Impact of light quality on leafy green vegetables

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Abstract

Fruits and vegetables are a vital part of the human diet, and their consumption is on the rise because they are considered as a healthy intake. However, ingredients of fruits and vegetables are vulnerable in postharvest handling like moisture loss, wrong handling, physical damage, and microbial contamination. Postharvest moisture loss in fruits and vegetables affects weight, texture, appearance, acridness, vitamins, sugar and phenolic.

Outbreaks of food borne related illnesses have been increasing during the last two decades. Many of the diseases have been associated with leafy green vegetables contaminated with human pathogens such as shiga toxin producing *Escherichia coli* O157:H7. To eradicate the pathogens from the surfaces of vegetables and fruits in postharvest handling, chlorine-based sanitizers treatments are widely used. But there are some complications of using chlorine-based sanitizers for example, producing harmful compounds like chloroform and its residual toxicity supposed to have negative repercussions on human health. Therefore, researchers are looking for alternative safe and less expensive methods for sanitization, one of which is the use of blue light. In the field of microbiology recent studies showed the potential of influencing the behavior of E. coli O157:H7 by using a certain wavelength of light within the visible spectrum can help to kill the bacterial pathogens with a positive effect on maintaining quality.

The main purpose of this study is to investigate the effect of blue light dose on spinach baby leaves in terms of freshness and weight retention during shelf life. To conduct this study, spinach leaves were exposed to blue light with a wavelength of approximately 460 nm for 0, 2, 4, 6 and 8 hours intervals. The weight and leaf firmness were measured after each time interval. In the control samples, the same methodology was applied on spinach leaves without being exposed to blue light dose. The results have shown no significant effect on spinach leaves weight and hardness under blue light dose exposure. However, a significant loss in weight was observed when spinach leaves were not exposed to blue light. This study has shown that there is a possibility of using blue light as a disinfectant, but more research is needed to optimize the dose and exposure duration of blue light so that the product

itself is not damaged in terms of quality and freshness taking into account various factors such as growing conditions, logistics and storage climate conditions etc. The combined effect of blue light with other light spectrums such as green and red could be a future action point.

Popular science summary

The consumption of fresh herbs and salads is becoming an increasing trend because they provide good nutrition to the human body and play a preventive role against progression of disease like obesity, diabetic, heart problems, blood pressure etc. There is also an increase in outbreaks of food borne illnesses because of the contamination of food by different human pathogens. To address these problems, the food industry uses sodium hypochlorite as a disinfectant agent for fruits and vegetables. But sodium hypochlorite with organic load can produce harmful toxic compounds like chloroform, chloramines and trihalomethanes. Its residual toxicity may have adverse impact on quality and human health. Therefore, researchers are looking for alternative disinfectant methods for fresh vegetables and fruits. One of the alternative methods is to use blue light as a disinfectant tool. The blue light treatment decreases the mesophilic and psychrophilic effects on spinach leaf surface as compared to sodium hypochlorite washing. Also, blue light has no residual impact on spinach leaves.

In this study, spinach leaves were exposed to blue light under different time intervals to investigate the impact on leaf weight and leaf hardness. The results showed that there is no effect of blue light on spinach leaves weight, however a slight positive effect was observed on the hardness of spinach leaves. On the other hand, a significant loss in weight was observed in control samples without exposure to blue light dose and more deterioration in hardness of spinach leaves. The exposure of blue light has a positive effect on spinach leaves weight and hardness. Therefore, blue light treatment can be used as a disinfectant tool instead of sodium hypochlorite during shelf period of product, but it requires more investigation and research for further optimization of dosage and intensity.

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Abbreviations

LED	Light Emitting Diode
UV	Ultraviolet

1. Introduction

Consumption of "ready-to-eat" salads gained popularity during the last decade. This diet provides not only nutrition to the human body but also plays a preventive role against different diseases. Consumption of "ready-to-eat" salads increased to 19% between 1970 to 2005 (Wells & Buzby, 2008) and it is predicted to boost in coming years (Lin et al., 2003). On the other hand, use of "ready-to-eat" salads has led to an increase in outbreaks of food borne illnesses because they are contaminated with food borne human pathogens (Brandl, 2006; Alsanius, 2014). Around 23% of the food borne illnesses from 1998 to 2007 were due to the consumption of fruits and vegetables (CSPI, 2009). The major contributors to the bacterial infections are mostly *Salmonella spp.*, *Camplyobacter* spp. and *Escherichia coli* O157:H7 which can stay in the food processing units and processed products such as fresh cut herbs and salads (Tauxe, 1997; WHO, 2020). In the food industry, sodium hypochlorite is used as a disinfectant tool for disinfecting fruits and vegetables. This chemical has been used to remove microbiological risks in vegetables and fruits and has proven to be effective. But these vegetables and fruits reach the consumers after undergoing chlorine based chemical sanitizer (Boyette et al., 1993). The effectiveness of chlorine based disinfectants got a boost by increasing the concentration of chlorine, but high-level of concentration may destroy products (Adams et al., 1989) and also leave sodium residue on the surface of products under treatment (Ritenour and Crisosto, 1996). These side effects bring down the shelf-life and quality of the product under treatment (Wiley, 1994).

As consumers are more aware of a healthy lifestyle, they prefer minimal processed fruits and vegetables. Therefore, to disinfect the fresh vegetables and fruits, alternative methods are needed which are chemical free and are less hazardous. An emerging alternative is to use light-based sanitation methods which are more sustainable and less expensive. The exposure of leafy vegetables to different wavelengths of blue light is helpful to retain the quality which is due to the continuation of photosynthesis process and low ethylene production after exposure to blue light (Ma et al., 2011, Craig et al., 2015). The antibacterial effect of blue light works mainly through the action of reactive oxygen state (ROS). A wide range of bacterial macromolecules such as lipids, cell wall, extracellular biofilm matrix and nucleic acids undergo damage because of ROS. In addition to that, many bacterial species including *E. coli* and *A. baumannii* have blue light receptors that are involved in the formation of biofilm and motility. Due to this, these can be a focused target to lower down their presence in such conditions.

1.1. Objectives

The primary objective of this study is to observe the effect of blue light with wavelength of 460 nm on weight and hardiness of spinach leaves. I formulate the following hypothesis:

1. Blue light imposes a negative impact on spinach leaves weight.

2. The leaf quality (measured in terms of hardness) is affected by exposure to blue light.

1.2. Limitations

The main limitation for this study is, unavailability of lamps with corresponding higher light Intensities, so I can take the reading for expanded exposure time to achieve higher doses of light intensities to check the behavior of spinach leaves in terms of weight and hardness.

2. Materials and Methods

To review literature, we used recommendations given for meta-analysis and systematic review that were covered in literature during the period of last 25 years. We used the web of knowledge (Wok) for literature searches. In search criteria combination of keywords like “Effect of LED/blue light as disinfectant”, “Shelf life of spinach under LED exposure”. As understanding the topic developed, I excluded the material only dealing with pathogen eradication only and included research papers that address product shelf life as well. I reviewed approximately 30-40 research papers, out of which 33 paper material was used in the thesis. The evaluation technique is based on experiment and statistical analysis. Finding of the evaluation is based on hypothesis “Effect of different LED dose on spinach leave weight and hardness” in terms of significance.

Following databases and search tools were used like SLU library, science direct, google scholar, web of science, Scopus etc.

2.1 Materials

Leafy greens spinach was purchased from the supermarket. For the experiment set-up climate chamber is used. In this climate chamber there are options of setting properties for moistness, temperature, humidity, time interval and different light qualities with different combinations. However, the light settings in the climate chamber only tells you which type of light and not the actual wavelength. In this case the chamber was put on 100% blue light.

2.2 Experiment Set-up

Four samples of spinach leaves were put on a tray covered with a bench cover (Fig. 1A). The fresh weight of the leaves was taken before the experiment (2 digits) and light spectra and intensity were measured in seven different spots with an Ocean Optics Spectrophotometer (Ocean Optics, Model Name/Number: USB 2000) when the climate chamber was set on 100% blue light (Fig. 1B).

Figure 1. Picture over the set up on the tray used in the climate chamber.

Before putting the tray into the climate chamber, the temperature in the chamber is set to10°C and the humidity to 98%. The light quality is set on 100 % blue light with the estimated wavelength of 460 nm. The fresh weight of spinach leaves was measured after 2, 4, 6, and 8h of blue light dose exposure and the spinach leaves hardness in Newton (N) was measured with Digital Fruit Firmness Tester. For this measurement five leaves were randomly chosen, and the measurement was taken on the middle part of the spinach leaves. The experiment is replicated three times. For control samples spinach leaves were treated in the same way as mentioned above but without any exposure to blue light dose.

2.3 Data Collection

For the quantitative analysis of our experiment on spinach leaves without effect of blue light and with effect of controlled and blue light environment, weights and hardness of spinach leaves were noted in excel sheets with proper labeling to be used for statistical analysis.

2.4 Statistical Analysis

The statistical analysis was performed in R Studio. A t-test is used to compare the means of differences between the two considering weights and hardness of spinach leaves. Anova was used to analyze the impact of blue light on weight and leaf hardness. Weight and leaf hardness after blue light treatment was used as response variable and time as co-variate. To investigate if there was a correlation between weight and hardness a spearman correlation test was used. The data from the Ocean Optics Spectrophotometer was also analyzed in R Studio with the package photobiology InOut.

3. Results

3.1 Literature Review

Before performing the experiment, a review of relevant literature was done in which traditional tools were used to decrease the devaluation and increase the shelf life of fruits and vegetables. following methods were used like sanitizers, cold storage, controlled environment by using natural and fabricated preservatives and drying (Pinheiro, Joaquina, et al., 2015). Most of these techniques are effective with some apprehension of decay in nutrients that leads to human health problems in terms of food borne diseases (Millan-Sango et al., 2016; Park et al., 2018). Mainly sodium hypochlorite (NaOCL) is being used for vegetable surfaces or green leaves for decontamination. Sodium hypochlorite can produce harmful compounds like chloroform, chloramines and trihalomethanes (Richardson et al., 1998), these compounds likely to have carcinogenic effects with demonstrated pernicious in kidneys and liver (Nieuwenhuijsen et al., 2000) because of sodium chlorite residual toxicity and harmful impact on vegetables and human health, researchers are interested in alternative strategy for decontamination of vegetables and green leaves, instead of sodium hypochlorite-based sanitizers. Also, consumers are getting more conscious about healthy lifestyle demands for fresh, "ready-to-eat" foods. In the past decade researchers considered and explored various non-thermal techniques for decontamination and better shelf life of produce. Few of these techniques are peroxyacetic acid, electrolyzed water ozone and antimicrobial coating described in detail by (Artés- Hernandez, 2009).

The blue region of the visible light spectrum is used to limit the bacterial population. This method has way fewer negative effects on the human body. It has therefore allowed its application on a wider scale in the food supply chain sector due to its safety (Bintsis et al., 2000). The antibacterial effect of blue light is reported to work better in wavelengths ranging from 400-450 nm (Gwynne & Gallagher, 2018). The longer wavelengths of blue light (460, 465 or 470 nm) have more antibacterial properties in some studies (Aurum & Nguyen, 2019; De Lucca et al., 2012). There are various types of endogenous photosensitizers present in different kinds of bacteria which on contact with blue light produce reactive oxygen species (ROS). These photosensitizers (protoporphyrin, coproporphyrin and uroporphyrin) are likely present in the cytoplasmic matrix of bacterial cells (Choby & Skaar, 2016). The photosensitizers process induced by blue lights depends on the availability of oxygen and it causes cytotoxicity such as apoptosis or necrosis. The single oxygen species induces oxidative stress, and it leads to cytotoxicity. The blue light exposure converts the normal ground state of photosensitizers to an excited state which has a short life span in case of singlet state and long-life span in case of triplet state (Dailey et al., 2017). These both states when achieved produce toxic oxygen forms like hydrogen peroxide, superoxide or hydroxyl radicals. These ROS cause damage to various bacterial cell parts involving destruction of cell membrane, cell wall and genome (Lukšienè, 2005; Hu, et al., 2018). The use of blue light to disinfect horticultural produce also depends on the produce type. It has been observed that photosensitizer mediated inactivation of blue light works more effectively on vegetables which have flat surface geometry such as lettuce, cucumber and tomatoes as compared to produce with uneven surface such as beans and lentils (Glueck et al., 2017). Another study conducted by Tortik et al., (2014) also showed the reduction of *S. aureus* bacterial load by using blue light on cucumbers and peppers (Buchovec et al., 2016).

3.2 Results Experiment

In this study the effect of blue light on spinach leaves was investigated. The ocular observation showed that the leaves that had not been exposed to blue light were more affected than leaves that had been exposed to blue light (Fig. 2)

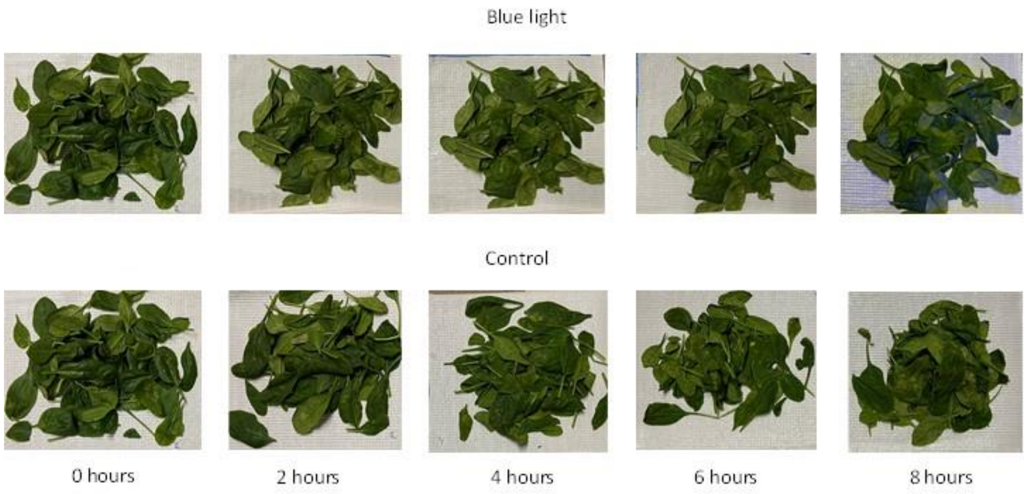

Figure 2. Picture of spinach leaves during the experiment. The upper pictures show spinach leaves being exposed to blue light. The pictures above spinach leaves that have not been exposed to blue light.

There was no significant difference in weight after 8 hours of exposure to blue light (Fig. 3)

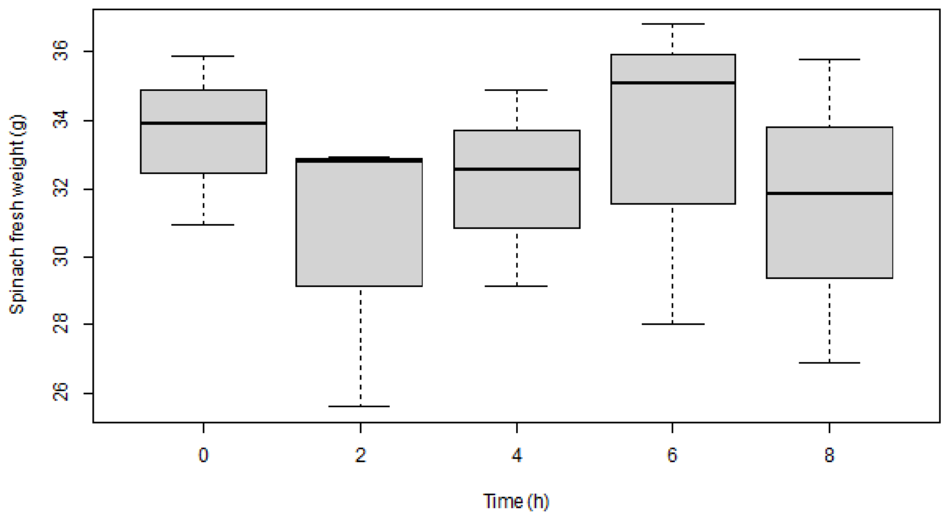

Figure 3. Impact of duration (h) of blue light treatment on fresh weight of baby spinach leaves (g) with standard error bars.

However, there was a significant difference in weight loss between the leaves exposed to blue light and the leaves that had been incubated in the climate chamber without the blue light (t = -4.045, df = 3.648, p-value = 0.01866, Fig. 4), showing larger weight loss in the control samples indicating that blue light could have a small positive effect on leaf freshness.

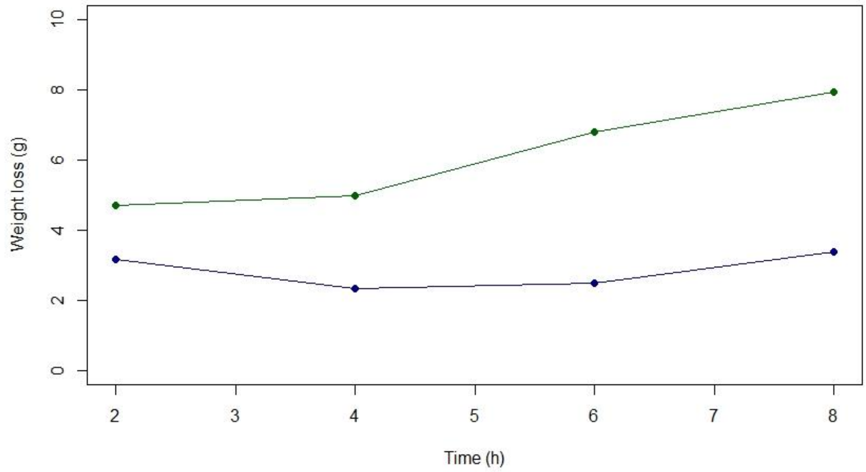

Figure 4. Weight loss of spinach leaves fresh weight (g) after incubation in the climate chamber. Green line shows weight loss of the control leaves which were incubated without blue light. Blue line shows weight loss of spinach leaves after blue light exposure.

To validate the impact of blue light on leaf quality, leaf hardness was measured. The results showed no significant impact of blue light on leaf quality (Fig. 5).

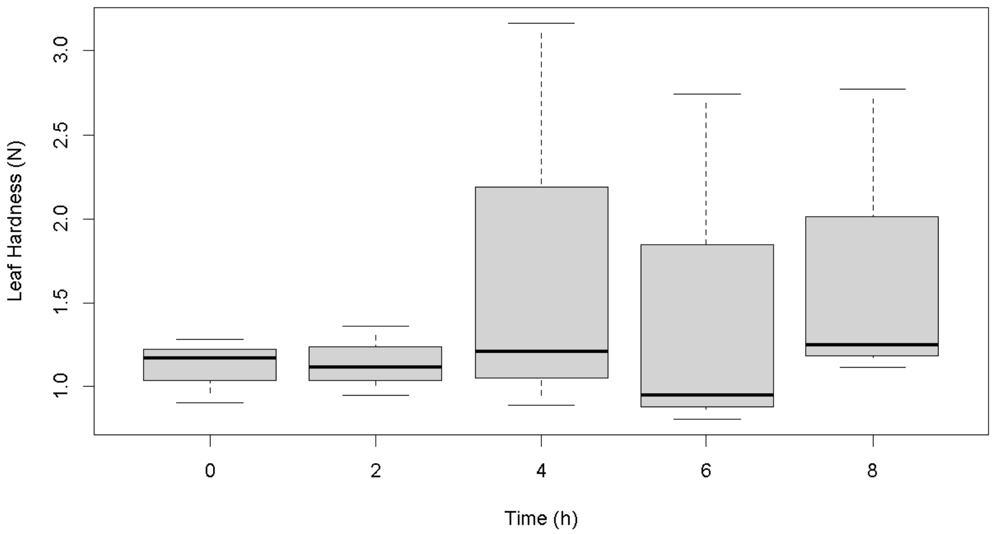

Figure 5. Boxplot showing leaf hardness measured in newton (N) after blue light exposure.

When compared to the control samples there was a significant difference between the two treatments (t = 4.6329, df = 6.7008, p-value = 0.002678). Where the control samples have much softer leaves and lower hardness value compared to leaves exposed to blue light. In order to investigate if the weight loss could have an impact of leaf hardness a correlation test was performed. The result showed a negatively significant correlation between weight and leaf hardness after blue light exposure (r = -60, p = 0.018, Fig. 6).

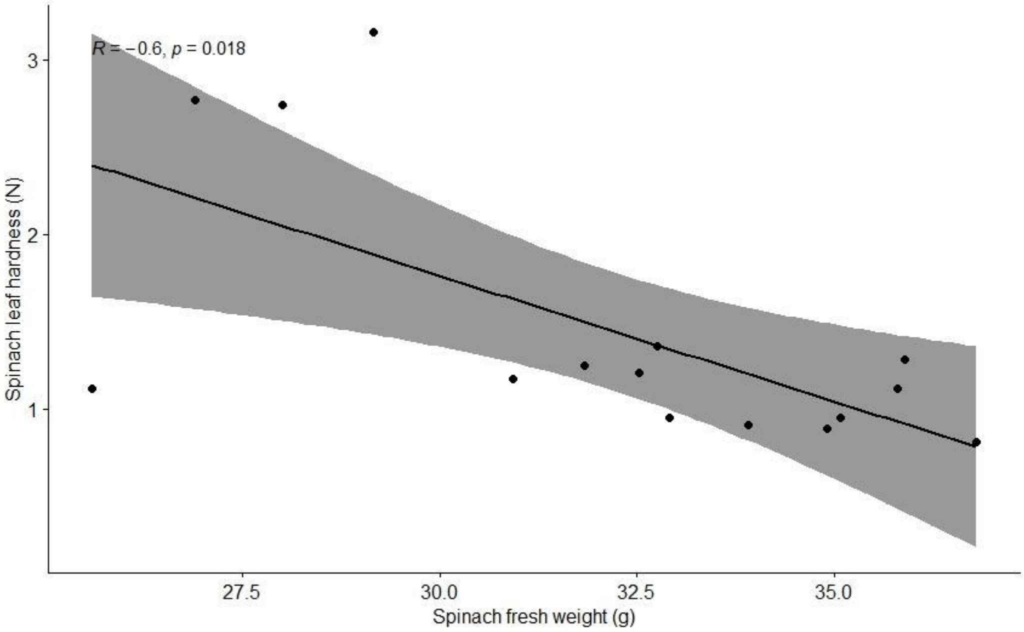

Figure 6. Correlation between spinach fresh weight (g) and spinach leaf hardness (N) after blue light exposure with the confidence interval.

4. Discussion

This study is performed to investigate the effect of blue light on spinach leaves in terms of weight and hardness. The prime focus of the experiment was to assess the quality of produce during shelf life.

When spinach leaves were not exposed to blue light, a significant difference in weight loss was observed. But overall, it was observed that the weight loss percentage was less in the spinach leaves exposed to blue light. The spinach leaves retained firmness after blue light exposure. The stomatal transpiration is mainly linked to loss of weight in harvested leaves which reflects the freshness in terms of hardness or firmness of spinach leaves. The transpiration rate is normally higher in harvested leaves which cause loss of water leading to shriveling and weight loss (Escalona et al. 2010; Gómez-López et al. 2013; Karaca and Velioglu 2014; Jinag et al., 2021). Jinag et al., (2021) reported that blue light can be helpful in maintaining the weight of Chinese kale leaves during postharvest storage. However, there are some studies of postharvest blue light exposure to broccoli that weight loss percentage increased after blue light exposure (Ma et al., 2014) but again the blue light exposure depends upon the structure and shape of produce also. Perhaps the spinach leaves have a more even surface than complex broccoli shape which favored the spinach leaves to retain weight than the control samples. Xu et al., (2014) also reported the same results while working with strawberries that blue light enhanced the quality of strawberry fruit, but the respiration rate was also found more during storage. The blue light exposure is also reported to delay the senescence and less browning of fresh cut pineapple fruit (Leong and Wan, 2019). Dhakal (2014) reported in his findings that single blue wavelength light extends the storage life of tomatoes by delaying the deepening and retaining the firmness. The combination of red and blue light can be helpful in maintaining the freshness and delaying the weight loss in leafy vegetables like spinach, but it needs further investigation.

5. Conclusion

- The blue light exposure has no effect on the weight of spinach leaves.
- Blue light maintained the freshness of spinach leaves by maintaining the firmness of leaves to a small extent.

To get more convincing results we required more data which is a limitation for our study. For future research we need to study the effect of light on spinach by using different spectrums with short and long-time intervals. The blue light exposure to fresh cut produce is highly dependent on various other factors like type of produce, growing conditions, storage temperature and humidity, light or dark storage etc. More research is needed to optimize the blue light intensity and investigate its effect on produce quality retention along with bacterial disinfestation.

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