Pectin based Edible coating with enhanced hydrophobic properties for shelf life extension of Button Mushrooms

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Abstract- The pectin-based edible coating solution was prepared by adding High methoxyl pectin (HMP), Candelilla wax and glycerol followed by their further homogenization. A total of seventeen different combinations with lower and higher levels of High methoxyl pectin powder concentration (1.5 and 2.5 %w/v), Candelilla wax concentration (0.4 and 0.8 %w/v) and glycerol concentration (1 and 2 %w/v) respectively, were used to prepare the coating solution. The effect of different concentration of ingredients upon the properties of the edible films was studied by analysing the response parameters like Water vapour transmission rate (WVTR), Percentage Moisture content (% MC) and Colour Analysis. The film with lowest value of WVTR, Lowest value of Moisture content and in range value for colour, was chosen to be the one with optimized concentration of ingredients containing pectin (2.1 %w/v), Candelilla wax (0.77 %w/v) and glycerol (1.83 %w/v). The optimized concentration of ingredients was further used in coating application study for the shelf-life extension of button mushrooms by analysing their physicochemical properties like weight loss, texture analysis, colour variation, content of total polyphenols, Polyphenol oxidase (PPO) and Peroxidase (POD) assay. Novelty Impact Statement: Incorporation of Candelilla wax (vegan based) within the pectin edible coating for improvisation of its water vapour transmission and moisture barrier properties has been studied. From the literature review performed, candelilla holding lowest water vapour transmission among different types of waxes and also being from a plant source was chosen as suitable hydrophobic agent. The composite pectin-candelilla coating was further used to achieve shelf life extension in button mushrooms.

Index Terms— Candelilla wax, Edible Coating, Pectin, Shelf-life

I. INTRODUCTION

Fruits and vegetables form the mainspring of a healthy diet. They are rich source of nutrients like vitamins, fibres and several essential minerals etc. Their high nutrient content makes them susceptible to faster perishability rates. Freshly harvested fruits and vegetables are predominantly comprised of water, having 90 - 95% moisture content. Hence, the loss of water is one of the most serious problem encountered after harvesting. Consequently, efforts must be taken to minimize the effects of these naturally occurring processes if the quality of harvested produce is to be maintained to the consumer's expectations. Because of these reasons, it makes their commercialization a little challenging. Different techniques that can be used for the extension of the shelf life includes chemical treatments, product irradiation, modified and controlled atmospheric packaging, etc. Amongst all the conventionally available techniques, edible coatings have come into limelight in the recent past and are being used extensively for preservation of fruits and vegetables (Yousuf et al; 2020).

Table1. Types of Edible Coatings

Polysaccharides	Vegetable Proteins	Lipids and Waxes	Agro-industrial Residue
Pectin	Soybean	Candelilla wax	Sugarcane bagasse
Alginate	Wheat gluten	Carnauba wax	Fruit and Vegetables residue
Chitosan	Corn zein	Paraffin wax	Residual waste from Wine Industry
Starch, Cellulose derivatives & Gums	Sunflower protein	Sunflower wax	Processing residue

Edible coating is primarily used to provide barrier against micro-organisms, moisture, gas and solute migration in foods. They effectively help in decreasing the water vapour transmission rate from the surfaces on which it is effectively coated upon, helps in retaining texture and preventing the metabolic alterations that can lead to accelerated rate of senescence (Cazon et al; 2022). Edible coatings also offer several advantages in terms of its eco-friendly nature, acting as effective carriers of several functional food additives, facilitating safety and enhancement of sensory and nutritional aspects of different food products. All these advantages of edible coatings make them provide their scope for application in shelf life enhancement of highly perishable fresh produce including mushrooms. The different materials used in preparation of edible coatings can be polysaccharides, proteins, fats and oils, used either in combination or as stand-alone.

Pectin is structurally and functionally the most complex polysaccharides, which is the major component of higher plant cell walls, especially citrus fruits, and apple pomace (Thakur et al; 2020). Pectin based edible coatings offer excellent mechanical properties, but being hydrophilic in nature, they show poor moisture barrier properties. Thus, addition of suitable hydrophobic substances into pectin based edible coatings can lead to enhancement in the moisture barrier properties of the coatings. The hydrophobic substances that can be used in the preparation of edible coatings include natural waxes, petroleum based waxes, vegetable oils and fatty acids.

The different types of methods that can be used for the application of edible coatings include dipping, spraying and brushing. Each method as per it's ease, convenience and requirement can be employed for the application of edible coating on different types of food products. The dipping method involves immersion of the fresh food produce into the edible coating to entirely coat the surface of the fresh produce and later is kept to drain in order to removed excess coating from the surface. Similarly, the spraying and brushing method involves controlled deposition of droplets of edible coating formulation over the substrate with the help of nozzles and brushing of edible coating upon the surface of the fresh produce respectively.

This study involves the preparation of stable pectin-based composite edible coatings with Candelilla wax as a hydrophobic substance where improved mechanical as well as moisture barrier properties played important role. The response parameters used for optimizing the concentration of ingredients for the films include water vapour transmission rate, colour of the films and the moisture content of the films. This is further followed with application of the edible coating in the shelf-life extension of button mushrooms.

III. METHODOLOGY

A. Materials required

High methoxyl pectin, Glycerol, Gallic acid monohydrate, Catechol, Guaiacol, and Hydrogen Peroxide were ordered from Himedia Pvt. Ltd., India. Materials like Candelilla wax of Tattvalogy, Muslin cloth, and polystyrene petri-plates of Omark Lifesciences Pvt. Ltd., were ordered via online platform. Fresh button mushrooms (*Agaricus bisporus*) were purchased from the local market of Matunga, Mumbai at the early morning and were later immediately used for the experimental study.

B. Preparation of Pectin-based Edible Coating

High methoxyl pectin (HMP) (2.1 g) was suspended in distilled water (DW) (100 mL) and allowed for dissolution at temperature of 90°C. To this pectin solution, 1.83% (w/v) of glycerol and melted Candelilla wax (0.7 g) is added and mixed using the magnetic stirrer. After complete dissolution, the solution is homogenized at 10,500 rpm for 5 mins. Further, homogenization of solution is followed with cooling to room temperature and allowing to degas. The solution (30 ml) was poured into 90 mm of Petri-plates and kept for drying in the oven at 45°C for overnight.

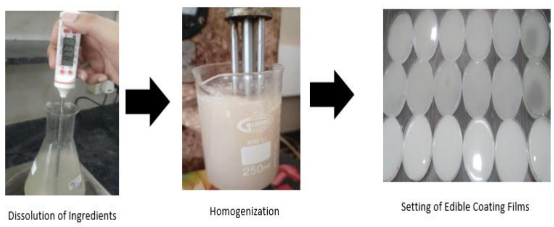

Figure1: Coating Preparation steps

C. Determination of Physical and Mechanical properties of the Films

Water Vapour Transmission Rate

The water vapour transmission rate (WVTR) of the films was measured according to the monograph of the European Pharmacopoeia (Sarwar, Niazi, Jahan, Ahmad, & Hussain, 2018). The scraped films were mounted on to the surface of beaker filled with distilled water, and sealed by parafilms. The beakers were then placed in an oven at 45°C for 24 hr. The WVTR of the films was calculated using the equation:

$WVTR = [(w_i - w_t)/ A \times 24] \times 10^6 \ g \ m^{-2}h^{-1}$

Where, A is the area of the beaker mount (m^2),
w_i and w_t are the weight of the beaker at time zero and the weight of the beaker after 24 h respectively.

Moisture Content

The moisture content of the films was measured by placing the films in oven at 105°C for 2 hrs until constant weight was recorded (AOAC, 1999). Three replications of each film treatment were used for calculating the moisture content. The water content was calculated as the percentage of the weight loss of the film during the drying process. The moisture content was further calculated using the following equation:

% Moisture Content= (w1-w2)/w1 x 100%

Where,
w1 = weight of sample before drying (g)
w2 = weight of sample after drying (g)

Colour Analysis

The colour analysis of the edible films was performed by using the Hunter lab Colorimeter. The major colour attributes i.e. L^*, a^* and b^* values were recorded for the films in triplicates.

Where,
L^*– darkness to whiteness
a^* - greenness to redness
b^* – blueness to yellowness

D. Physicochemical properties of Coated and Uncoated Mushrooms during storage

Weight loss

The loss in weight for both the uncoated (control) and the coated mushrooms was measured periodically on 0^{th}, 2^{nd}, 4^{th}, 6^{th}, 8^{th} and 10^{th} day along the storage period. The percentage weight loss was calculated using the following equation:

Weight loss = $[S_1 – S_2 / S_1]$ x 100
S_1 – Initial weight of sample
S_2 – Final weight of sample

Firmness Loss

The texture of both the coated and uncoated mushrooms is measured used TA. XT texture analyser. It was measured periodically on 0^{th}, 2^{nd}, 4^{th}, 6^{th}, 8^{th} and 10^{th} day along the storage period. Probe of 2mm was used for penetration within the mushrooms and two readings per sample were recorded on different surfaces. Texture profile analysis (TPA) for the uncoated and coated mushrooms was performed with the calibration parameters as return distance of 60 mm, return speed of 2mm/sec and contact force of 50g.

Colour Analysis

The mushroom samples were analyzed for their colour at periodic intervals along their storage period using the Hunter Lab Colorimeter. The colour measurements for each sample were carried out in triplicates by recording the L^*, a^* and b^* values. The values were taken periodically as for other parameters during the storage period.

Where,
L^*– darkness to whiteness
a^* - greenness to redness
b^* – blueness to yellowness

Polyphenol oxidase and Peroxidase enzyme assays

The enzyme extracts used for the assays of polyphenol oxidase (PPO, EC 1.10.3.1) and peroxidase activities (POD, EC 1.11.1.7) were obtained according to the method described by Zhao et al. (2011) with modifications. For both the Polyphenol oxidase assay and Peroxidase assay, the coated and the control mushrooms (1 gm) were extracted with 1 ml of Enzyme extract solution (EES) [25ml SSP buffer + 1g Polyvinylpolypyrrolidone (PVPP) + 250µl Triton X100 + 1.461g NaCl] by storing at 4^0C for 30 mins on shaker. The homogenate was centrifuged at 10,000×g for 30 mins at 4 °C, and the supernatant was further used as crude enzyme extract for the assessing the enzymatic activities. PPO and POD activities were determined using catechol and Guaiacol as the substrates respectively. The absorbance was measured at 420nm and 470nm for PPO and POD assays respectively. One unit of PPO was defined as the amount of enzyme that causes an increase of 0.1 absorbance per minute. Similarly, one unit of POD was defined as the amount of enzyme that causes a change of 0.1 absorbance per minute at 470 nm.

Total Phenolic Content Estimation

The total phenolic content of the extract was determined by the Folin–Ciocalteau method with slight modifications. During mixing of the reactants, 100 µl of extract was taken in 1.5 ml Eppendorf tube and was followed with the addition of 100 µl 80%

methanol, 100 µl of Fc reagent and 700 µl of 20% Na_2CO_3. The reaction-solution mixture is later allowed to incubate for 20 mins and was followed with centrifugation at 10,000 rpm for 3-5 mins. The absorbance is recorded at 735 nm using 1 ml cuvette. The values were taken periodically as for other parameters during the storage period.

IV. RESULTS AND DISCUSSION

The effect of different levels of coating materials (Pectin concentration, Candelilla wax concentration and glycerol concentration) along with storage days on quality parameters of coated button mushroom has been checked by One-way ANOVA. The edible films were analyzed for their mechanical properties and was followed with the analysis for assessing the effect of coating materials on edible coated button mushroom was carried out for initial 10 days at 4 °C.

Optimization of the Edible Coating Formulation

The concentration of ingredients present within the initially casted edible films was optimized based upon the values of water vapour transmission through the surface of the edible films, analysis of colour and the content of moisture present within the film. Specific ranges of each ingredient were chosen for different experimental sets and fed into the RSM software for further analysis and optimization. The 17 experimental runs in the design include 5 repetitions at the centre points and 12 factorial runs (±1 level). The average was taken into account for modelling, and each experiment was carried out in triplicate. A random order was used for the trials in order to prevent the block effect. The best solution sets were suggested by the software out of which, the edible film with lowest value of water vapour transmission rate, lowest value of moisture content and specified in range value for colour (L and b value) was chosen as the film with optimized concentration of ingredients and further analyzed for values.

Table 1. Established ranges for all Ingredients

Ranges → Ingredients ↓	Low (%w/v)	High (%w/v)
Pectin	1.5	2.5
Candelilla wax	0.4	0.8
Glycerol	1.0	2.0

Table 2. Input of recorded values into Box Behnken Design

Std.	Run	Type	Factor A: Pectin %	Factor B: Candelilla wax %	Factor C: Glycerol %	Response 1: WVTR g mm/m2 day	Response 2: L	Response 3: b	Response 4: Moisture %
1	17	IBFact	1.50	0.4	1.5	1.35	25.02	2.08	21.9
2	2	IBFact	2.50	0.4	1.5	1.32	24.11	1.85	45.16
3	8	IBFact	1.50	0.8	1.5	1.4	17.12	0.31	34.7
4	9	IBFact	2.50	0.8	1.5	1.5	21.135	1.44	24.3
5	1	IBFact	1.50	0.6	1.00	1.5	21.11	1.45	22.9
6	6	IBFact	2.50	0.6	1.00	1.43	22.1	1.957	21.5
7	7	IBFact	1.50	0.6	2.00	1.7	20.5	0.87	21
8	12	IBFact	2.50	0.6	2.00	1.13	31	2.11	28.1
9	10	IBFact	2.00	0.4	1.00	1.29	20.17	1.24	24.11
10	16	IBFact	2.00	0.8	1.00	1.32	15.38	0.75	37.17
11	3	IBFact	2.00	0.4	2.00	1.47	20.49	1.43	26.03
12	13	IBFact	2.00	0.8	2.00	1.2	18.34	1.32	28.5
13	15	Center	2.00	0.6	1.5	1.00	15.47	0.275	25.65
14	11	Center	2.00	0.6	1.5	1.5	19.9	2.63	24.1
15	4	Center	2.00	0.6	1.5	1.5	20.89	0.43	23.9
16	14	Center	2.00	0.6	1.5	0.93	25.1	1.55	18.12
17	5	Center	2.00	0.6	1.5	1.9	19.12	0.6	34.08

Table 3. Suggested Best Combinations from Box-Behnken Design

No.	Pectin	Candelilla	Glycerol	WVTR (g mm/m2 day)
1	2.1	0.77	1.83	1.07
2	1.96	0.73	1.99	1.15
3	1.63	0.71	1.78	1.20
4	1.81	0.64	1.68	1.31
5	1.54	0.48	1.74	1.92

For some top best suggested combinations, formulation sets were again prepared and analysis of the response parameter WVTR was performed as it primarily affected the moisture barrier properties of the coating. It was observed that the increase in concentration of Candelilla wax also affected the water vapour transmission values. The wax being hydrophobic in nature, increase in its concentration gradually enhanced the moisture barrier properties with decrease in WVTR. It's not just one that affects, instead all ingredients significantly create their combined effect upon the response parameters

In the suggested optimized sets, set 1 was suggested as the best set containing Pectin (2.1 gm), Candelilla (0.77 gm), Glycerol (1.83 gm) with WVTR of 1.07 g mm/m2 day. From the table we can understand, as the concentration of Candelilla wax reduces within the film, it negatively affects the values of water vapour permeability. As the hydrophobic agent (Candelilla) wax concentration is reduced, the film starts losing its moisture barrier properties and thus the water vapour can easily permeate through the film thereby increasing its WVTR values. But if the concentration of Candelilla wax within the film gradually increases, it results in lowering the values of WVTR.

Nextly, Glycerol acting as a plasticizer positively affects the values of WVTR. Plasticizers boost the amount of space between polymer chains, which increases chain mobility and improves film flexibility. But so also it increases available space in between the polymer chain units and thus water vapour transmission rate shows an increasing trend with respect to increase in concentration of Glycerol within the edible film formulation. So an increase in the concentration of pectin also affects the film thereby improving its mechanical properties.

Application of Optimized Edible Coating on Button Mushrooms

The optimized edible coating formulation was applied upon the mushrooms for performing shelf life studies. The application was done using the dipping method, mushrooms were dipped within the coating formulation and left for 3 mins and later removed. The mushrooms were stored at 4 °C and the parameters which were analyzed periodically on 0^{th}, 2^{nd}, 4^{th}, 6^{th},8^{th} and 10^{th} day included weight loss of mushrooms, Texture analysis, Color analysis, Total phenolic content, PPO and POD Assays. The Control (CON) uncoated mushrooms and Coated (COA) both were compared based upon the selected parameters in order to analyze the efficiency of the edible coating for enhancing shelf life.

Figure 2: Dipping of Mushrooms in Coating Solution

Figure 3: Control and Coated Mushrooms

Analysis of Physicochemical parameters of mushrooms

This section involves analysis of parameters including estimation of weight loss, firmness of the mushrooms, browning index of mushrooms during storage, total phenolic content, polyphenol oxidase and peroxidase assay.

Estimation of weight loss in mushrooms

Weight loss in the fresh fruits and vegetables forms one of the major parameters of concern post their harvest period. This happens primarily due to the loss of moisture from the surface of the fresh produce.

The trend of variation in the weight loss for the control as well as coated mushrooms was monitored along the storage period of 10 days. The weight loss percentage in coated mushrooms 29.19% was significantly less ($p<0.05$) in comparison to that of the control ones with total weight loss of 58.29%. The edible coating over the surface of coated mushrooms restricts direct contact of the surface with the atmosphere and thus prevents the heavy loss of moisture through transpiration that happens in the control samples (uncoated) at elevated levels with respect to that of the coated samples when calculated for weight loss percentage along the span of 10 days.

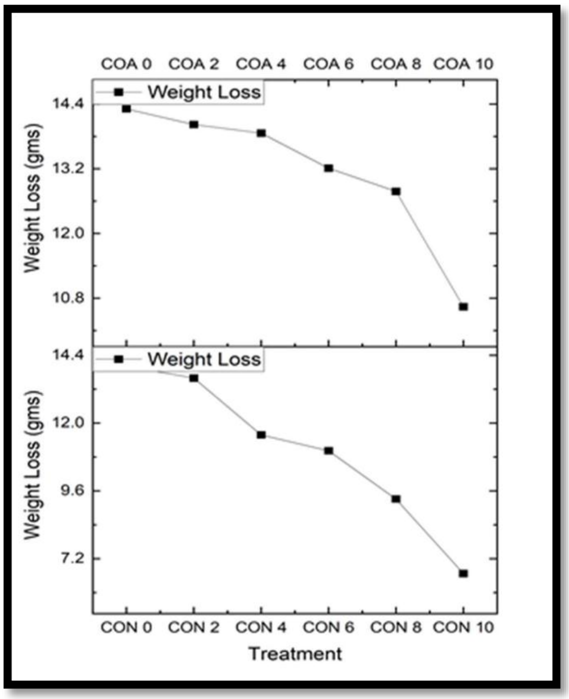

Figure 4: Estimation of weight loss over storage period

Estimation of Firmness Loss in Mushrooms

The amount of force needed by the probe to pierce the sample's surface is referred to as firmness. Both the control and coated mushroom samples nearly displayed similar range for firmness on the first day. Then, from the second to the tenth day, both the control and coated mushroom samples showed a decline in the turgidity of the mushrooms, with a percentage of firmness loss of 36.21% for the control samples and 10.51% for the coated samples. But because the coated mushrooms had edible covering on their surface, they were able to retain more moisture than those in the control group, which kept their turgidity and firmness. And loss was significantly less in coated samples ($p<0.05$) in comparison to that of the control ones.

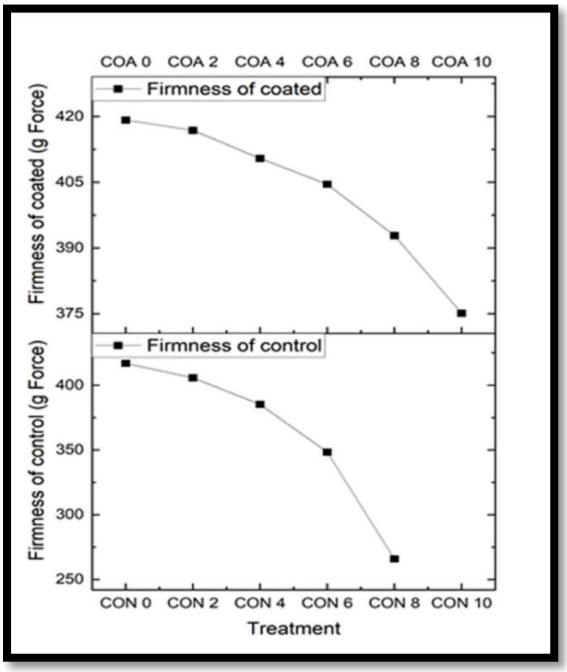

Figure 5: Estimation of firmness loss over storage period

Effect of Edible Coating on Browning index in Mushrooms

Browning index refers to the degree of discoloration of the product during the storage period. During the storage period, the browning index showed an increase in both the control as well as the coated mushrooms due to the degradative activities of the enzymes polyphenol oxidase and peroxidase. During the 0th to 10th day of storage, the activity of polyphenol oxidase and peroxidase enzymes showed an increase due to the oxidation of polyphenolic compounds into Quinone's.

When monitored upon 2nd, 4th and 6th day of storage, the PPO and POD activities showed an increasing trend with BI of 5.53, 7.09 and 9.98 for the control samples and 4.84, 5.04, 5.95 for the coated sample. Thus, from the 8th day the activity of enzymes showed a decreasing trend due to less availability of enzymes and so also the polyphenolic substrates for further oxidation process. Thus the rate of browning was significantly higher in the control ones ($p<0.05$) when compared with that of the coated mushrooms.

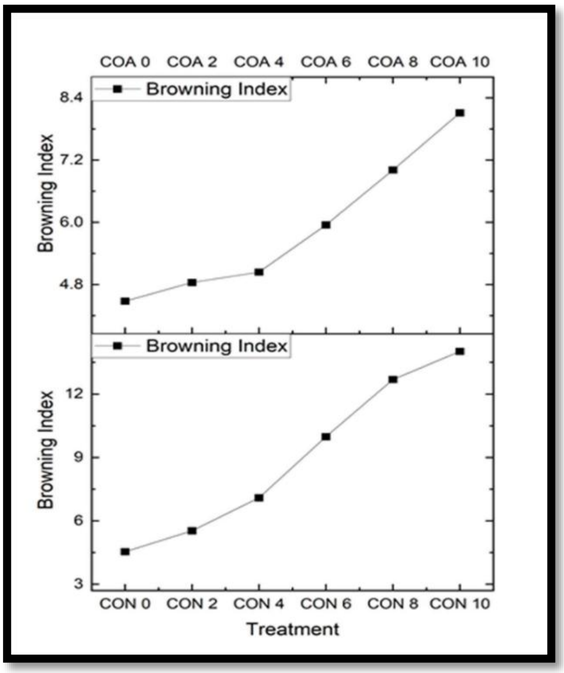

Figure 6: Browning Index over storage period

Estimation of Total Phenolic Contents during storage period

Using Gallic acid as the reference, the total phenolic contents of several extracts of both the control and the coated mushroom was assayed using the Folin-Ciocalteau (F-C) method. The calibration curve was built using the absorbance values that were obtained at various Gallic acid concentrations. The calibration curve's regression equation ($Y= 0.007X+0.186$; $R2 = 0.992$) was used to compute the total phenolic content of the samples, which was then represented as mg of Gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g) Both the control as well as the coated samples showed high content of polyphenols at the beginning. The decline in concentration was seen faster in case of the non-coated mushrooms than with respect to the coated ones. ($p<0.05$) due to the volatile nature of the polyphenols as they can escape faster from the surface of uncoated ones. On the other hand, the coated ones possessing a layer of edible coating upon their surface, restrict the easy escape of polyphenols. The percentage reduction in control and the coated mushrooms at the end of the storage period was 78.57% and 38.93% respectively. The results were further analyzed using one way-ANOVA with ($p<0.05$). The results suggested that coating effectively worked upon the mushrooms by acting as a sacrificial moisture agent and helped in retaining the phenols within.

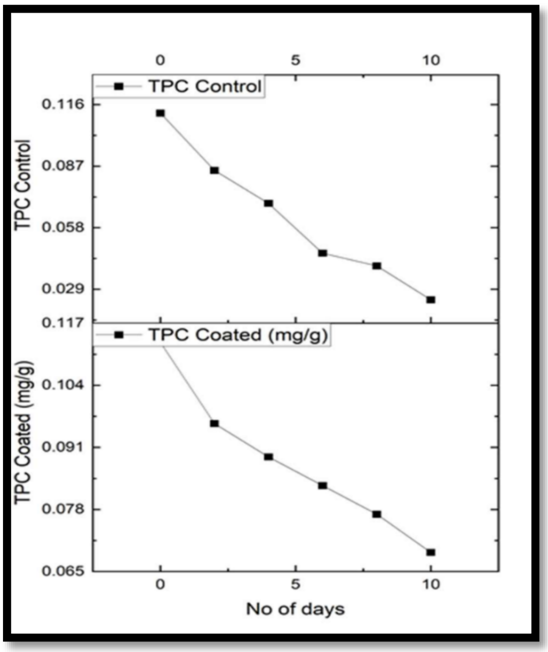

Figure 7: Total phenolic content estimation during storage

Effect of Edible Coating on activities of PPO and POD during storage

PPO and POD are two major enzymes responsible for catalysing the oxidation of polyphenols into chains of Quinones. The intensity of oxidation of phenols into Quinones can be analyzed by measuring the absorbance of the samples at 420 nm and 470 nm for PPO and POD assay respectively. In case of the coated samples for PPO assay, it gets clear from the graph that activity of degradative enzymes initially was higher due to greater availability of the substrate. Upon application of edible coating, the coated mushrooms show decreased activity of these degradative enzymes due to restriction of direct contact of the mushroom surface with air thus reducing the oxidation of the substrates. In Case of the control ones, due to the direct exposure of mushroom surface to the atmosphere, activity of PPO and POD significantly increased along the storage period in comparison to that of the coated ones.

The percentage increase in POD activity was 52.41% and 17.94% in the control and coated ones respectively at the end of the storage period. And similarly the percentage increase in PPO activity was 42.99% and 21.58% for control and coated mushrooms respectively at the end of storage period. Thus, Coated ones showed less degradative enzyme activity with respect to that of the control ones ($p<0.05$).

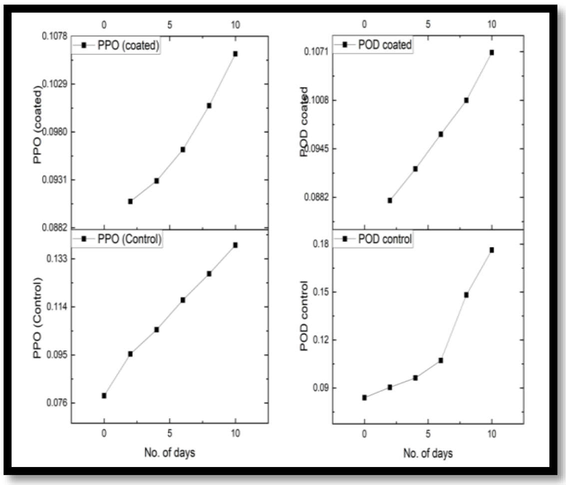

Figure 8: PPO and POD assay values during storage

V. CONCLUSION

In the present study, the pectin-based edible coating was initially casted into films to analyze its water vapour transmission rate, content of moisture and the colour of the films. The different concentration of ingredients within the optimized coating formulation individually cast their effect upon the moisture barrier properties of the coating. Incorporation of hydrophobic Candelilla wax in the coating formulation enhances hydrophobicity of the coating. Therefore, it was essential to incorporate Candelilla wax into the coating and use it for further shelf-life application studies. The optimized edible coating film containing pectin 2.1 (%w/v), Candelilla 0.77 (%w/v) and glycerol 1.83 (%w/v) possessed WVTR of 1.07 g mm/m2 day. The coating significantly showed promising results with less weight loss, firmness loss, browning Index in coated ones with respect to that of the control samples. The degradative activity of PPO and POD showed increased rate in the control mushrooms than the control. So also, content of total polyphenols was higher in the coated ones than the control at the end of the storage period. Thus the edible coating helped in retaining the mechanical as well as physicochemical properties of mushrooms along the storage thereby extending their shelf life.

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