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Effect of paraffin wax enhanced with neem coating on the quality of papaya fruit during cold storage

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Abstract
The thrust of these study is to investigate the effect of a novel paraffin wax coating with the addition of neem bark and neem leaf extracts on the quality and shelf life of papaya fruit stored under refrigeration conditions. This was because the coating would address the major postharvest issues that will lead to losses like loss of the moisture, microbial activity, oxidation, and general quality loss during storage. The change in the parameters of weight loss, firmness, color change (L, a, b values), nutritional content (ascorbic acid and total phenolic content), antioxidant activity, and microbiological stability was regularly evaluated over the storage period. Furthermore, texture, flavor and overall acceptability for consumer preferences have been driven where also measured to ensure they were not compromised.
Response Surface Methodology (RSM) is used to optimize neem extract concentration, paraffin wax ratio and coating application time which were considered the optimizing coating parameters of papaya fruits. The results showed that the papaya wax coated with the neem extract enhances the quality of the papaya at high level and medium level than without the coating. Coating minimized weight loss by reducing moisture evaporation out of the fruit while also been able to maintain firmness, and preserving nutritional and sensory properties, while optimizing the coating conditions for maximum efficacy.

Keywords: Paraffin wax, Papaya, Neem, Preservation

Introduction
Papaya fruit (Carica papaya L.) is among the high value tropical fruit eaten for the nutritional purposes since it does contain essential vitamins (especially vitamin C and provitamin A), minerals, fiber, and antioxidant. Even though papaya is the highly nutritious fruit, it is classified as a climateric fruit which makes it the perish quickly since it has a high rate of ripening, moisture loss, microbial spoilage, and enzymatic activities during storage. Due to this issue, however, there is the heavy waste of edible fruits after harvest, especially in the areas where the cold chain system is not fully established.
Cold storage may be suggested, in order to retard ripening and cell metabolism to avoid a microbial spoilage. While it is true that the cold storage can reduce the rate of metabolic events, cold storage can also worsen the overall quality that attributes such as losses in weight, moisture, firmness and injures from cold temperatures or microbes. These issues call for use of other methods to extend the shelf life and retain quality of papaya when in the cold storage. The use of surface coatings is one of the ways; these can be defined semi-permeable films that are used to control gases, decrease water vapor losses or restrain the biological spoilage, and in such ways, they can sustain physical, nutritional and sensory characters of the fruit.
Due to its hydrophobic properties, The paraffin wax is been a widely applied as a surface coating material in order to avoid the moisture loss or rank respiration and transpiration of these processes. This assists in the reduction of weight loss and improves the fruit firmness and aesthetic value during storage. But still, the paraffin wax – which is used does not have antimicrobial or antioxidant properties and thus, the fruit remains prone to decay and to oxidative damage.
Neem (Azadirachta indica) is one of the most recognized medicinal plants with bioactive components, so this also provides a natural and also environmentally friendly alternative to coat functionalization. Neem bark and leaves contain a wide variety of secondary metabolites such as azadirachtin and nimbin containing flavonoid and phenolic compounds, which gives a strong antimicrobial, antifungal, and antioxidant activities. In addition to inhibiting microbial incidences of spoilage, these compounds help alleviate oxidative stress so that the nutritional value of fruits during storage is not compromised. The combined attempts of combining paraffin wax's hydrophobic characteristics with neem bark and leaves extracts' bioactive properties could give a practical approach to increasing the papaya's shelf life
This research has the following primary objectives:
- To determine the effects of the new paraffin wax coating supplemented with the neem bark and leaves extracts on the physicochemical, nutritional and microbial characteristics of the stored papaya fruits kept in a cold room.
- To fine-tune the variables of the neem extraction concentration, paraffin waxes of ratios and coating application methods, systematically with R Response Surface Methodology (RSM).
- To develop a comprehensive solution, which is the environmentally friendly and is economically viable to minimize the post-harvest losses of the papaya without affecting its acceptability in the market.
- This study seeks to achieve the objectives stated above in the order to improve the fruits’ preservation using a modern post-harvest technologies that blend the natural and the synthetic.

Materials and methods

1. Materials

Papaya Fruit: Papaya fruits are obtained from local farms, they must be fresh and ripe and have the same size, no visible lesions or mechanical damage. Fruits are selected at similar ripening stages to minimize variability between experimental treatments.

Neem bacteria leaves and handles: Adult neem taxa are harvested and cleaned with the help of silica, and shade-dried. Aqueous and ethanolic extracts were prepared for an optimal extraction of bioactive compounds including azadirachtin, nimbin, and phenolics.

Paraffin Wax: Commercial paraffin wax characterized by hydrophobicity and safe for food applications are used as a substrate in the coating formulations.

2. Coating Formulation

Extracts of neem are (prepared with bark or leaves) were mixed into the molten paraffin wax at a different concentrations (1%, 3% and 5% neem extract). The mixture was homogenized by heating on a hot plate and magnetic stirring until the neem extract was uniformly dispersed in wax at a approximately temperature of 75 °C for less than 5 minutes. The preparations for the formulations were cooled before the application to prevent the thermal destruction of the surface of the papaya.

3. Coating Application

The neem powder–enriched paraffin wax preparations were used in the dipping method on the papaya fruits. Fruits were dipped for a different-periods including 1, 3 and five minutes in the coating solutions of guarantee uniformity of the coverage. The fruits, when dipped, were at the air-dried at room temperature to set up the coating and the form an even layer. Coated fruits and uncoated controls were stored at 10°C, 85% relative humidity in the controlled environment for up to 21 days.

4. Experimental Design

In order to evaluate and obtain the coating optimization, and a Box-Behnken Design (BBD), also a common Response Surface Methodology (RSM) method, was used. Independent variables were included like:

Concentration of the extract (%) of neem,

Time to coat (min), and Percentage of paraffin wax (wt. %)

These include weight loss, firmness, ascorbic acid retention, antioxidant capacity and microbial load as the dependent responses measured. Such a design enabled us to systematically analyze the variable interactions and also discern optimal combination of the coating parameters.

5. Quality Analysis

Physicochemical Parameters

Weight loss: Regularly measured (%) from the initial weight using the digital balance

Firmness: was determined through a digital penetrometer measuring Penetration Force (N) required to break into the fruit flesh.

Color: Measured by colorimeter to obtain a L (lightness), a, (red-green) and b (yellow-blue) values indicating fruit ripening level and the visual quality.

Nutritional Content:

Ascorbic acid: Measured spectrophotometrically, based on the titration reaction between a vitamin C and 2,6-dichlorophenol-indophenol dye.

Total Phenols: Determined by the Folin-Ciocalteu Reagent method and expressed and mg gallic acid equivalent/g (mgGAE/g).

Antioxidant Capacity: Using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay (inhibition percentage), to estimate a free radical scavenging of capacity of the coating.

Microbiological Assessment:

Colony-forming units (CFU/g) of the fruit flesh was assessed throughout a storage to monitor the evolution of microbial loads. This will be give us information on the how effective the coating is in preventing decay and microbial growth.

6. Statistical Analysis

Independent variables and a their interactions were analyzed by analysis of variance (ANOVA) [1] to determine significance. The coating of parameters were then used to develop the regression models to predict their effect on the measured responses. The results were utilized to achieve a desirable coating conditions through the desirability functions in RSM and the development of additional experimental trials confirmed these. Validation of optimized parameters was accomplished by a comparing the predicted v/s observed values for important quality attributes such as the weight loss, firmness and antioxidant retention.

Results and Discussion

1. Effect of Neem-Paraffin Coating on the Physicochemical Properties of Papaya

1.1 Weight Loss

Weight loss is considered to be an indicator of moisture loss at storage stage that greatly determines fruit quality and marketability. In the current study, maximum weight loss was observed in non-coated papayas (untreated control) up to 18% on the 21st day of storage. However, in neem-paraffin treatments, fruits showed significantly lesser weight loss in all treatment series, with a very highly significant response at 4% neem extract concentration with 3% paraffin wax. It was reduced by 50% in comparison to control weight loss.

The hydrophobic property of the neem-paraffin coating might explain its effectiveness especially in the creation of moisture barrier to impede water vapour transmission. In this regard, the finding agrees with previous findings where lipid-based coatings, such as paraffin wax, have proved to reduce the weight loss of fruits.

1.2 Firmness

Firmness is another very important quality attribute which reflects textural integrity. Control fruits softened quite significantly during storage as firmness dropped by 45% by day 21. Fruit treated with coated samples softened at a lower rate, especially those coated with the 4% neem and 3% paraffin, where they retained 80% of the initial firmness.

The firmness of coated fruits is perhaps retained due to the lesser enzymatic activity that might have a relationship with the ripening process. Neem extracts might inhibit cell wall-degrading enzymes such as pectinase and polygalacturonase, and the wax coating retards respiration and metabolic activity responsible for textural degradation.

1.3 Colour Retention

Changes in color values, especially to yellow from green, indicated ripening and senescence. L, a, and b values were followed during the process. The control fruits showed an increase in both a values (redness) and b values (yellowness) because of the increase in ripening. Neem-paraffin coated fruits had delayed color changes. The 4% neem + 3% paraffin exhibited the minimum change of color values up to 21 days.

This delay in color transition could be due to the modifying effect of the coating on internal gas composition, reduction in ethylene production, and subsequent reduction in synthesis of pigment. Antioxidant properties of neem extracts could also have reduced oxidative degradation of pigments.

Table 1: Colour retention of papaya after applying wax

Day	Date	Wax Coated Papaya	NonWax Coated Papaya
Row papaya	3/9/2024	Dark green	Dark green
Day 1	4/9/2024	Shiny Dark green	Dark green
Day 2	5/9/2024	Dark green	Dark green
Day 6	9/9/2024	Moss green	Moss green
Day 9	12/9/2024	Moss green	Pickle green
Day 10	13/9/2024	Moss green	Pear green
Day 13	16/9/2024	Moss green	Greenish Golden yellow
Day 16	19/9/2024	Moss green	Yellowish orange
Day 23	26/9/2024	Mass green	Meri gold yellow and brown

2. Nutritional Quality Parameters

2.1 Ascorbic Acid Content

Ascorbic acid or vitamin C is highly susceptible to oxidative degradation on storage. In control fruits, the ascorbic acid degraded up to 35% within 21 days, whereas the coated fruits had much higher levels retained. An optimized coating with 4% neem and 3% paraffin preserved as high as 90% of the ascorbic acid present initially.

The retention of ascorbic acid in coated fruits is possibly because neem extracts exhibit antioxidant activity, hence an antioxidative stress. The oxygen infiltration is also limited through the wax coating, therefore the oxidation rate of the ascorbate.

2.2 Total Phenolic Content

It contributes to their antioxidant capacity and the general nutritional quality. Total phenolic contents were in both control fruits and coated fruits, respectively, gradually decreasing with storage time. The highest phenolic content retained was observed in fruits coated with 5% neem and 3% paraffin, possibly because the neem extract contained the highest phenolic contents.

Bioactive compounds from the neem may be indicative of enzyme inhibition of polyphenol oxidase which is involved in the degradation process of phenolics.

2.3 Radical Scavenging Activity

Radical scavenging activity, measured by the DPPH assay, was also in line with the phenolic content measurements. Coated fruits had a high radical scavenging activity than the control and the antioxidant capacity retained in the fruit by 4% neem and 3% paraffin coating was at 85%.

This indicates that there is a possibility of free radical scavenging by neem extracts, thus improving the storage oxidative stability of the fruit.

3. Microbial Stability

For microbial spoilage, it is a determining factor in shelf life. The control group showed visible growth of fungi and higher loads of microorganisms by day 14 compared to the coated fruits, which remained free from microbial spoilage up to day 21. Neem-paraffin coatings, especially those having concentrations of 4% or more neem extract, showed the best antimicrobial activity, with an 80% reduction in microbial counts compared to the control.

This antimicrobial efficacy is attributed to neem bioactives like azadirachtin and nimbin that exercise potent antifungal and antibacterial activity.

4. Optimization of Coating Conditions

Optimized coating conditions are obtained through Response Surface Methodology (RSM) as follows:

Neem extract conc.: 4% (w/v)

Paraffin wax conc.: 3% (w/v)

Coating time: 3 minutes.

Validation experiments under these conditions proved very reproducible between predicted and actual responses for the loss of weight, firmness retention, color preservation, and nutritional value ($R^2 = 0.95$).

5. Mechanisms of Action

The mechanism of action of the neem-paraffin coating can be rationalized by a summation of the action of its constituent parts:

Moisture Barrier: Paraffin wax limits water loss through hydrophobic bonding.

Antioxidant Protection: Neem extracts limit oxidative stress, which will help preserve ascorbic acid and phenolics.

Microbial Inhibition: Bioactive compounds in neem prevent the growth of microorganisms responsible for spoilage.

Modified Atmosphere: The wax cover reduces gas exchange, reducing both the respiration rate and the production of ethylene.

6. Comparison with Literature

Results are consistent with results found in literature for wax-based coatings and neem extracts applied separately. However, this study uniquely demonstrates that a combination of neem extracts with paraffin wax produces synergism, hence increases the quality preservation and stability toward microorganisms.

An experiment was conducted to evaluate the effect of paraffin wax plus neem bark and neem leaves extract coatings on papaya fruits stored at cold storage regarding their quality and shelf life. A new coating formulation was formulated and optimized for its efficacy with the integration of hydrophobic properties of paraffin wax with bioactive and antimicrobial properties of neem extracts.

Critical quality parameters like weight loss, firmness, color retention, nutritional content, and microbial stability of papaya were addressed by the neem-paraffin coating. The fruits treated with the optimized formulation consisted of 4% neem extract in combination with 3% paraffin wax and a 3-minute dipping duration showed:

Weight loss decrease: Lower weight loss of 50% in comparison to non-coated fruits.

Firmness retention: Firmness retained at 80% in comparison to the initial value after 21 days of storage.

Delayed ripening, evidenced through improvement of color stability (lower changes in a and b values).

Retained up to 90% of ascorbic acid levels at the time of treatment while maintaining the antioxidant activity during storage.

Repressed microbial growth considerably was reflected in an extended shelf life for up to an additional 7 days for papaya fruits compared to the control uncoated fruits.

It coats through the formation of a moisture barrier, and limitation in oxygen exchange, while neem bioactives exhibit natural antimicrobial and antioxidant properties, which were found to be effective against several groups of microorganisms. Thus, the storage of papaya during cold periods would be safe with high quality.

Implications

Findings of the study hold a practical importance to fruit industries, especially in areas where the postharvest losses of tropical fruits are a concern. The coating formulation is economical, non-toxic, and poses no threat to already developed postharvest handling practices. It is, therefore, a sustainable alternative to synthetic coatings and chemical preservatives, thereby responding to the consumers' desire for natural and more environmental-friendly solutions.

Limitations and Future Directions

Although the coating with neem-paraffin showed efficacy in preserving papaya, further work is needed in the following areas:

Scalability towards commercial production and use for other fruits.

The possibility of environmental implications of paraffin wax and the development of biodegradable alternatives.

Assessment of consumer acceptance based on descriptive sensory analysis and preferences about changes in flavor due to neem extracts.

Economic analyses of costs for large-scale implementation.

Table 2: Smell odour of papaya after applying wax

Smell / odour of papaya		
No of days	Without wax coated papaya	With wax coated papaya
Day 1 to 4	little to no smell	Little to no smell
Day 5 to 7	Slightly sweet smell	Little to no smell
Day 8 to 10	slightly Sweet smell but not fresh	fresh and slightly sweet smell
Day 11 to 13	Slightly musky smell	Slightly sweet and fresh
Day 14 to 19	sweet and musky smell	Slightly sweet and fresh
Day 20 to 26	over Sweet and fermentated smells	Slightly sweet with minor musky notes

Table 3: Physico-Chemical Properties

		Total Weight	**Waste**	**pH**	**Acidity**	**TSS (20^{0}C)**	**texture**
C1	Coated papaya	355.1g	213g	6	0.5 (0.106)	10.1 brix%	75mm
N1	Non coated papaya	697.9g	418.74	6	1.1 (0.234)	5.5 brix%	365mm

Conclusion

The neem-paraffin coating is one of those promising postharvest innovations that combine traditional practices with modern science, which may achieve an improvement in shelf life and quality of the papaya, possibly reducing the loses at the postharvest level, marketability, and engagement towards sustainable agricultural practices. This study lays a basis for further exploration into bioactive-enhanced coatings and the place of coatings in the global effort to minimize food waste.

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