

CHEMICAL INVESTIGATION OF PROPOLIS AND ITS UTILISATION IN EDIBLE COATING ONTO *CUCUMIS SATIVUS* VAR. MALINI (CUCUMBER)

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Article Received on 29/07/2017

Article Revised on 19/08/2017

Article Accepted on 09/09/2017

ABSTRACT

Propolis is a popular bee product because of its antimicrobial and antioxidant activities. Its chemical composition depends on the species of bee that collects it, the environment in which the bee thrives and plants on which it forages. Until now, not much research has been done to discover the chemical composition of Indian propolis and its application as edible coatings, hence, two propolis samples were procured from Punjab and Delhi respectively for the study. ¹H NMR and GC-MS analysis revealed the chemical heterogeneity of both the samples and the results of ICP-MS analysis points towards the high amount of heavy metal content in both the samples. Ethanolic Extract of Propolis (EEP) was used along with chitosan and acacia gum to formulate edible coating solutions which were coated on cucumbers. Trehalose was incorporated as an active ingredient as it contributes to the freshness of cucumbers for a longer period of time. Cucumbers were assessed for quality parameters like weight loss percentage, decay percentage, juicability, Total Soluble Solid (TSS) content, pH and Titratable Acidity (TA). Edible coating increased the shelf life of cucumbers by 7 days.

KEYWORDS: Propolis, Trehalose, Edible Coating, Environmental Pollution, Environment-Friendly.

INTRODUCTION

Propolis or "bee glue" is a resinous substance collected by bees from flowers and leaf buds and carried on their hind legs.^[1] A word of Greek origin, propolis means "in front of the city" ("*pro*": in front & "*polis*": city)^[2] which is apt to describe the protective role it plays in the beehive. Propolis is utilised by the bees to seal small cracks in their hive and maintain a constant temperature and moisture in it.^[1] It also has antiseptic properties, thereby, protecting the larvae, honey and comb from microbial infection.^[3] The chemical composition of propolis depends on the species of honeybee, the geographical and botanical origin of honeybees' collection and its environment.^[1] Commonly found compounds in propolis includes flavonoids, terpenoids, phenolic esters and hydrocarbons. While terpenoids are generally responsible for its characteristic odour, both flavanoids and terpenoids contribute to its pharmacological properties.^[4] Most phenolic compounds serve as an antioxidant. Due to these properties, propolis has been incorporated as an active ingredient in cosmetic products, medicinal products, especially for the treatment of skin burns and diseases of the teeth and dietary supplements.^[1,3]

Since not much research has been done to elucidate the chemical composition of Indian propolis, propolis samples were taken from two states of Northern India and their chemical composition was analysed. Moreover, the efficacy and durability of edible coating containing propolis along with chitosan and acacia gum were also analysed.

Chitosan is the de-acetylated form of chitin composed of D-glucosamine and N-acetyl D-glucosamine with β -1,4 linkage. It is obtained from the exoskeletons of crustaceans like shellfish or cell wall constituents of fungi and green algae.^[5] Chitosan based edible coatings are considered to be one of the best preservative coating as it offers several functional advantages like firmness retention, slower respiration rate and even controls the proliferation of microbes.^[5]

Gum arabic or acacia gum is an edible polymer derived from the exudates of mature trees of *Acacia senegal* and *Acacia seyal*.^[6] The exudate is emanated under stress condition from the stems and branches of the trees. It is a branched-chain polysaccharide that exists as a calcium, magnesium or potassium salt of arabic acid. The backbone and side chain of gum acacia is composed of 1,

3-linked b-D-galactopyranosyl units, joined to the main chain by 1, 6-linkages.^[7] It has been widely used as a stabilizer, thickening agent and an emulsifier in the food industry.^[6]

Trehalose, a non-reducing disaccharide formed by α, α -1,1 linkage of two D-glucose molecules^[9] was also incorporated as an ingredient of the edible coating solution due to three reasons. First, it has exclusive water-retention properties that even help desert plants to survive complete desiccation.^[9] Second, it shows masking effect, i.e. it suppresses the bitter taste of a food item.^[10] Third, its glycosidic bond is more flexible as compared to other disaccharides and so it fits more closely on an irregular surface, making it a useful component for forming films.^[11]

MATERIALS AND METHODS

Samples of raw propolis were collected from two companies based in Patiala (Punjab) and New Delhi respectively. Chitosan and acacia gum were obtained from HiMedia Laboratories, Mumbai and trehalose was procured from Bakersville India Pvt. Ltd.

Cucumis sativus Var. Malini (cucumber) was used to study the effect of edible coatings and was procured from Safal Fruits and Vegetables (Mother Dairy) store in Dwarka, Delhi. The samples were used for the application of edible coating within 48 h of their harvest. The samples were thoroughly washed under tap water, followed by dipping in 0.1% sodium hypochlorite solution for 2 min and subsequent rinsing with distilled water.^[12] The samples were allowed to dry at room temperature before being used for coating. After washing and drying steps, the cucumbers were divided into four groups containing equal number of cucumbers. Three different formulations of edible coating were applied on three of these groups and one of the groups was left uncoated to serve as a control.

2.1 Preparation of Ethanolic Extract of Propolis and Aqueous Extract of Propolis

Ethanolic Extract of Propolis (EEP) was prepared by mixing 10g of raw propolis in 100 ml ethanol (99.99%) by continuous shaking for 7 days at 20°C using incubator shaker (Orbitek LT Orbital Laboratory Incubator Shaker) in dark conditions in airtight containers. The mixture was then filtered through a Whatman No. 1 filter paper to discard any wax that was insoluble in ethanol or any material insoluble in water.^[1,13,14]

2.2 Assessment of propolis

2.2.1 ¹H NMR: Liquid state ¹H Nuclear magnetic resonance was done for proton characterisation of propolis sample. 2mL of EEP was taken in a glass tube and left to dry overnight in an incubator at 40°C. The dried sample was dissolved in 600 μ l deuterated chloroform (CDCl₃). The ¹H-NMR spectra was recorded on a 400 MHz instrument (JEOL, ECS-400 NMR).

2.2.2 Gas Chromatography-Mass Spectrometry (GC-MS):

EEP samples were left to dry overnight at 37°C and then dissolved in methanol. The samples were then loaded in GC-MS (Shimadzu GCMS-QP2010 Plus) for obtaining the results. The samples were injected in split mode at 300°C. Oven was programmed from 40°C (1 min) to 300°C (28 min). The column flow rate was 1.20 ml/min with Helium being the carrier gas. Metal quadrupole mass filter with pre-rod with a range of m/z 1.5–1000 was used in scan mode. Electron Ionisation (EI) was used as the ionization mode. The compounds were identified by means of their retention time by comparison of their mass spectra with National Institute of Standard and Technology (NIST) library data and literature.

2.2.3 Inductively Coupled Plasma-Mass Spectrometry (ICP-MS):

The samples were milled and homogenized and were submitted to digestion by microwave. The determination of the metals in the samples was determined by ICP-MS (Perkin Elmer SciexElan II DRC). The mass analyser was scanned by “peak-hopping” method.

2.3 Preparation of edible coating:

All solutions were prepared by dissolving the gum powder or EEP in distilled water with the help of hot plate magnetic stirrer (2MLH, Remi Equipments) for half hour. The solution containing chitosan was kept for continuous shaking overnight at room temperature in an incubator shaker (Kuhner LT-W Lab Therm).

Table 1: Different edible coating formulations.

Chitosan: EEP (Coating Formulation 1)	
Distilled water	100 ml
Chitosan	1 g
Ethanolic Extract of Propolis	3 ml
Glacial acetic acid	250 μ l
Glycerol	350 μ l
Trehalose	0.4 g
Acacia gum: EEP (Coating Formulation 2)	
Distilled water	100 ml
Acacia gum	3 g
Ethanolic Extract of Propolis	3 ml
Glycerol	350 μ l
Trehalose	0.4 g
EEP (Coating Formulation 3)	
Ethanolic Extract of Propolis	50 ml

2.4 Film Preparation: 10ml of each coating solution were poured into polystyrene Petri-dishes (9 cm diameter) such that a thin layer of solution is formed at the base of Petri-dish. These Petri-dishes were then incubated at 40°C for 24 h for films to dry and yield a self standing film of uniform thickness.^[15]

2.5 Film Analysis

The surface morphology of the films was studied by Scanning Electron Microscope (Zeiss EVO 18). FT-IR

spectra were recorded by FT-IR spectrometer (Shimadzu).^[16,17]

2.6 Application of edible coating: The coating solutions were applied on the cucumbers by using sterile cotton dipped in the solution. The coated cucumbers were allowed to dry for at least 15 to 20 min at room temperature.

2.7 Storage of cucumbers

The treated cucumbers were placed in an incubator shaker (Orbitek LT Orbital Laboratory incubator shaker) at 20°C along with the uncoated or a control group of vegetables till the duration of the experiment i.e. 21 days.

2.8 Assessment of cucumbers: The cucumbers were assessed initially (Day 0) and after every 3 days up to the completion of the experiment for noting the following observations: Change in rind/peel colour, weight loss, decay percentage, juice content, Total Soluble Solids (TSS), Titratable Acidity (TA), pH and shelf life.^[12]

2.8.1 Change in Visual appearance

The changes in visual appearance, such as freshness, peel colour etc. were examined for each vegetable.

2.8.2 Weight Loss

Weight of each cucumber was taken initially and after every three days and the weight loss percentage was calculated as follows^[12]

$$\text{Weight loss \%} = [(W_i - W_f) / W_i] \times 100$$

Where, W_i is the initial weight and W_f is the weight at sampling period.

2.8.3 Decay percentage

Any cucumber that showed fungal or microbial contamination or appeared shrunken with many wrinkles was considered to be decayed. These decayed cucumbers were eliminated from further studies. Decay percentage was calculated as follows

$$\text{Decay \%} = [\text{No. of damaged vegetables at sampling period} / \text{Total no. of vegetables}] \times 100$$

2.8.4 Juice content

One vegetable from each group was weighed on a digital weighing balance (GC-1603 P, Sartorius), chopped into small pieces and then ground in a mixer-grinder. The juice was then filtered using a muslin cloth and its amount was measured using a measuring cylinder. Juicability was calculated as follows: Juicability = Juice content (in ml) / weight of cucumber (in g).

2.8.5 Total Soluble Solids (TSS)

The sugars and acids along with small amounts of vitamins, minerals and amino acids constitute the TSS of a fruit or vegetable. This value is expressed as % Brix (AOAC, 2000)^[25] and was determined by using a portable refractometer (ERMA). Juice extracted from one vegetable of each group was used for this analysis.

2.8.6 pH and titratable acidity (TA)

The pH of the juice was measured by using a digital pH meter (HICON) and trend in the change was analysed (AOAC, 2000).^[25] The titratable acidity was calculated as the volume of Sodium Hydroxide (0.1 Mol/L) required for titrating 1mL of the diluted juice which was homogenized to 10mL of distilled water for titration. The results are expressed as percentage of citric acid since citric acid is the predominant organic acid in cucumber. Its milli-equivalent factor is 0.064.^[12] TA was calculated using the following formula (AOAC, 2000)^[25]

$$\% \text{ Citric Acid} = \frac{\text{Vol. of NaOH used (in ml)} \times 0.1 (\text{Molarity of NaOH}) \times 0.064}{\text{Weight of sample (in g)}} \times 100$$

2.8.7 Statistical Analysis

Experiments were measured in triplicate and the results are reported as Mean±SD. Student's T-test was used to analyse the statistical significance. The differences between experimental groups are considered significant when the p-value < 0.05.

RESULT AND DISCUSSION

3.1 ¹H-NMR (400MHz, CDCl₃) analysis.

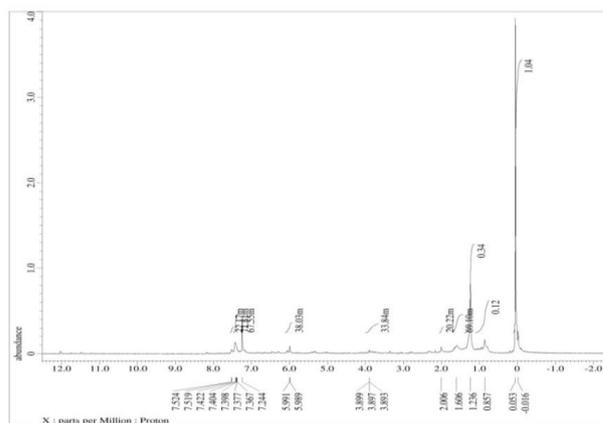


Table 3: Compounds obtained in GC-MS spectrum of propolis (from Delhi) as identified by NIST Library Search.

S. No	Name	R.Time
1.	2,4-Di-tert-butylphenol	17.28
2.	Dodecanoic acid	18.035
3.	Propenoic acid, 3-(cycloheptatrien-7-yl)-, methyl ester	23.25
4.	1-Phenanthrenemethanol, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-	24.427
5.	Phenanthrene, 1,2,3,4,4a,9,10,10a.alpha.-octahydro-1,1,4a.beta.-trimethyl	24.65
6.	4H-1,2,4-Triazole-3-thiol, 4-(2,3-dihydro-1H-inden-1-yl)-5-(1-methylethyl)	24.712
7.	Bicyclo[4.3.0]nonan-2-one, 8-benzylidene	24.901
8.	Linoleic acid ethyl ester	25.903
9.	Ethyl oleate	25.976
10.	Benzene, 2-ethenyl-1,4-dimethyl-	26.33
11.	2-Methylaminomethyl-5-nitrobenzophenone	26.39
12.	Naphtho[2,3-b]furan-4,9-dione, 2-isopropyl-	26.612
13.	Benzene, 1,1',1''-(ethoxymethylidyne)tris-	26.895
14.	3-(2,5-Dimethoxyphenyl)-2-hydroxy-2,4,6-cycloheptatrien-1-one	27.373
15.	Tetracosane	27.443
16.	Fumaric acid, 3-phenylpropyl dodec-2-en-1-yl	28.013
17.	Ethanone, 1-(5-ethyl-2-hydroxy-4-methoxyphenyl)-2-(3,4-methylenedioxyphenyl)-	28.57
18.	Cinnamylcinnamate	28.792
19.	7-Chloro-3-m-tolylimino-1,3-dihydroindol-2-one	29.365
20.	4H-1-Benzopyran-4-one, 2,3-dihydro-5,7-dihydroxy-2-phenyl-	29.47
21.	Naringenin	30.135
22.	Medicarpin	30.338
23.	Chrysin	31.371
24.	4H-1-Benzopyran-4-one, 2,3-dihydro-5-hydroxy-7-methoxy-2-phenyl-	31.595
25.	1(2H)-Naphthalenone, 3,4-dihydro-2-(1-naphthalenylmethylene)-	31.684
26.	Galangin	31.848
27.	Benzoic acid, 4-propyl-, 4-cyanophenyl ester	32.425
28.	(Z)-3-(Heptadec-10-en-1-yl)phenol	33.283
29.	Benzofuran-3-one	33.752
30.	1-Hexacosanol	34.727
31.	Stigmast-5-en-3-ol, oleate	35.074
32.	gamma.-Sitosterol	37.797
33.	beta.-Amyrone	38.05
34.	beta.-Amyrin	38.523
35.	Lup-20(29)-en-3-one	38.779
36.	24-Norursa-3,12-diene	39.276
37.	Lup-20(29)-en-3-ol, acetate	40.788

GC-MS analysis of propolis sample from Delhi showed the presence of flavanoids, phenolics, terpenoids and fatty acids (Table 2). The propolis extract from Punjab showed the presence of polycyclic hydrocarbon derivatives in addition to flavanoids, phenolics, terpenoids, steroids and fatty acids (Table 3). Polycyclic hydrocarbons are generally found near the sites of coal mining or coal burning. It is quite possible that polycyclic hydrocarbons were present on the flowers as fly ash deposits and thus incorporated in propolis. The presence of such compounds may indicate environmental pollution.

It can be deduced that phenolic esters and flavanoids like galangin, naringin, pinocembrin, chrysin are responsible for its antimicrobial and anti-oxidant properties [4].

Presence of 1-Hexacosanol, a waxy substance, may also contribute in enhancing the protective nature of propolis.

3.3 Inductively Coupled Plasma-Mass Spectrometric analysis.

Table 4: Metal content in propolis samples

Metal	Propolis: Punjab Concentration (in ppm)	Propolis: Delhi Concentration (in ppm)
Chromium (Cr)	Not detected	92.65
Cobalt (Co)	0.54	87.49
Nickel (Ni)	11.15	95.14
Arsenic (As)	2.36	88.40
Cadmium (Cd)	0.36	93.54
Antimony (Sb)	23.69	118.46
Lead (Pb)	2.93	89.87
Mercury (Hg)	1.64	99.14

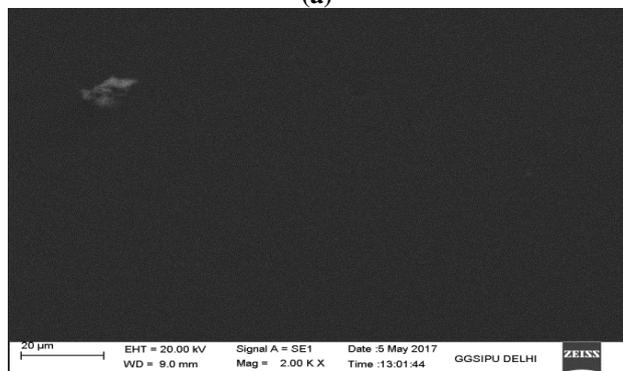
Propolis sample from Delhi contained high levels of heavy metal content, whereas the sample from Punjab was relatively less contaminated (Table 4). It can be inferred that large concentrations of heavy metals were found to be in bee products from hives located in areas of high industrial activity. Presence of industries, vehicular emissions, combustion of poor quality coal and irrigation of the soil with water containing industrial effluent are some of the factors that pollute the soil and the environment which then pollutes the plant products^[20] and subsequently the bee products. Bee pollen and propolis are far less transformed by bees than wax and honey.^[21] Thus, propolis more precisely reflects the environmental contamination.^[22] It is also possible that propolis readily adsorbs heavy metal on its surface.

3.4 Film analysis

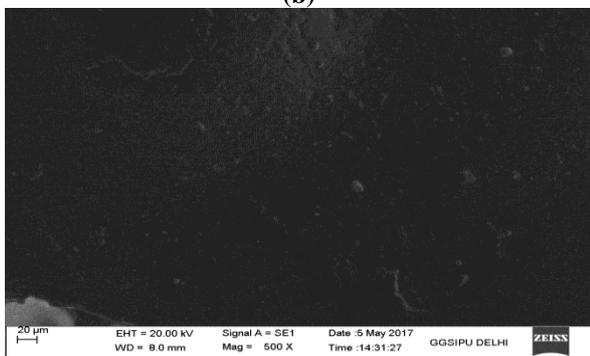
3.4.1 Surface Morphology



(a)



(b)



(c)

Fig. 3: SEM images of edible coatings (a) Acacia gum: EEP (b) Chitosan: EEP (c) EEP.

All films were non porous and homogenous. The surface structures of all films were smooth and even (Fig. 3 a, b and c). These observations suggest that the edible coating material forms a uniform layer around the fruit or vegetable. By forming this protective layer, the edible coating may work as a physical barrier to keep away microbes, dust or any other particles.

3.4.2 FT-IR analysis

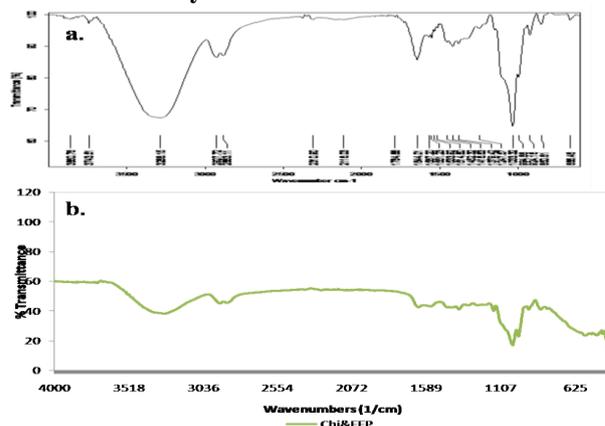


Fig. 4: FT-IR spectrum (a) Chitosan film (b) Film composed of chitosan and EEP.

Fig. 4a represents the spectrum of chitosan. The broad vibrational band at 3288 cm^{-1} is due to the OH bond stretching. The peak at 1644 cm^{-1} is due to carbonyl group. The peaks at $1514\text{--}1567\text{ cm}^{-1}$ were the NH bending (amide II) along with a small band near 1567 cm^{-1} could be due to carbonyl (amide I) stretching of chitosan. Propolis addition did not have a significant effect on the chitosan spectrum (Fig. 4b). Thus, it can be said the new film formed retained the qualities of chitosan.

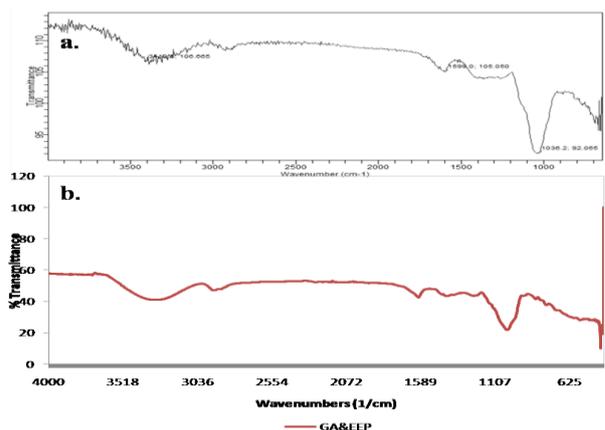


Fig. 5: FT-IR spectrum (a) acacia gum film (b) Film composed of acacia gum and EEP.

Peak at 3400 cm^{-1} is indicative of stretched hydroxyl group, whereas the peak at 1036 cm^{-1} shows bending vibration of hydroxyl group. Peak at 2900 cm^{-1} shows asymmetric stretching of CH groups. The peaks at 1589 cm^{-1} were due to NH bending (amide II) (Fig. 5a). Addition of propolis did not alter the spectrum of Gum

acacia significantly. Some additional peaks may be attributed to the compounds in propolis (Fig. 5b). Thus, it can be said that the new film formed retained the qualities of gum acacia.

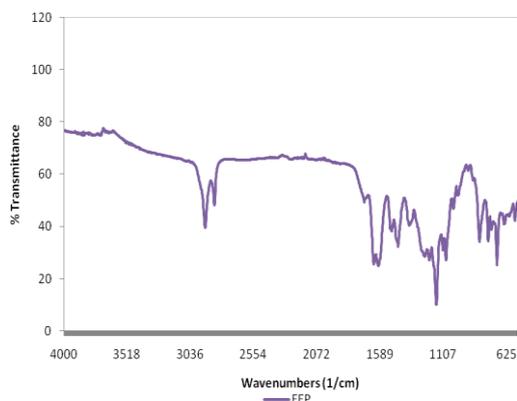


Fig. 6: FT-IR spectrum of Ethanolic Extract of Propolis.

Fig. 6 shows the spectrum of EEP. Peak at 2900 cm^{-1} suggests the presence of aromatic rings. The peak at 1600 cm^{-1} was due to carbonyl group. The peaks at 1589 cm^{-1} were the NH bending (amide II) and a small band near 1540 cm^{-1} could be due to carbonyl (amide I) stretching of chitosan. The peak at 1107 cm^{-1} indicates the presence of stretched hydroxyl group. The peak at 625 cm^{-1} may be due to stretched methyl groups.

3.5 Effect of Edible coating on cucumbers

Initially, all cucumbers were fresh, uniformly sized and light green in colour. Their skin was smooth and they appeared to be juicy. With time, wrinkles began to appear on the cucumber and corners became softer and shrunken. Within 6 days of incubation, wrinkles first appeared on the centre and the edges of the control group of cucumbers, followed by group of cucumbers coated with acacia gum:EEP (Day 9), then chitosan:EEP (Day 12), and EEP (Day 15). The cucumbers coated with EEP had a characteristic shine on the rind. This could be because EEP is a brownish liquid and masked the true colour of cucumbers lending it a shiny appearance. The cucumbers coated with chitosan were also shiny. Both these groups would appeal to the customers and were indicators of good quality of coating material. The addition of trehalose might also be responsible for maintaining this freshness on the outside. The development of wrinkles was delayed on coated vegetables as compared to uncoated ones. On touching, the cucumbers coated with chitosan:EEP and EEP were firm for a longer period of time. The peel colour showed a gradual decrease from green colour to yellow. By day 9, fine yellow spots began to appear on the peel, indicating the loss of chlorophyll (green colour) from the peel in all the groups. By day 15, the fruit appeared to be yellow, except for some green areas and by day 23, the cucumbers had lost their marketability. The coated vegetables showed a slower increase in the loss of colour.

3.5.1 Physiological Weight Loss.

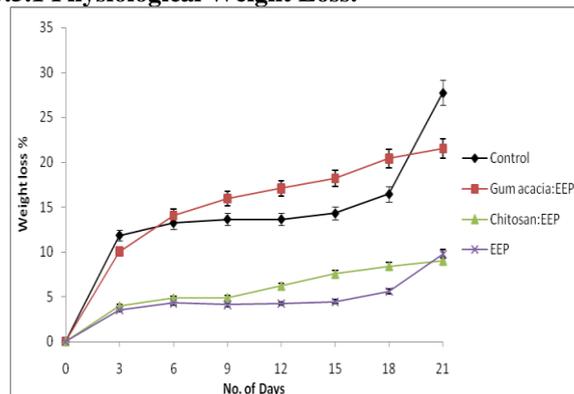


Fig. 7: Weight loss in uncoated and coated cucumbers over the duration of the experiment.

Weight loss in fresh fruits is an indicator of respiration rate and moisture evaporation from the fruit which depends on the postharvest treatment as well as storage temperature.^[5] Weight loss increased throughout the storage period in all groups.

There was a steady weight loss in cucumbers belonging to the Control group. Over the duration of the study, the control group lost around 27% of their initial weight (Fig. 7). Acacia gum:EEP lost around 21% of its initial weight. Cucumbers coated with chitosan:EEP and EEP showed minimum weight loss, 8% and 9% respectively. It can be inferred that all the coating materials provide an excellent moisture barrier and slow down the loss of water. Chitosan:EEP and EEP as a coating material are very effective. These results are consistent with those of previous studies demonstrating the effectiveness of propolis as an edible coating material.^[23,13]

3.5.2 Decay percentage

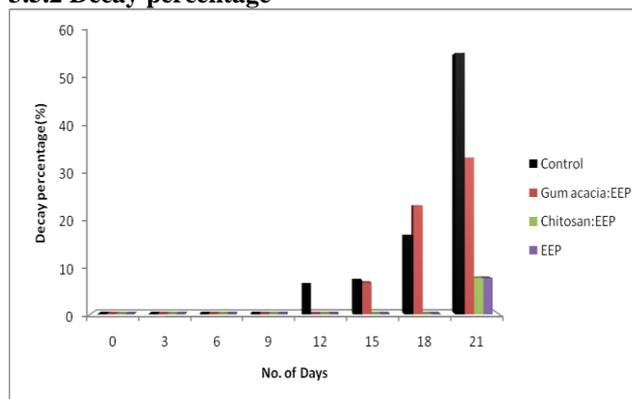


Fig. 8: Increase in decayed cucumbers (coated and uncoated) over the duration of the experiment.

The results of Decay percentage showed that propolis can be a very promising component of edible coating. Only 7% decay was seen in cucumbers coated with EEP and chitosan as compared to 60% decay of uncoated ones by the end of storage period (Fig. 5). It can be inferred that propolis plays a significant role in preventing

moisture loss and also significantly keeps microbial infection at a distance.

3.5.3 Juicability.

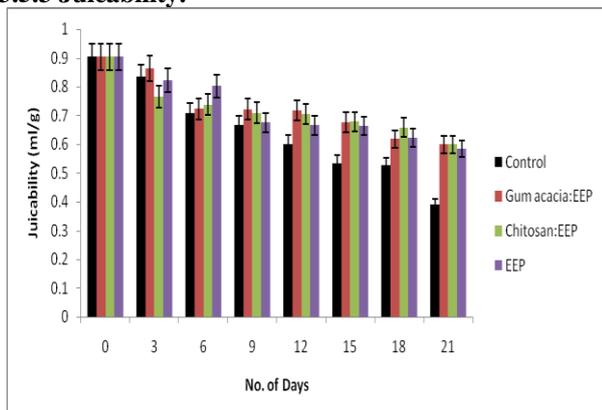


Fig. 9: Change in juice content of cucumbers with increase in the storage time.

It was observed that the juice content of the cucumbers decreased with the increase in storage time in both coated and uncoated cucumbers. This is because water is lost through the fruit in transpiration process. Since, cucumber is rich in vital vitamins and minerals; loss of water deteriorates its overall quality. The decline in juicability in uncoated group was maximum (around 50%) whereas a decline of about 35% was reported in coated fruits (Fig. 9). This difference can be attributed to the fact that the edible coating material forms a semi-permeable barrier around the fruit wall and prevents moisture loss, thereby, helping in retaining the overall quality of the fruit.^[26] Out of all the coated ones, those coated with chitosan:EEP showed the least decrease in juicability.

3.5.4 Total Soluble Solids (TSS)

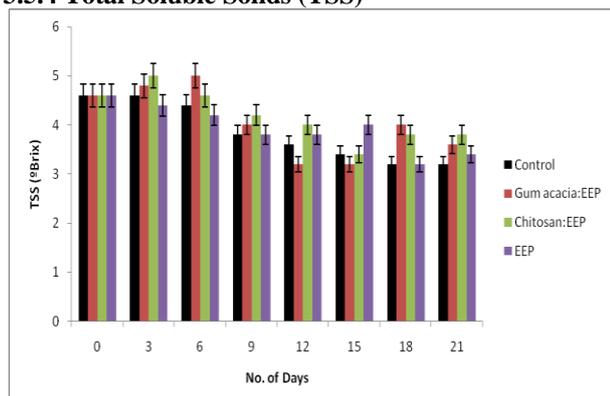


Fig. 10: Change in TSS with increase in the storage time.

The changes in TSS were not very significant. TSS value increased with increase in storage period in the fruits under study. This increase could be attributed to hydrolysis of starch into sugars during ripening.^[12] After 6 to 9 days, the TSS started decreasing (Fig. 10). In 2002, Kluge *et al.* suggested that the decrease in TSS may be due to oxidative decomposition of complex

substances such as polysaccharides, sugars, organic acids, proteins, lipids present in fruits into simple molecules and energy.^[12] The results are in tandem with previous researches carried out by Saha *et al.*, 2016. It can therefore be inferred that the edible coating may lower the metabolism rate by lowering the respiration rate of the fruit which ultimately leads to a greater retention of soluble solid content of the coated fruit.

3.4.5 pH and titratable acidity (TA)

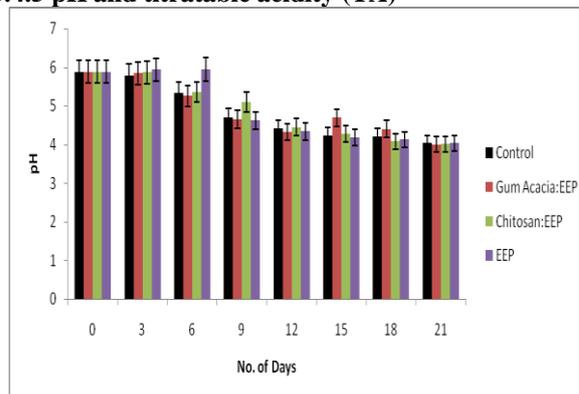


Fig. 11: Change in pH with increase in the storage time.

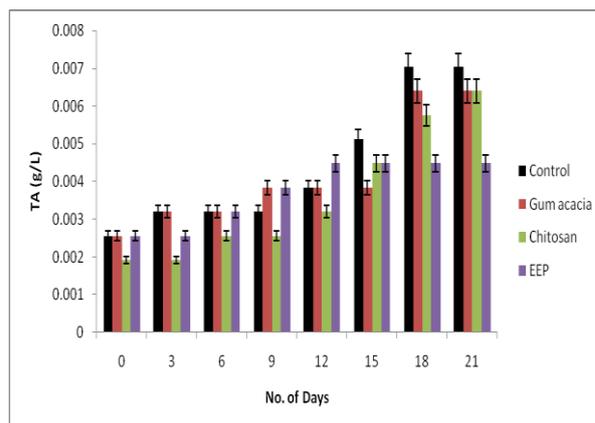


Fig. 12: Change in titratable acidity (TA) with increase in the storage time.

The developed edible coatings did not have any significant effect on the pH (Fig. 11). With an increase in the storage period, the pH of the cucumbers decreased in both the coated and uncoated fruits. This decrease in pH can be attributed to the utilization of accumulated citric acid in the cucumber. The values of titratable acidity of cucumber samples showed inverse behaviour to that of pH. Titratable acidity increases non-significantly, with storage time in both control and coated cucumbers (Fig. 12). However, the control group showed a greater variation in the titratable acidity. The coating samples definitely slowed down the changes in TA of cucumber by effectively delaying fruit ripening. Since acidity loss has been associated with quality loss during post harvest storage of fruits and vegetables, the retention of acidity contributed towards increasing the shelf life of cucumber.^[12]

CONCLUSION

Phytochemical profiling for the samples of propolis obtained from Patiala (Punjab) and Delhi was done for the first time. Also, the use of Etanolic Extract of Propolis (EEP) along with trehalose was a novel approach used for extending the shelf life of fresh vegetables. Our study concluded that the cucumbers coated with EEP and the ones coated with EEP and chitosan could be stored for 7 more days when compared to the uncoated ones. This approach could be extended for coating other fruits as well. Moreover, only 10g of raw propolis was used for coating 10kg of cucumbers. Thus, this approach seems to be a viable and economical option for Indian farmers to reduce postharvest losses of fresh produce. However, further research focused on commercial scale needs to be done so that more realistic information can be used to commercialize this environment-friendly technique.

ACKNOWLEDGEMENT

The authors would like to thank Mr. Subodh from Shimadzu Analytical (India) Pvt. Ltd, New Delhi for allowing them to use the GC-MS facility. They would also like to thank Dr. Priti Gupta from Footwear Design and Development Institute, NOIDA for providing the ICP-MS facility and Guru Gobind Singh Indraprastha University for providing the lab facilities.

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