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Analysis of chlorophyll degradation of leafy vegetables and green chilies by coating with *Terminalia arjuna* (Kumbuk) plant mucilages

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Abstract
The objective of the present study was to investigate the possible application of the edible grade plant mucilaginous of *Terminalia Arjuna* (Kumbuk) over selected high respiratory leafy vegetable and green chilies as a thin coat. In this study five types of high respiratory vegetables names as *Centella asiatica* (Gotukola), *Alternanthera sessilies* (Mukunuvenna), *Ipomoea aquatic* (Kankun),*Capsicum annum* (Green chilies) and *Allium ampeloprasum* (leeks) were taken and coated with mucilaginous materials extracted from Kumbuk (*Terminalia Arjuna*).The selected vegetables were coated with Kumbuk and their initial and final chlorophyll concentrations were determined by method described by (Nayek Sumanta *et al*. 1989) and results were compared. The chlorophyll degradation rate was reduced in the samples coated with Kumbuk mucilage solution when compared with the control samples.

Keywords: chlorophyll degradation, edible, kumbuk, mucilaginous, *Terminalia Arjuna*

1. Introduction
Leafy vegetables deteriorate rapidly after harvest as compared with other fruits and vegetables. One of the main factors causing deterioration of freshness is yellowing of leaves. The yellowing phenomenon, the degradation of chlorophylls is involved one or more of following reasons. Pheophytin formation from chlorophyll by organic acids, chlorophyllide formation by chlorophyllase, bleaching reaction of chlorophyll by oxidative reactions, the formation of pheophytin is particularly found in processed foodstuffs. Although the chlorophyllase has been thought to catalyze the degradation of chlorophylls, a function for the enzyme in the synthesis of chlorophylls has also been postulated [1]. Post-harvest chlorophyll degradation is a serious problem for green leafy vegetables and yellowing is common symptom of their senescence process which is greatly affected by the storage temperature. One of the advantages in using edible coatings and films is the reduction of water loss, considered one of the main factors in the deterioration of perishable foods. In fact, this thin layer protects fruits and/or vegetables against moisture loss, maintaining the texture and extending the shelf-life of the product, forming a protective barrier. On the other hand, when edible coatings are poor in water vapor barrier properties, a weight or moisture loss of the product could be recovered [2]. *Terminalia Arjuna*, commonly known as arjuna, belongs to the family of Combretaceae. Its bark decoction is being used in the Indian subcontinent for anginal pain, hypertension, congestive heart failure, and dyslipidemia, based on the observations of ancient physicians for centuries [3]. Bark of *T. arjuna* contains a very high level of flavonoids, namely arjunolone, flavones, luteolin, baicaleiin, quercetin, kempferol, and pelargonidin evaluated with other medicinal plants particularly having favorable effects on cardiovascular diseases. Aqueous extract of *T. arjuna* contains 70% polyphenols having a molecular weight greater than 3.5 kDa and they are confirmed by the HPLC and LC-MS. The aqueous extract contains flavon-3-ols, such as (+)-catechin, (+)-gallocatechin and (−)-epigallocatechin; gallic acid, ellagic acid and its derivatives such as 3-O-methyl ellagic acid 4-O-β-d-xylopyranoside and 3-O-methyl ellagic acid 3-O-rhamnoside [4].

2. Materials and Methods
2.1 Collection of plant materials
Leaves of Kubuk (*Terminalia Arjuna*) were collected from Kalutara, Sri Lanka.

2.2 Collection of vegetable varieties
Five types of high respiratory vegetable varieties were collected from the local market

and subjected to the study. Those are Gotuloka *(Centella asiatica)*, Mukunuvenna (*Alternanthera sessilis*), Kankun (*Ipomoea aquatic*), Leaks *(Allium schoenoprasu*), Green chili (*Capsicum annum*).

2.3 Extraction of mucilaginous materials from Kumbuk leaves

Fresh matured Kumbuk leaves were initially washed and air dried. The extraction of mucilaginous materials was carried out by making the minor modifications to the procedure developed by [5].One hundred grams of leaves from each mucilaginous material source were taken and steam blanched in 1% SMS solution for 10 minutes. Just after blanching leaves were washed with cold distilled water. Leaves were mashed manually in 1% citric acid solutions (leaves: water 1:10 ratio). The Extract was filtered through six layers of muslin cloths. 500 ml of mucilaginous material solutions were prepared by the mucilaginous source.

2.4 Properties of prepared mucilaginous solutions

2.4.1 Determination of the viscosity of the prepared Kumbuk mucilaginous solutions

500 ml of prepared mucilaginous materials solutions were taken in to a beaker. Then the sample was placed on the plate of viscometer. Then the viscosities were measured at various speeds using LV spindle. Temperature of the solutions were maintained at 25 ^{0}C using adjustable water bath.

2.4.2 Determination of the film foaming ability of the prepared mucilaginous solution

Initially two petri dished were taken and they were cleaned well and dried well using the hot air oven. Thereafter one petri dish was dipped in mucilaginous material solutions and the other was kept as the control. Then the two dishes were put into the moisture oven for 3 hours. Then they were taken out and their appearance was compared visually (Transparency and opacity of the dishes)

2.4.3 Preparation of mucilage treated vegetable samples

The application of gum was done by dipping the vegetables in a very dilute mucilaginous solution for 30 seconds and letting them to drain.

2.4.3.1 For the Gotukola, Mukunuvenna and Kankun

Initially 30 g of cleaned green leafy vegetable samples were prepared as bundles and the bundles were dipped in mucilaginous material solutions separately for 30 seconds. Control sample was dipped in distilled water for 30 seconds. Then the excess mucilaginous gum was drained off properly. The coated leaves bundles were dried in a force air dryer at 25 ^{0}C for 30 minutes. Then the dried leaves bundles were packed in polyethylene bags and were sealed and kept under ambient conditions (Temperature -25 ^{0}C and 85% RH). All polyethylene bags were punctured to get five holes to facilitate the air movement. Thereafter weight loss and keeping quality of each vegetables. (How long leafy vegetables were taken to turn yellowish color) were recorded daily. All treatment were triplicated.

2.4.3.2 For Green chilies

Well matured green chili pods at same size were taken and the weights of the samples were recorded and the same procedure done for green leaves were carried out.

2.4.3.3 For Leeks

Leeks trees at the same size were washed with distilled water and the same procedure done for green leaves were carried out.

2.5 Determination of the chlorophyll degradation of the mucilaginous material coated vegetable samples and control samples.

Chlorophyll concentrations were determined as the method of [6].

All the samples were subjected to this procedure daily and the rates of the chlorophyll degradation of each treatment were analyzed and compared with the control sample using the pre-determined equations derived by [7].This procedure was triplicated for all the samples.

$$\mathbf{Ch\ a + b = 17.76\ A^{646.6} + 7.34\ A^{663.6}}$$

Ch –chlorophyll
A – Absorbance

3. Results and Discussion

3.1 Determination of the viscosity of the mucilaginous solution prepared from Kumbuk leaves

Viscosity mucilaginous solution prepared from Kumbuk leaves was 13.2 Cp (30 rpm).

Kubuk mucilaginous material contain complex polysaccharides which can be formed a hydrocolloid solutions binding with the water molecules of the solution. This polymeric substances are soluble in water and capable of displaying colloidal properties. These mucilaginous material are the product of the plant metabolic reactions. This mucilaginous material are widely used in the food industry since the capability of mucilaginous materials to enhance the viscosity of a solution. The mucilage materials are accumulated in between the cell wall and the plasma membrane. Some mucilaginous materials are vacuoles also. Mucilage is in most cases produced in Golgi bodies, from which vesicles filled with polysaccharides move towards the plasma lemma and fuse with it. The mucilage accumulates between the plasma lemma and the cell wall [8].

3.2 Determination of the film foaming ability of the prepared mucilaginous solution

It was observed that a transparent film has been formed on thee petri dsh dipped in the mucilage solution.

3.3 Determination of the chlorophyll degradation rate of the mucilaginous material coated vegetable samples and control samples

The Chlorophyll content in vegetables at the beginning and after three days of treatment were determined. When C*entilla* is concerned the initial chlorophyll content of the vegetable sample was 13.17±0 mgg^{-1}. After three days the chlorophyll content of the control sample, refrigerated sample and the coated sample were 1.21 ± 0.3 mgg^{-1}, 8.75 ± 0.1mgg^{-1} and 8.20 ± 0.1 mgg^{-1}. When *Capsicum* is concerned the initial chlorophyll content of the sample was 0.31±0.01 mgg^{-1}. After three days the chlorophyll content of the control sample, refrigerated sample and the coated sample were 0.18 ± 0.3 mgg^{-1}, 0.22 ± 0.2 mgg^{-1} and 0.28 ± 0.2 mgg^{-1}. When *Alternanthera* is concerned the initial chlorophyll content of sample was 9.8±0.1 mgg^{-1}. After three days the chlorophyll

content of the control sample, refrigerated sample and the coated sample were 4.1 ± 0.2 mgg^{-1}, 9.4 ± 0.1 mgg^{-1} and 6.1 ± 0.1 mgg^{-1}. When *Allium* is concerned the initial chlorophyll content of the sample was 11.1±0.1 mgg^{-1}. After nine days the chlorophyll content of the control sample, refrigerated sample and the coated sample were 4.0 ± 0.2 mgg^{-1}, 9.4 ± 0.1mgg^{-1} and 6.3 ± 0.2 mgg^{-1}. When *Ipomoea* is concerned the initial chlorophyll concentration was 13.5 ± 0.2. After three days the chlorophyll content of the control sample, refrigerated sample and the coated sample were 4.1 ± 0.1 mgg^{-1}, 9.4 ± 0.1 mgg^{-1} and 6.1 ± 0.2 mgg^{-1}. Altogether it is clear that the chlorophyll degradation rate was minimum in refrigerated samples. The highest chlorophyll degradation rate was in the control sample. The bleaching of chlorophylls seems to involve lipoxygenase and peroxidase. Orthoefer and Dugan Jr. observed that chlorophyll was bleached in a system which consisted of linoleic acid and lypoxygenase [1]. The yellowing phenomenon, the degradation chlorophylls is involved one or more of following reasons. Pheophytin formation from chlorophyll by organic acids, chlorophyllide formation by chlorophyllase, bleaching reaction of chlorophyll by oxidative reactions, the formation of pheophytin is particularly found in processed food stuffs. Although the chlorophyllase has been thought to catalyze the degradation of chlorophylls, a function for the enzyme in the synthesis of chlrophylls has also been postulated [1]. When the internal O_2 composition is lowered respiration causing senescence decreases, preserving the quality of the produce during storage, however, special care has to be taken in order to avoid a very low internal O2 concentration since it can cause anaerobic respiration with consequent ethanol production and off flavour formation [9]. Films and coatings applied to fruit surfaces reduce water loss, lower internal O_2, and increase internal CO_2 concentrations. They may also reduce respiration rate, retard ripening and alter the level of other physiologically active compounds [10]. In addition to primary metabolism, secondary biochemical reactions occur in plant tissue which could contribute to both synthesis of certain desirable substances as well as to deterioration in quality. They include loss of chlorophyll (loss of green colour), formation of pigments by carotenoids and phenylpropanoid synthesis [11].

4. Conclusion

The coating of vegetables by plant mucilaginous material can be applied successfully for some vegetable varieties. By this coating a micro film is formed over the vegetables creating a barrier for the moisture and air migration through the surface while lowering the rate of respiration and evapo- transpiration of the vegetable and the chlorophyll degradation rate can be retarded by it.

5. References

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