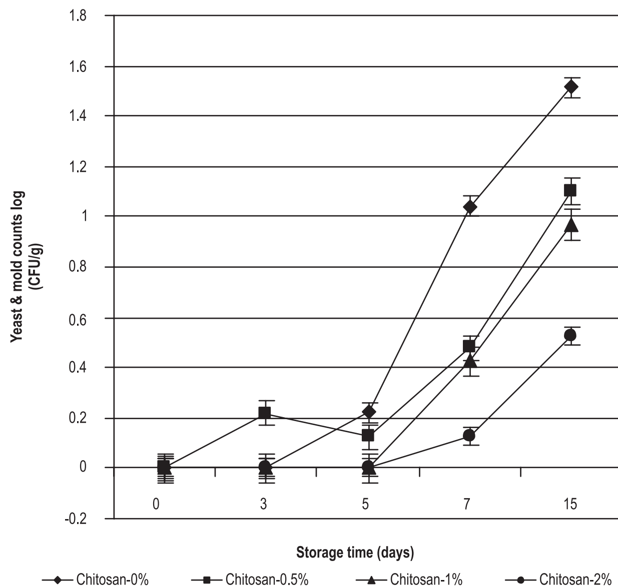

FIGURE 11. Effect of chitosan coating on yeast & mould counts log (CFU/g) in fresh-cut mushroom during 15 days of storage at 4°C.

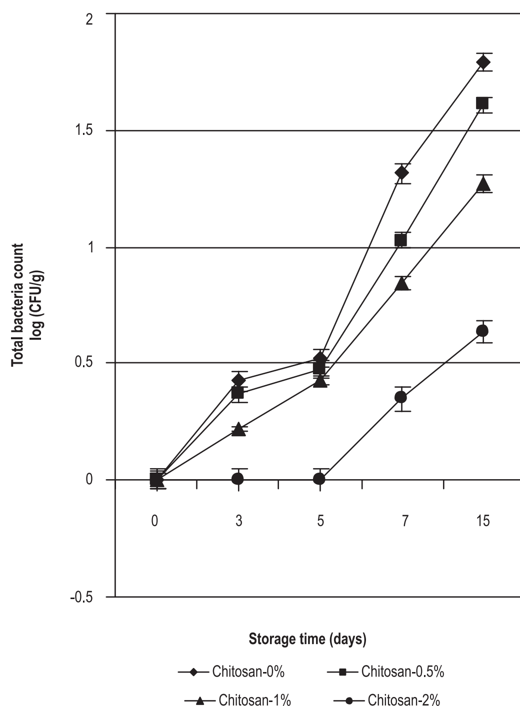

FIGURE 12. Effect of chitosan coating on total bacteria counts log (CFU/g) in fresh-cut mushroom during 15 days of storage at 4°C.

Effect of chitosan coating on chemical properties of fresh-cut mushroom

The mushroom treated with 20 g/kg chitosan had a higher TSS, total acidity and ratio of TSS /Total acidity than either of the other treatments or the control, which can be attributed to low changes (Table 1). Also, an increase in chitosan concentration resulted in an increase in TSS, total acidity and ratio of TSS/total acidity. The pH values of mushroom were slightly affected by chitosan treatment (Table 1).

Total acidity values are presented in Table 1, indicating an effect of chitosan treatment. This is in agreement with the finding by Imeri & Knorr [1988] on the effects of chitosan treatment on the reduction of total acidity provide a potential for acidity control in other food systems.

Application of chitosan coating exhibited a beneficial effect on the contents of total soluble solids, pH and total acidity (mushroom quality). The fresh-cut mushroom treated with chitosan coating had higher contents of total soluble solids (TSS), pH and total acidity, which is consistent with the decline in sensory quality (Table 1).

Total acidity and TSS/acid ratio increased by increasing chitosan concentration in mushroom coated, as seen in Table 1. This indicates that chitosan-coated mushroom may be advisable for fresh use. However, it might be processed into acceptable quality mushroom.

Effect of chitosan coating on colour characteristics and parameters of fresh-cut mushroom

The effect of chitosan on the colour of mushroom during storage at 4°C for 15 days is shown in Table 2. The result indicated no effect of chitosan on L*, a* and b* values at zero time of storage. During storage, the L* value of the control sample decreased compared with the sample coated with chitosan. It may be caused by the water–binding capacity, so it could repress the drip loss of mushroom resulting in increased transparency and lowering lightness [Darmadji & Izumimoto, 1994]. The a* and b* values of all mushroom samples increased during storage and the increase in control samples was higher compared with samples coated with chitosan. The greater a* value corresponded to the increasing enzymatic browning during storage.

An analysis of data revealed that the H*, C* and BI values were affected by chitosan concentration and storage for 15 days at 4°C, as seen in Table 2. The H* values decreased at all the conditions during storage, indicating a shift from the yellowish-green region of the L*, a* and b* colour solid [Anonymous, 1988] towards the yellow region. This effect was also evident from the increase in the average b* measurements from about 13.41 for the mushroom at zero time to 15.14 after 15 days of storage. During storage at 4°C for 15 days, the C* and BI values of the control mushroom sample increased compared with the mushroom sample coated with chitosan (Table 2). The greater BI value corresponded to the increasing enzymatic browning in control samples compared with the samples coated with chitosan during storage. This observation suggests that coating with chitosan resulted in colour and quality preservation of fresh-cut mushroom.

DISCUSSION

In our studies, the application of chitosan coating delayed the change in eating quality and partially inhibited the increase of oxidative enzymes activity (PPO, POD, PAL, LAC and CAT) of mushroom which is associated with discolouration. This implies that the chitosan coating may form a protective barrier on the surface of the mushroom and reduce the supply of oxygen. Also, it delayed texture changes by inhibiting the increase of the enzyme activity of cellulose in chitosan-treated samples compared with the untreated sample.

TABLE 2. Effect of chitosan coating on colour characteristics and parameters in fresh-cut mushroom during 15 days of storage at 4°C.

Storage time (days)	Chitosan treatments	b*	a*	L*	C*	H*	BI
Fresh mushroom	Untreated	12.83	2.25	78.46	13.025	80.05	35.695
Zero time or control	Control 0.0%	13.00	2.59	78.25	13.255	78.73	36.855
	Chitosan 0.5%	13.78	2.29	68.12	13.969	80.56	44.858
	Chitosan 1.0%	13.41	2.78	67.64	13.695	78.29	44.979
	Chitosan 2.0%	13.84	3.11	67.22	14.185	77.33	47.398
3 – days	Control 0.0%	13.00	2.59	78.25	13.255	78.73	36.855
	Chitosan 0.5%	13.89	2.94	68.11	14.197	78.05	46.534
	Chitosan 1.0%	14.13	3.10	67.46	14.466	77.63	48.161
	Chitosan 2.0%	14.37	3.28	67.23	14.739	77.14	49.522
5 – days	Control 0.0%	22.01	5.67	63.88	22.728	75.55	87.433
	Chitosan 0.5%	14.07	3.25	68.03	14.440	76.99	47.813
	Chitosan 1.0%	14.35	3.29	67.24	14.722	77.09	49.466
	Chitosan 2.0%	14.34	3.31	67.15	14.717	77.00	49.547
7 – days	Control 0.0%	25.09	6.90	63.00	26.021	74.62	105.31
	Chitosan 0.5%	14.22	3.43	67.73	14.627	76.44	48.913
	Chitosan 1.0%	14.51	3.39	66.94	14.900	76.85	50.464
	Chitosan 2.0%	14.49	3.28	67.02	14.856	77.24	50.104
15 – days	Control 0.0%	29.57	8.61	61.34	30.798	73.77	135.32
	Chitosan 0.5%	15.14	4.28	67.19	15.733	74.21	54.217
	Chitosan 1.0%	14.96	3.93	66.48	15.467	75.28	53.522
	Chitosan 2.0%	14.52	3.45	66.81	14.924	76.63	50.731

In some research, chitosan coating has inhibited the growth of some fungi and delayed the increase in decay of stored longan fruit [Jiang & Li, 2001], indicating that the chitosan coating reduced pathogen growth in some way. Since attack by pathogens is also a major factor causing discolouration of the mushroom, inhibiting decay could be partially beneficial in delaying browning of mushroom.

However, food nutrient metabolism in vegetables is known to be under the control of various enzymes. Baardseth [1979] stated that amylase, cellulase, lipase, proteinase and respiratory enzymes control the levels of carbohydrates, lipids and proteins, while polyphenoloxidase, peroxidase and catalase activities alter vegetable flavour.

Fasidi & Kadiri [1991] found that the matured mushroom is the most nutritious compared to mushrooms in the three mushroom development stages employed, would be expected to undergo a faster decline in flavour and food nutrients and to show more browning than the other two stages during storage or after harvest. This is due to their having greater activity of amylase, peroxidase, catalase and polyphenoloxidase.

Furthermore, increasing the concentration of chitosan coating markedly enhanced the beneficial effects.

CONCLUSIONS

We suggest that the application of chitosan coating (with optimum concentration 20 g/kg) could be beneficial and considered for commercial application in extending the shelf-life and maintaining quality and, to some extent, controlling decay of mushroom. In using chitosan for decay control, we consider that it may be suitable in the treatment of mushroom stored for shorter periods (*e.g.* 3 days) or for short-distance transport and distribution. However, for longer storage and marketing, chitosan coating to control discolouration and decay in mushroom could be better.

REFERENCES

1. Aebi H.F. Catalase. 1983, *in*: Methods in Enzymatic Analysis, vol. 3 (Ed. H.U. Bergmeyer). Verlagchemie, Dearfield Beach, Florida, p. 273.
2. Amerine M.A., Ough C.S. Methods for Analysis of Musts and Wines. 1980, Ch.7, A Willy- Interscience Publication, Univ. of California, NewYork, pp.175-199.
3. Annese M., Manzano M., Nicoli M.C., Quality of minimally processed apple slices using different modified atmosphere conditions. J. Food Quality, 1997, 20, 359–370.
4. Anonymous. Handbook of Food Preservation. 1999 (ed. M.S. Rahman). Marcel Dekker, Inc., p. 809.
5. Anonymous. Precise colour communication. 1988, Minolta Pamphlet PCC 807E-EI, p. 11.
6. Baardseth P., Enzymatically induced quality changes in fresh and frozen carrot. Acta Hortic., 1979, 93, 67-71.
7. Baldwin E.A., Nisperos-Carriedo M.O., Baker R.A., Use of edible coatings for lightly processed fruits and vegetables. Hortic. Sci., 1995, 30, 35–38.
8. Betty R., William H., Jill K., Dawley R. Tyrosinase, Laccase and peroxidase in mushrooms (Agaricus, Crimini, Oyster and Shiitake). J. Food Sci., 1994, 59, 824-827.

9. Biljana B., Kulevanova S., Stefov V., Infrared analysis of Macedonian mushroom dietary fiber. Nahrung / Food, 2002, 46, 238-239.
10. Buta G.J., Moline H.E., Spaulding D.W., Wang C.Y., Extending storage life of fresh-cut apples using natural products and their derivatives. J. Agric. Food Chem., 1999, 47, 1–6.
11. Chefetz B., Chen Y., Hadar Y., Purification and characterization of Laccase from *Chaetomium thermophilium* and its role in humification. Appl. Environ. Microbiol., 1998, 64, 3175-3179.
12. CIE. 1978, Recommendations on Uniform Colour Spaces-Colour Difference Equations, Psychometric Colour Terms. Supplement No. 2 to CIE Publication No. 15 (E-1-3.1) 1971/ (TC-1-3). Paris: CIE.
13. Darmadji P., Izumimoto M., Effect of chitosan in meat preservation. Meat Sci., 1994, 38, 243-254.
14. Devece C., Neptuno J., Lopez R., Lorena G., Tudela J., Enzyme inactivation analysis for industrial blanching applications: Comparison of microwave, conventional, and combination heat treatments on mushroom polyphenoloxidase activity. J. Agric. Food Chem., 1999, 47, 4506-4511.
15. El Ghaouth A., Arul J., Ponnamapalam R., Boulet M., Chitosan coating effect on storability and quality of fresh strawberries. J. Food Sci., 1991, 56, 1618–1620.
16. Fasidi I., Kadiri M., Changes in enzyme activities of *Termitomyces robustus* (Beeli) Heim and *Lentinus subnudus* Berk during sporophore development. Food Chem., 1991, 39, 109-116.
17. Food & Drug Administration. Chemical preservatives. Code of Federal Regulation, title 21 part 182. 1989, Washington, DC: US-GPO.
18. Friedman M., Food browning and its prevention, an overreview. J. Agric. Food Chem., 1996, 44, 631–653.
19. Gonulalan Z., Kose A., Yetim H., Effects of liquid smoke on quality characteristics of Turkish standard smoked beef tongue. Meat Sci., 2003, 66, 165-170.
20. Gorny J.R., Summary of CA and MA requirements and recommendations for fresh-cut fruits and vegetables. 1997, *in*: CA 97 Proceedings: Fresh cut fruits and vegetables and MAP, vol. 5 (ed. J.R. Gorny). CA: University of California, pp. 30–33.
21. Imeri A., Knorr D., Effects of chitosan on yield and compositional data of carrot and apple juice. J. Food Sci., 1988, 53, 1707 – 1709.
22. Jiang Y., Li Y., Effects of chitosan on postharvest life and quality of longan fruit. Food Chem., 2001, 73, 139-143.
23. Jiang Y.M., Purification and some properties of polyphenoloxidase of longan fruit. Food Chem., 1999, 66, 75–79.
24. Ke D., Saltveit M.E., Wound-induced ethylene production, phenolic metabolism and susceptibility to russet spotting in iceberg lettuce. Physiologia Plantarum, 1989, 76, 412–418.
25. Lavid N., Schwart A., Yarden O., Tel-Or E., Accumulation of heavy metals in epidermal glands of the water lily (*Nymphaeaceae*). Planta, 2001, 212, 323-331.
26. Lee J., Sung T., Lee K., Kim M., Effect of Gamma-irradiation on color, pungency, and volatiles of Korean red pepper powder. J. Food Sci., 2004, 69, 585-592.
27. Li H., Yu T., Effect of chitosan on incidence of brown rot, quality and physiological attributes of postharvest peach fruit. J. Sci. Food Agric., 2000, 81, 269–274.
28. Li P., Barth M.M., Impact of edible coating on nutritional and physiological changes in lightly processed carrots. Postharv. Biol. Technol., 1998, 14, 51–60.
29. Lister C.E., Lancaster J.E, Walker J.R.L., Phenylalanine ammonia—lyase activity and its relationship to anthocyanin and avonoid levels in New Zealand grown apple cultivars. J. Am. Soci. Hortic. Sci., 1996, 121, 281–285.
30. Loaiza-Velarde J.G., Saltveit M.E., Heat shock applied either before or after wounding reduce browning of lettuce leaf tissue. J. Am. Soci. Hortic. Sci., 2001, 126, 227–234.
31. Lopez J., Fenoll L., Tudela J., Devece C., Thermal inactivation of mushroom polyphenoloxidase employing 2450 MHz microwave radiation. J. Agric. Food Chem., 1999, 47, 3028-3035.
32. Lopez-Serrano M., Barcelo R., A H_2O_2-mediated pigment decay in strawberry as a model system for colour alterations in processed plant foods. J. Agric. Food Chem., 1999, 47, 824–827.
33. Luo Y., Barbosa-Canovas G., Preservation of apple slices using ascorbic acid and 4-hexylresorcinol. Food Sci. Technol. Int., 1996, 2, 315-321.
34. Malik C., Singh M., Plant enzymology and histo enzymology. 1980, *in*: Plant Enzyme Assays – Some Examples. Kalyani Publishers, 24, dar yaganj, Nuw. Delhi. 11002, UK.
35. Mayer A., Harel E., Polyphenoloxidase and their significance in fruit and vegetables. 1991, *in:* Food Enzymology, Ch. 9 (ed. F. Fox). Elsevier, New York, pp. 373-398.
36. Mihai N.-L., Chevolot L., Iwahara S., Takegawa K., Furmanek A., Lienart Y., High performance liquid chromatography and photodiode array detection of ferulic acid in *Rubus* protoplasts elicited by O-glycans from *Fusarium* sp. M7-1. Acta Biochimica Polonica, 2002, 49, 1019-1027.
37. Olmos E., Piqueras A., Martinez-Solano J.R., Hellin E., The subcellular localization of peroxidase and the implication of oxidative stress in hyperhydrated leave of regenerated carnation plants. Plant Sci., 1997, 130, 97–105.
38. Palou E., Lopez-Malo A., Barbosa-Canovas G., Chanes-Welti J., Swanson W., Polyphenoloxidase and colour of blanched and high hydrostatic pressure treated banana puree. J. Food Sci., 1999, 64, 42-45.
39. Perry C., Smith M., Britnell C., Wood D., Thurston C., Identification of two laccase genes in the cultivated mushroom, *Agaricus bisporus*. J. Genatic. Microbiol., 1993, 139, 1209-1218.
40. Rattanathanalerk M., Naphaporn C., Walaiporn S., Effect of thermal processing on the quality loss of pineapple juice. J. Food Eng., 2005, 63, 259-265.
41. Saltveit M.E. Physical and physiological changes in minimally processed fruits and vegetables. 1997, *in*: Phytochemistry of Fruits and Vegetables (ed. F.A. Tomas-Barberan). Oxford, UK: Oxford University Press, pp. 205–220.
42. Saltveit M.E., Wound induced changes in phenolic metabolism and tissues browning are altered by heat shock. Postharv. Biol. Technol., 2000, 21, 61-69.
43. Salunkhe D.K., Bolin H.R., Reddy N.R., Storage methods. 1991, *in*: Storage, Processing and Nutritional Quality of Fruits and Vegetables. vol 1. (2nd ed.) CRC Press Inc., Florida, pp. 243-294.
44. Sapers G., Douglas F., Measurement of enzymatic browning at cut surfaces and in juice of raw apple and pear fruits. J. Food sci., 1987, 52, 1258-1262, 1285.
45. Scott D., Oxidoreductase. 1975, *in*: Reed Gerald, Enzymes in food processing. Academic Press, NY and London, pp. 219-254.
46. Son S.M., Moon K.D., Lee C.Y., Inhibitory effects of various antibrowning agents on apple slices. Food Chem., 2001, 73, 23–30.

47. Su X.G., Zheng Y.H., Zhang L., Wang F., Zhang Y.M.m Effects of chitosan coating on postharvest quality and decay of vegetable soybean pods. Acta Phytophysiologica Sinica, 2001, 27, 467–472.
48. Sun N., Song K., Effect of nonthermal treatment on the molecular properties of mushroom polyphenoloxidase. Food Chem. Toxicol., 2003, 68, 1639-1643.
49. Tung-Sung C., Siddiq M., Sinha N., Cash J., Commercial pectinase and the yield and quality of stanley plum juice. J. Food Proc. Preserv., 1995, 19, 89-101.
50. Van Der Lubben I.M., Verhoef J.C., Borchard G., Junginger H.E., Chitosan and its derivatives in mucosal drug and vaccine delivery. Eur. J. Pharmac. Sci., 2001, 14, 201–207.
51. Wilson M., Amylase synthesis and stability in crested wheat grass seeds at low water potentials. Plant Physiol., 1971, 48, 541-546.
52. Yaropolov A.I., Skorobogatko O.V., Vartanov S.S., Varfolomeyev S.D., Laccase – Properties, catalytic mechanism, and applicability. Appl. Biochem. Biotechnol., 1994, 49, 257–280.
53. Zhang X., Flurkey W., Phenoloxidases in portabella mushrooms. J. Food Sci., 1997, 62, 97-100.

Received December 2006. Revision received May and accepted July 2007.