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Original article

Extending of Shelf Life of Mushroom and Asparagus using Rice bran Protein edible coating

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Abstract

Button mushrooms and asparagus have a very short shelf life after harvesting and are sensitive to mechanical damage, browning, moisture loss and chlorophyll degradation. An edible coating based on rice bran protein (RBP) was tested in order to determine the shelf-life extension period of mushroom and asparagus. Weight loss, color browning, ascorbic acid, total chlorophyll, pH, total titratable acidity, polyphenol oxidase activity and peroxidase activity of mushrooms and asparagus were studied. Also, microbiological analysis of the coated samples was studied. The results revealed that, weight loss and firmness of samples decreased with of using RBP coating compared to the control samples. Also, RBP coating maintain the pH values for samples during storage compared to control. Ascorbic acid decreased during storage for both coated and uncoated while, the uncoated samples decreased more. Also, RBP coating was effective to maintain of the color compared to control. Finally, the study showed also that RBP coating with bacteria, molds and yeasts of mushroom and asparagus samples compared to control.

Keywords Mushroom; Asparagus; Rice bran protein; Coating; Storage; Quality

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Introduction

Mushrooms are used not only as food but also as functional food and medicines due to high content of proteins and minerals, low cholesterol and starch, and also different bioactive compound (1). Mushrooms have a very short shelf life after harvesting of about 3-5 days at 1- 4 C ºand 70 – 75% relative humidity (2).Thus, it is important to extend the shelf life of fresh mushrooms, while preserving their quality, is desirable to export and import grocery companies (3). Serious problems contribute to the postharvest deterioration of mushroom such as browning, moisture loss, softening, high respiration rate and microbial attack (4). From the point of view of post-harvest physiology, special protection techniques are washing them with citric acid, ethylene diaminetetraacetic acid (EDTA), hydrogen peroxide, and sodium hypochlorite or using ultrasound and high-pressure argon, pulsed light or using coatings required maintaining their quality and freshness (1).

As one of the important fresh vegetables, green asparagus (*Asparagus officinalis L.*) is becoming increasingly popular due to its special flavor, taste, low calories content, high nutritional value and the high economic value in terms of export in recent years**.** Anthocyanins are one of the important groups of phenolic compounds, presenting in fruits and vegetables. They contribute to the characteristic color and have been linked to antihyperglycemic, anticancer, and antimutagenic health benefits. Asparagus has a limited shelf-life of less than 5 days at ambient temperatures, mainly due to its high respiratory rate after harvesting **(5)**. During storage, asparagus undergoes undesirable physiological and compositional changes such as moisture loss, chlorophyll degradation, and lignifications **(5**, 6**)** that lead to a deterioration of the overall quality of the vegetable.

In recent years, the increasing consumption of fresh produce worldwide has led to the necessity for alternative methods with a high efficiency, with a low residue rate, that are non-toxic, environmentally and economically friendly, and which do not threaten human health. Edible coatings applied as a thin layer on the product's surface are biodegradable materials that have no adverse effects on human health and are environmentally and economically friendly. In this respect, edible coating materials are promising treatments for extending the commercial storage life of fresh fruit and vegetables **(7).**

Edible coatings are traditionally used to enhance postharvest food appearance and preservation, as edible coatings provide products with sheen and make them more attractive to consumers **(8)**. Moreover, they maintain the phytochemical (antioxidants, phenolics, and color) and physicochemical (weight loss, respiration rate, and ethylene production) properties for a longer period, and some edible coatings act as a natural antimicrobial and antifungal compound in many vegetables such as mushroom and asparagus **(9).** Edible coatings generally act as a barrier to gas exchange properties and thus prolong the storage life of fruit and vegetables **(10 and 11).** Edible films can be used as semipermeable barriers in food with various purposes, such as: control the respiration rate, retard moisture loss and color variation, improve texture and maintain mechanical integrity, help retain flavor and inhibit growth of micro-organisms (**12)**. Thus, the development of edible or biodegradable films arises from the demand for high quality and safe food, as well as from environmental concerns with the disposal of non-renewable materials. It is also an opportunity to create a new market of raw materials for packaging**.** Proteins of plant origin are more often used than animal proteins for films production due to availability and lower cost **(13, 14).** The byproducts from the cereal agro-industrial processing may be a source of protein that can be recovered for the production of protein-based films. An example is rice bran, resulting from the processing of the grain, which proteins were extracted to produce bio-based films (**13**, **15).**

Rice bran Protein has the ability to develop protein films with good physical, mechanical and exposure to light properties. Deamidation process improves protein structure, solubility and increase the strength of hydrogen bonds between protein polymers, hence production of rice bran protein films with suitable physical, mechanical, Exposure to light properties and crystalline structure. That can be widely used as edible film for packaging food products. Hence, deamination process can be applied on another protein material from food and agricultural manufacturing residues to increase their use as distinctive edible coating.

The aim of this study is to evaluate Rice bran protein film to extend shelf life of Mushroom and Asparagus Also, study the changes of some properties during storage.

Materials and Methods

Materials

White mushrooms (*Agricus bisporus*) and green asparagus spears (*Asparagus officinalis L.*) were harvested from a commercial farm in Wadi El-Natrun City, El- Beheira Governorate, Egypt. Samples were transported to the laboratory at 4 Cº. Mushrooms and asparagus with homogeneous color and size and free of injuries were selected. Rice bran protein extracted from rice bran (Giza 178 variety) was obtained from Rice Research Center at Sakha, Agricultural Research Center, Egypt during 2021Season.

All solvents and chemicals such as glycerol, peptone, plate dextrose agar and plate count agar were in analytical grade and purchased from El- Gomhoria Co. for Chemicals and Drugs, Tanta, Egypt.

Methods

Sample preparation

White mushrooms (*Agricus bisporus*) and green asparagus spears (*Asparagus officinalis L.*) of uniform size and free of physical damage, absence of external injuries and fungal infection were chosen and washed with tap water directly before dipping.

Preparation of the edible coating solution

Extracted, freeze-dried rice bran protein at concentration1, 2 and 3% were used. RBP samples were dissolved in distilled water, stirred at 80˚C for 30 min. Glycerol was added at 40% level and pH was adjusted to 8. Citric and ascorbic acids were added as antibrownning and antimicrobial agents at 1% w/v. The solutions were heated under constant agitation until they reached to 80˚C, After that, the coatings were allowed to cool down to room temperature.

Application of coating on samples

Mushroom and asparagus were immersed in a 0.1 % NaClO solution for 1 min for surface-sterilization and air-dried at room temperature for 30 min. Then, the mushrooms and asparagus dipped for 1 min into rice bran protein solutions at levels 1, 2, 3 % (w/v). After coating, the mushrooms and asparagus were left to dry at ambient temperature. All coated samples were placed into macroperforated polypropylene film bag. The treated samples and control were stored in a refrigerator at 5±1˚C and 70 – 75% RH. Samples were stored for 14 and 17 days for mushrooms and asparagus; respectively. Sample withdrawn every 2 days for physical, chemical and microbial properties.

Physical determination

Weight loss

Weight loss was calculated as percentage loss of initial weight, as reported by Han et al., **(16).**

Firmness

The firmness of fresh vegetables was determined by measuring the compression force of the samples using a texture analyzer (QTS-25) fitted with a Kramer shear cell **(17**).

Total soluble solid (TSS)

TSS was measured in the juice pressed from the sample by the refractometeric (RR 12, Nr 05116, 0-35% at 20 ˚C, Made in Poland) method at room temperature according to the methods outlined in the **AOAC** (18 **).**

Chemical properties

Moisture content

Moisture content was determined according to the methods of **(18).**the samples moisture content (approximately 2gm) was determined by measuring the weight loss of samples before and after drying in laboratory oven at 105 C^{o} until constant weight.

$$\%\text{Moisture content} = W_{O-}W_F/W_O \times 100$$

Where W_O was the sample weight before drying, W_f was its weight after drying.

Measurement of pH

pH meter(Fisher Scientific accument pH meter 25 USA) was used to measure pH the extracted juices as the methods described by **(18).**

Total titratable acidity (TTA)

Total titratable acidity of juices was determined according to the method of **(18).**The (TTA) was expressed as percentage of malic acid in mushroom; while expressed as percentage of galic acid in asparagus.

Ascorbic acid contents

2, 6 dichloro-phenolindophenol titrimetric method was used to determine ascorbic acid in juices as described in **(18).** The results were expressed in milligrams ascorbic acid per 100 ml of vegetables juice.

Chlorophyll content

Chlorophyll content in the asparagus was determined according to the methods described by **(19**) with slight modification. In brief, 2 g asparagus was ground in a mortar and extracted in 10 mL % (v/v) ethanol (95%) and centrifuged at 6000 rpm for 15 min at 4 ºC. The supernatant was used to determine the chlorophyll content. Chlorophyll quantification was performed spectrophotometrically using a spectrophotometer (Varian, Melbourne, VIC, Australia) at 665 and 649 nm, and the chlorophyll content was expressed as chlorophyll mass on a fresh weight (FW) basis (mg/kg FW). The calculation of chlorophyll amount was described by **(20).**

Oxidative Browning

Browning

A colorimeter (CR 300; Minolta, Japan) was used to measure L, a, and b values of the middle part of the A. bisporus cap. The browning index was calculated according to **(21)** the following Equations:

$$\mathbf{X} = a + 1.75L/(5.645L + a \times 3.012b)$$

$$\text{Browning index} = (100 \times (X - 0.31))/0.172$$

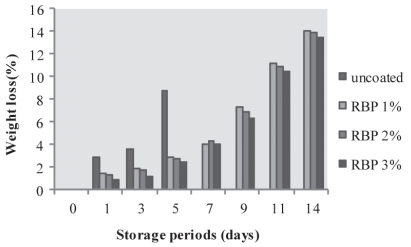

Fig (1): Weight loss (%) of mushroom coated with different levels of RBP coatings, stored at 5±1˚C and 70 – 75% RH for 14 days

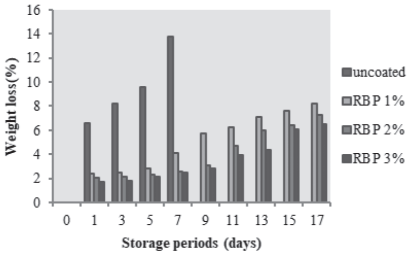

Fig (2): Weight loss (%) of asparagus coated with different levels of RBP coatings, stored at 5±1˚C and 70 – 75% RH for 17 days

Polyphenol Oxidase

Extraction was performed with phosphate buffer at 0.1 M (pH 6.8) using 10 g of each sample; then, 0.5 mL of catechol 0.01M was then added to 5 mL of phosphate buffer solution. The sample was immediately analyzed at an absorbance of 420 nm. Also, absorbance was measured after 2 min. The change in absorbance per minute is 0.01 as a polyphenol oxidase activity unit (U) and the result is expressed in U kg^{-1}1 FW (**22**).

$$\text{Polyphenol oxidase activity} = \\ = \text{DOD420} \times \text{V}/ (0.01 \times \text{Vs} \times \text{t} \times \text{m})$$

Where:

V = Total volume of enzyme solution (mL).

Vs = volume taken during determination (mL).

T = reaction time (min).

m = fresh weight of sample (kg).

Peroxidase enzyme (POD) activity

POD enzyme activity was determined using the methods reported by **(23).**

Microbiological analysis

Total viable bacteria counts:

Total viable bacteria counts per one of sample were determined using standard techniques on nutrient agar medium. Incubation was carried out for 48 hrs, at 32^0C according to **(24).**

Mold and yeast counts

Mold and yeast counts were determined by plating one ml of diluted sample suspension on acidified potato- dextrose agar medium. Triplicate plates were incubated at 25^0C for 5 days **(25).**

Results and discussion

Effect of edible coating on physical properties

Weight loss:

The weight loss is indicator for vegetables dehydration process due to transpiration and involves water transfer from the cell to surrounding atmosphere, thus representing a way to evaluate coating efficiency in preservation of quality **(26).**

Data presented in Figures (1 and 2) show the effect of edible coatings prepared using different levels of rice bran protein (1, 2 and 3%) on weight loss of mushroom and asparagus during cold storage for 14 and 17 days, respectively.

The results indicated that weight loss significantly increased during storage of both uncoated and coated samples. All coated samples significantly reduced weight loss during

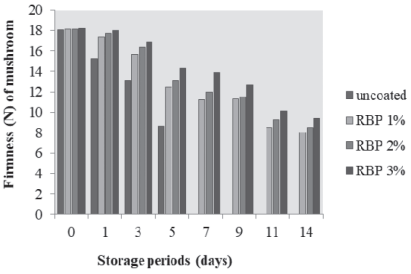

Fig (3): Firmness (N) of mushroom coated with different levels of RBP edible coatings, stored at 5±1˚C and 70 – 75% RH for 14 days

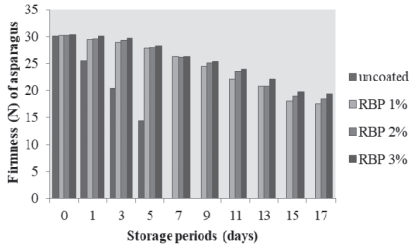

Fig (4): Firmness (N) of asparagus coated with different levels of RBP edible coating of rice bran protein stored at 5±1˚C and 70 – 75% RH for 17 days

cold storage compared to uncoated samples. Coatings are clearly effective in conferring a physical barrier to moisture loss and therefore retarding dehydration and vegetables shriveling **(27).**

The increase in physiological weight loss of fresh mushroom and asparagus during storage period may be due to the loss of moisture content.

It is showed from Figures (1 and 2) that, coated samples with 3% RBP were found to be the best among all of the used coating levels, which reduced the weight loss of mushroom and asparagus to 13.5% and 6.52% after 14 and 17 days; respectively compared to uncoated samples 8.53% for mushroom and 13.80% for asparagus stored for 5 and 7 days; respectively.

Firmness

The data in Figures (3 and 4) revealed that the firmness significantly decreased as a function of storage conditions for both uncoated and coated samples. All coated samples showed significantly beneficial effect on firmness maintenance compared to control.

Firmness measurements of coated and uncoated samples are showed in Figures (3 and 4). Uncoated mushroom lost about 50 % of firmness after 5 days of cold storage; while uncoated asparagus lost about 59% of firmness after 7 days of cold storage, whereas the loss of firmness with other coated samples arranged between 16 to 20 % and 13 to 14 % for mushroom and asparagus after the same period of cold storage. During storage, firmness decreased to 48 % compared to initial of firmness for mushroom and 36% for asparagus; while uncoated samples were removed after 5 and 7 days for mushroom and asparagus; respectively because it were spoiled. The results showed similarity to (29) and (30). According to **(30),** vegetables softening are attributed to the degradation of cell wall components, mainly pectin, due to the action of specific enzymes such as polygalacturonase. The edible coatings showed a good result with respect to the retention of vegetables firmness probably because this coating might be slowed down metabolism and prolonged the storage life, same effect was previously observed by **(31).**

Total soluble solid (TSS)

Data in Figures (5 and 6) shown total soluble solid (TSS) of pressed juices from uncoated and coated mushroom and asparagus during cold storage. The results indicated that TSS which is an indicator of sugar content significantly increased in all samples as a function of storage time. This increase might be due the weight loss during storage especially moisture loss.

TSS of uncoated mushroom and asparagus samples were significantly higher than those of coated samples. It may be explained by decreasing of moisture content of coated samples compared to uncoated samples.

TSS increased during the storage time (Figures 5 and 6) from 9.61 to 12.54% during storage from zero to 5 days for mushroom, while increasing from 13.29 to 34.88% during storage from zero to 7 days; respectively for control asparagus samples (uncoated samples). TSS of samples coated with RBP coating was more than that of control. So, mushroom and asparagus coated with 3% RBP coated can be freshly stored for 14 and 17 days with high juiciness.

TSS increases during storage and ripening of fruits and vegetables. This could be attributed mainly to the breakdown of polysaccharides components into simple sugars during ripening along with a proportional increase in TSS, but further hydrolysis could decrease the TSS during storage and it is caused by a decline in the amount. The results obtained are agreement with investigations by **(32) and (33).** The coated samples showed a lesser increase in TSS than the uncoated samples. This could be due to the reason that coating reduces the production of ethylene and the rate of respiration **(34)** and reduces the rate synthesis and utilization of metabolites, delaying nutrient decomposition and as a result, a lower TSS values in coated samples were there as compared to non-coated samples **(35).** The increment of total soluble solids at the time of storage period is natural as sugar the basic constituent of the TSS is used in respiration process for metabolic activities of the fresh fruits and vegetables **(36).**

Chemical properties

Moisture contents

Figures (7 and 8) show the changes in moisture contents of coated and uncoated mushroom and asparagus during cold storage. Moisture content of mushroom and asparagus significantly decreased as a function of storage time for both uncoated and coated samples.

All coatings provided a beneficial barrier for moisture and prevent weight loss during storage. It may be due to that coatings fill pores and crakes on skin, so prevent moisture loss. The results revealed that uncoated samples can be stored for 5 days and 7 days for mushroom and asparagus; respectively. From the aforementioned results 3% RBP was more effective in reducing moisture loss and low water permeability until day 14^{th} for mushroom and 17^{th} for asparagus followed by 2% and 1%; respectively. The semi permeable barrier provided by edible coatings is aimed to extend shelf life by reducing moisture and solutes migration, gas exchange, respiration and oxidative reaction rates, as well as suppress physiological disorders on fresh vegetables **(37 and 38).**

pH and total titratable acidity (TTA) values

The results are shown in Figures (9 and 11) for mushroom and Figures (10 and 12) for asparagus revealed that pH values of uncoated samples increased as function of storage time. The increase of pH values of coated samples was lower compared to uncoated samples. Increase of pH values (decrease of acidity) demonstrates maturation development **(31).** The coating treatments delayed maturation process. It might be due to the effect of coatings in reducing vegetables respiration rate; therefore they delay the utilization of organic acids as substrates for enzymatic reactions **(39).**

No significant differences in pH values were found between coated samples during storage period, while pH increased in uncoated sample with increasing storage period.

Figure (11) show the total titratable acidity (TTA, expressed as % of malic acid) of both uncoated and coated vegetable. TTA of uncoated sample for mushroom significantly decreased as a function of storage time. No significant

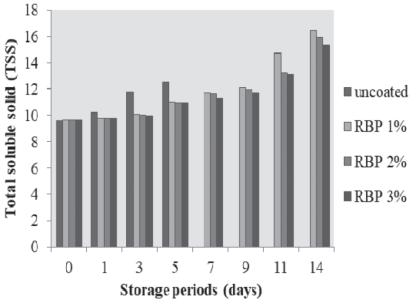

Fig (5): Total soluble solid (TSS) of juices from mushroom coated with RBP edible coatings, stored at 5±1˚C and 70 – 75% RH for 14 days

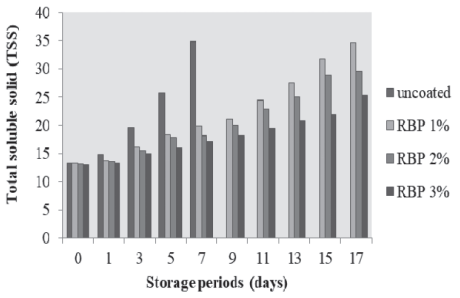

Fig (6): Total soluble solid (TSS) of juices from asparagus coated with RBP edible coatings, stored at 5±1˚C and 70 – 75% RH for 17 days

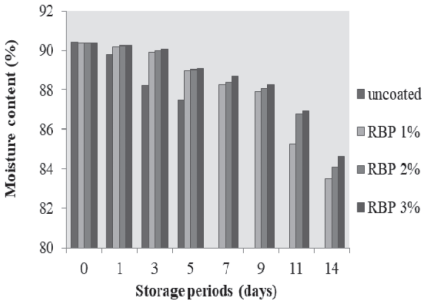

Fig (7): Moisture content of mushroom coated with RBP coating stored at 5±1˚C and 70 – 75% RH for 14 days

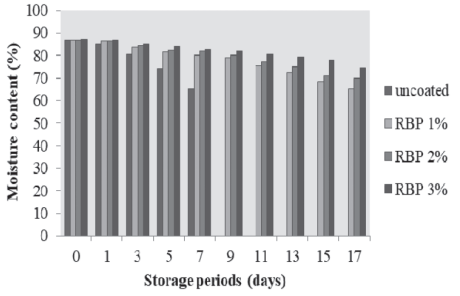

Fig (8): Moisture content of asparagus coated with RBP edible coating stored at 5±1˚C and 70 – 75% RH for 17 days

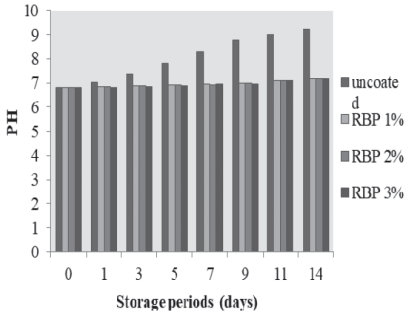

Fig (9): effect of edible coating with different levels of rice bran protein on PH of mushroom at 5±1˚C and 70 – 75% RH for 14 days

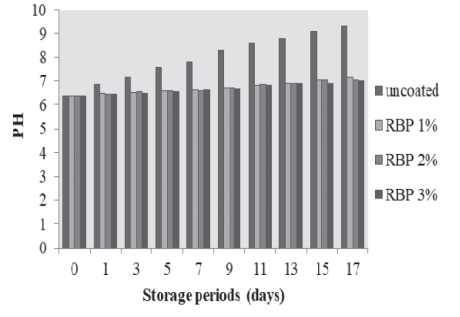

Fig (10): effect of edible coating with different levels of rice bran protein on PH of asparagus at 5±1˚C and 70 – 75% RH for 17 days

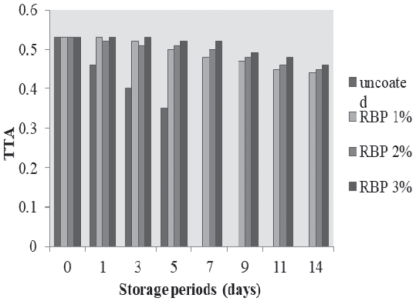

Fig (11): Effect of RBP coating total titratable acidity (TTA) of mushroom stored at 5±1˚C and 70 – 75% RH for 14 days.

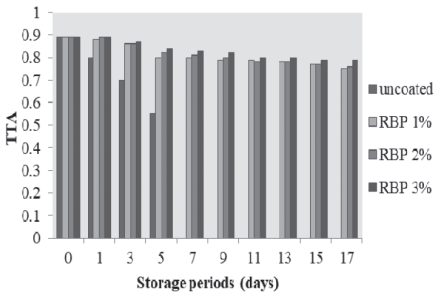

Fig (12): Effect of RBP coating on total titratable acidity (TTA) of asparagus stored at 5±1˚C and 70 – 75% RH for 17 days.

differences were found in pH between all of coated samples and other treated samples during cold storage from zero to 14th.

Results presented in Figures (10 and 12) showed both pH and total titratable acidity values of pressed juices from asparagus samples. No significant differences in pH values were found among coated samples during storage period; while pH increased in uncoated sample with increasing storage period for asparagus samples. Figure (12) show the total titratable acidity of both uncoated and coated samples. TTA of uncoated sample for asparagus significantly decreased as a function of storage time. No significant differences were found in pH among all of the coated samples during storage from zero to 17th.

(40) found that the decrease in titratable acidity and the slight increase in pH of coated samples for mushroom and asparagus might be due to the delay in maturation process and the metabolic activity within the tissue.

Since organic acid are substrates for enzymatic reaction of respiration, a reduction in the acidity and increase in pH values are expected of uncoated samples. Coatings reduce the respiration rate; therefore delay the utilization of organic acids. These results are in agreement of the results of **(41).**

The coating acts as a semi- permeable membrane against respiration, which reduces the rate of respiration and further reduces the consumption of organic acid acids; therefore, the titratable acidity decreased **(42).**

The low change of pH due to the edible coating forming a semipermeable membrane on the surface of fruits and vegetables which modified the internal atmosphere *i.e.,* the endogenous CO_2 and O_2 concentration, thus retarding the ripening process.

(43) reported that the pH seems to be influenced by the concentration of SPI (Soy Protein Isolate) and that of HPMC (Hydroxypropyl methylcellulose). According to this study with the increased edible coating concentration the pH is lowered in pears. **(44)** found that pH of control pears samples significantly increased (P<0.05) as compared to coated samples with alginate and carrageenan 0.5% during storage. **(45)** found similar results in chitosan- *Aloe vera* coated cucumbers and cornstarch and carboxy-methylcellulose coated cucumbers, respectively. **(46)** also reported the similar result with guar based edible coating in cucumber decreased the pH as compared to uncoated cucumber. Titratable acidity retention was reported by **(47),** using strawberry fruits coated with gluten film. Slowing down the strawberry respiration rate by means of an edible coating could explain the delay in the use of organic acid in the enzymatic reactions of respiration, **(48).**

Effect on ascorbic acid

It should be noticed from Figures (13 and 14) that ascorbic acid contents in both coated and uncoated mushroom and asparagus significantly decreased during cold storage. Ascorbic acid losses of coated samples were lower than that of uncoated samples during storage. It may be due to adding of ascorbic and citric acids in to coating solutions which plays important role as antioxidants and role of coating in reduction of oxygen permeability. **(49)** mentioned that the decrease of oxygen passes through the edible coating decrease the rate of oxidation of ascorbic acid.

Coating process maintains about 15.36 to 17.74 mg/ 100g of total ascorbic acid after 5 days of cold storage for mushroom, while the remained of ascorbic acid of control after 5 days was about 5.04 mg/ 100g. After 14 days of cold storage, ascorbic acid was 7.16 – 11.50mg / 100 g; while control sample was spoilage after 5 days. As for asparagus, ascorbic acid recorded about 173.95 to 180.97 mg/100g for coated samples; while it recorded 100.77 mg/100g for uncoated samples during storage to 7 days. After 17 days, ascorbic acid reached about 120.66 – 142.12 mg/100g as af-

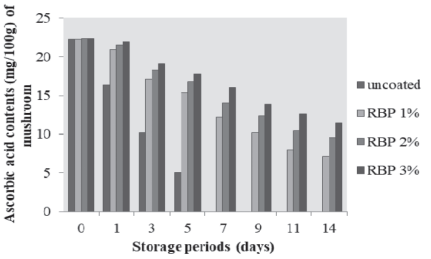

Fig (13): Ascorbic acid contents (mg/100g) of mushroom coated with RBP coating stored at 5±1˚C and 70 – 75% RH for 14 days.

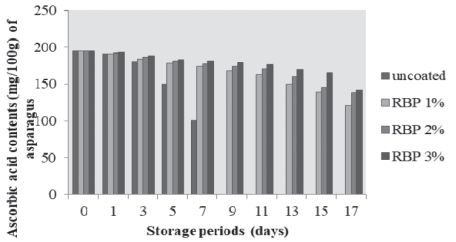

Fig (14): Ascorbic acid contents (mg/100g) of asparagus coated with RBP coating stored at 5±1˚C and 70 – 75% RH for 17 days

fected by coating process, but control sample spoiled after 7 days only of storage.

From the previous results, it found that 3% RBP coated samples were the best in maintenance V.C in mushroom and asparagus. The edible coating acts as a covering layer that prevents the autoxidation process, and maintained of ascorbic acid content **(9).**

Effects on total chlorophyll of asparagus:

In Figure (15) showed that significant difference ($p<0.05$) between coated and uncoated asparagus in total chlorophyll content.

A statistically significant decrease was noticed in total chlorophyll content with time during storage (Figure 15). Data showed that all coated samples had significantly highest chlorophyll content. Coating process maintains about 73.17 to 78.51 mg/ 100g of total chlorophyll after 7 days of cold storage; while total chlorophyll of control after 7 days was about 58.98 mg/ 100g. After 17 days of storage, total chlorophyll was 61.05 to 69.36 mg / 100 g, while control spoiled after 7 days.

From the previous results, with asparagus coated 3% RBP had significantly the highest value of total chlorophyll content among all of others levels during cold storage.

This decrease of chlorophyll content could be attributed to gradual increase of destruction by chlorophyll degrading peroxidase activity and also transformation chloroplasts to chromoplasts by chlorophyllase activity **(50).**

The reduction of chlorophyll loss of asparagus during storage using RBP coating may be attributed to reducing of respiration rate, resulted in lower activity of chlorophylls and consequence reduced color changes **(51).**

Effects on browning in mushroom:

The color of the button mushroom is an important parameter, since color relates directly to the perception of quality and acceptability by the consumer **(52).**

Figure (16) shows of the color parameters (L, a, b, ΔE and BI) for coated and control mushrooms.

The L values decreases, and ΔE and BI increased with storage time for coated and control mushrooms. From the day 3 it is that there was observed differences ($p<0.05$) between two groups, being the coated mushrooms those with higher whiteness, less color difference and browning. Being in agreement with a study realized by **(53),** where the L values of mushrooms coated with 1% , 2% and 3% rice bran protein were significantly ($p<0.05$) lower than that uncoated at 5 ºC after 14 days of storage.

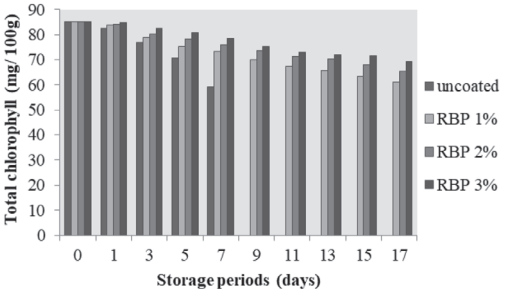

Fig (15): Effect of RBP coating on total chlorophyll of asparagus (mg/ 100g) stored at 5±1˚C and 70 – 75% RH for 17 days.

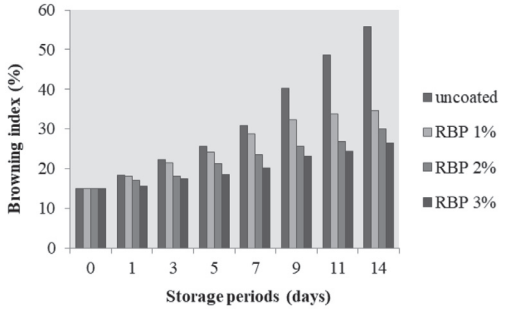

Fig (16): Effect of RBP coating on browning index (%) of mushroom stored at 5±1˚C and 70 – 75% RH for 14 days.

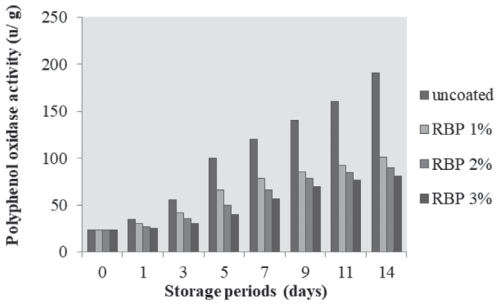

Fig (17): Effect of RBP coating on polyphenol oxidase activity(u/ g) of mushrooms stored s at 5±1˚C and 70 – 75% RH for 14 days

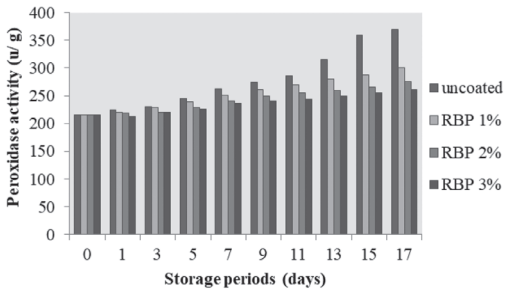

Fig (18): Effect of RBP coating on peroxidase activity (u/ g) of stored at 5±1˚C and 70 – 75% RH for 17 days

During storage, low changes of color were shown of coated mushrooms compared to control. The browning index at the end of storage increased from 15.0 to 55.76, from 15.0 to 34.63,from 15.0 to 30.06 and from 15 to 26.46 for control and coated mushrooms at 1 , 2 and 3% RBP; respectively. Samples coated with 3% RBP was the best reducing browning index for mushroom at the end of storage period, while control spoiled after 5 days of storage.

Protein coatings probably delay browning by preventing the oxidative process. An important factor implied in the inhibition of the oxidative browning is that the coating by protein solution represents an efficient barrier to oxygen.

Effect of RBP coatings on polyphenol oxidase of mushroom

PPO is the major contributor for the browning of fresh white mushrooms, due to their influence in the oxidation of phenolics, resulting in the formation of brown-colored substances. Figure (17) show the PPO activity during storage, which increases for both coated and uncoated samples. Significant difference (p<0.05) were observed between coated and control, being the coated mushrooms is best.

At zero time, no significant differences in PPO activity were found between uncoated and coated samples.

PPO activity increases from 23.67 to 40.15U/g fresh weight for samples coated with 3% RBP, from 23.67 to 49.60U/g fresh weight for coated with 2% and from 23.67 to 66.44 for coated with 1% RBP, while PPO control mushrooms increase from 23.67 to 100.53 after 5 days increased from 23.67 to 190.93 U/g fresh weight at the end of storage. On the other hand PPO activity increased from 23.67 to 80.57, from 23.67 to 89.63 and 23.67 to 100.98 u/g for samples coated with RBP at 1,2 and 3% respectively Generally, 3% RBP coated samples had the best in maintenance PPO activity in mushroom.

It may also be observed that the PPO activity increased more on control than that of coated mushrooms. The rapid increase of PPO activity possibly accelerate the oxidation of polyphenols presents on the mushrooms. However, on coated mushrooms the coating seems inhibit the PPO immediately on the day of coating application (day zero). This difference can be explained by the fact that the coating (that contain an inhibitor of the enzyme tyrosinase, cinnamic acid) inhibited the PPO, reducing drastically its activity.

In addition, and as stated before, the RBP coating forms a protect barrier on the surface of the fresh produce, reducing supply of O2 which can help reducing the PPO activity.

Han (16) found that protein and peptides in rice bran protein can affect the polyphenol oxidase activity by reacting with chelating on copper at active site of PPO, also it has reported that RBP contains anti-oxidative peptides and sulfur amino acids and thus these peptides might be the cause of browning inhibitor.

Effect of RBP coating on peroxidase activity of asparagus:

Figure (18) indicated that peroxidase (POD) activity of asparagus spears increased along storage period; these results are compatible with **(54)** on asparagus. The increasing of POD activity is caused delaying senescence. **(54)** found that POD enzyme cause catalyzes of corruption H2O2, this causes senescence of product. So, POD enzyme destroys pigments of chlorophyll and is considered an indication of senescence and intense stress**.**

Data reveled that all coating level significantly decreased activity of peroxidase compared with control; however asparagus spears coated with 3% RBP had significantly the lowest e of peroxidase activity, followed by 2% CMC then 1% CMC, while the highest peroxidase activity was obtained from control.

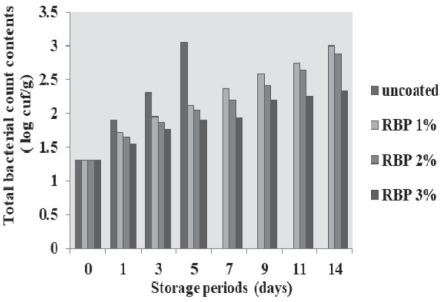

Fig (19): Effect of RBP coating on total bacterial count contents (log cuf/g) of mushroom stored at 5±1˚C and 70 – 75% RH for 14 days.

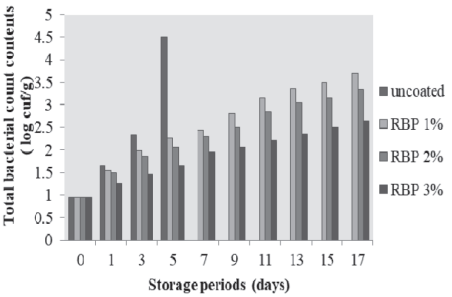

Fig (20): Effect of RBP coating on total bacterial count contents (log cuf/g) of asparagus stored at 5±1˚C and 70 – 75% RH for 17 days.

Effect of coatings on microbial growth

Total bacterial counts

Figures (19 and 20) showed the effects of RBP coating at levels 1, 2 and 3% on total bacterial count of mushroom and asparagus during storage for mushroom and asparagus, at 5± 1ºC and 70 – 75%.

The results pointed to, significant differences were found in total bacterial count between coated and uncoated samples. The same Figure illustrated that, total bacterial count significantly increased gradually with the increasing of storage period in all samples. Where, all coating levels were effective in reducing total bacteria count compared to control. These findings are in agreement with investigations by **(55).** It should be observed also from Figures that, total bacteria count decreased gradually with the increase of RBP levels comparing to control. However ,samples coating with RBP level 3% was the best among all of the coated samples reducing levels of total bacterial count to 2.33 and 2.65 (Log CFU/g) for mushroom and asparagus; respectively at the end of storage period. These results agreed with those reported by **(56).**

Mold and yeast counts:

Figures (21 and 22) showed the effects of edible coating of RBP at levels 1, 2 and 3% on mold and yeast counts of mushroom and asparagus during storage for mushroom and asparagus, at 5± 1ºC and 70 – 75%. .

The results reflected that, significant differences were found in molds and yeasts count between coated and uncoated samples. The results revealed that molds and yeasts count significantly increased gradually with the increase of storage period in all samples. Where, all coated samples had lower mold and yeast counts than control. These findings are in agreement with investigations by **(56).**

It should be observed also that mold and yeast decreased gradually with increasing of RBP levels comparing to control. Samples coated with 3% RBP was the best coating in reducing levels of mold and yeast to 2.35 (CFU/ g) for mushroom and 2.65 (CFU/ g) for asparagus at the end of storage period; While control sample was spoiled after 5 days for mushroom and 7days for asparagus.

(57) showed higher microbial counts with uncoated sample whereas all coated samples showed low total micro-

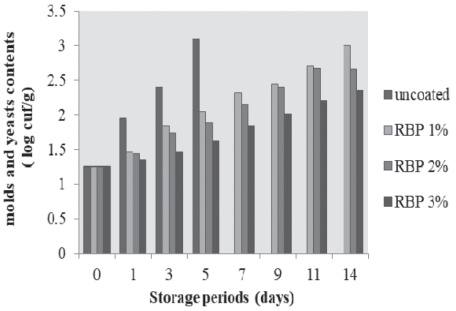

Fig (21): Effect of RBP coating on molds and yeasts contents (log cuf/g) of mushroom stored at 5±1˚C and 70 – 75% RH for 14 days.

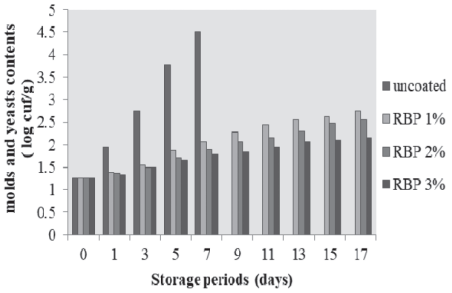

Fig (22): Effect of RBP coating on molds and yeasts contents (log cuf/g) of asparagus stored at 5±1˚C and 70 – 75% RH for 17 days

bial counts. Also, **(45)** showed that during the storage coating decreased aerobic yeast and mold count for cucumber compared with control. Moreover, **(14)** mentioned that edible coating applied on fresh cut apples had marked effect in reducing psychrophilic counts as compared to uncoated apple pieces.

Conclusion

Regarding of this work it can be concluded that the developed coating improved the quality of mushrooms and asparagus. The application of the coating showed to prolong the shelf life of mushrooms and asparagus, decreasing weight loss, reducing changes in color, titratable acidity, pH and TSS during refrigerated storage. The coating was effective as a barrier in the reduction of weight loss during storage and also had beneficial effects in delaying the ripening process and improved the appearance of the coated mushrooms and asparagus when compared with the uncoated.

The color is related to the age of the mushrooms and it has been used as an indicator to quantify the shelf life. The microbial population could affect the color change of fresh mushrooms and asparagus as well as the action of polyphenol oxidase (PPO) in browning for mushroom and peroxidase activity in degradation of chlorophyll for asparagus.

Rice bran protein (RBP) coating at level 3% was more effectively to increase shelf – life of mushroom and asparagus to 14 and 17 days; respectively. Also, protect these samples from microbial contamination compared to control. Also, some physical and chemical properties for coated samples were bitter than that of control. So, 3% RBP coating could be recommendation to maintain of mushroom and asparagus quality during storage for 14 and 17 days at 5 ºC.

These results suggest that the edible coating used in this work may be a promising method of maintaining the quality of the button mushrooms and asparagus that can be used to increase shelf-life during refrigerated storage. The use of this coating may have commercial importance, since it is necessary small amounts of the active compounds to obtain positive results.

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