A Study on the Effect of Storage of Betel Leaves at Ambient Temperature

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Authors’ contributions
This research work was carried out in collaboration among all authors. The work is based on author SS, Master of Technology (Agricultural Engineering) in Processing and Food Engineering entitled ‘Storage Studies on Betel Leaves (Piper betel)’ under supervision of co-authors at University of Agricultural Sciences (UAS), Bangalore, India. All authors read and approved the final manuscript.

Article Information
DOI: 10.9734/EJNFS/2020/v12i130183

ABSTRACT

A research study was conducted for studying the storage of Madras and Kolkata varieties of betel leaves under ambient temperature with different diffusion storage systems were observed for different treatments. In the treatment T1, the concentration of oxygen, O2 reduced to a minimum of 14.27 per cent and carbon dioxide, CO2 concentration increased to 4.80 per cent on the 14th day of storage in ambient condition (28°C). It was also reported that by the 14th day, betel leaves stored at ambient condition maintained satisfactory quality in the entire diffusion channel chambers. For the Kolkata leaves the treatment T2, the concentration of O2 was reduced to 12.0 per cent and CO2 concentration increased to 7.87 per cent on the 18th day of storage. Compared to other treatments, the O2 concentration was found to be very low and CO2 concentration was high on the 18th day of storage in T2 diffusion channel. Similarly, on the 18th day, betel leaves stored at ambient
condition maintained satisfactory quality in all the treatments. Hence, it is clear that from this present study, different varieties of betel leaves will have different rates of respiration for a particular size of the diffusion channel and temperature. In other words, varietal variation occurs concerning respiration rate under identical condition.

Keywords: Betel leaves; temperature; storage; diffusion channel.

1. INTRODUCTION

The Betel (Piper betle) is a leaf of a vine belonging to the Piperaceae family, which includes Pepper and Kava, is a shade-loving perennial root climber. It is valued as a mild stimulant and for its medicinal properties. It has an immense medicinal, social, religious and export value. Betel leaves are mostly consumed in Asia and in other parts of the world by some Asian emigrants. India, Bangladesh, Pakistan, Malaysia, Indonesia, Srilanka, Thailand, Bourbon and West India are the countries which are commercially cultivating the betel leaves. India is the largest producer and Bangladesh being the second largest producer. Srilanka also produces in large quantities [1]. In India, betel leaves are extensively cultivated in the states of Bihar, Gujarat, Karnataka, Kerala, Maharashtra, Madhya Pradesh, Assam, Orissa, Rajasthan, Tamilnadu, Uttar Pradesh and West Bengal [2].

Based on shape, size, brittleness, and taste of leaf blade, betel leaves are classified into pungent and non-pungent varieties. Important betel vine varieties cultivated in Andhra Pradesh are Karapaku, Chennor, Tellaku, Bangla and Kalli Patti. Important betel vine varieties cultivated in Assam are Assam Patti, Awani pan, Bangla and Khasi pan. Important betel vine varieties cultivated in Bihar are Desi pan, Calcutta, Paton, Maghai and bangle, while important betel vine varieties cultivated in KarnataKa are Kariyale, Mysoreale and Ambadiale. Important betel vine varieties cultivated in Kerala are Nadan, Kalkodi and Puthukodi and Important betel vine varieties cultivated in Madhya Pradesh are Desi Bangla, Calcutta and Deswari. Important betel vine varieties cultivated in Maharashatra are KalliPatti, Kapoori and Bangla (Ramtak) while important betel vine varieties cultivated in Orissa are Godi Bangla, Nova Cuitak, Sanchi and Birkoli [3]. Important betel vine varieties cultivated in Tamil Nadu are pachai Kodi and Vellaikodi and important betel vine varieties cultivated in Uttar Pradesh are Deswari, Kapoori, Maghai and Bangla. Important betel vine varieties cultivated in West Bengal are Bangla, Sanchi, Mitha, Kali Bangla and Simurali bangle [4].

An analysis of the betel leaf shows it to consist of moisture 85.4 per cent, protein 3.1 per cent, fat 0.8 per cent, minerals 2.3 per cent, fiber 2.3 per cent and carbohydrates 6.1 per cent per 100 grams. Its minerals and vitamin contents are calcium, carotene, thiamine, riboflavin, niacin and vitamin C. Its calorific value is 44 [5].

Betel leaves consumption is mainly in fresh form. However, limited shelf life is a great detriment in the sale of leaves which are not sold fresh. These leaves either remain unsold or to be sold at a throwaway price. Due to the low keeping quality and faulty transportation system, betel leaves worth millions of rupees go as waste every year [6]. Hence, improvements in the existing packaging and storage systems are to be explored for preserving the nutritional and medicinal qualities of the leaves. With this background information, the objective was to study the effects of different treatments and storage of betel leaves at ambient temperature.

2. MATERIALS AND METHODS

2.1 Measurement of Gas Composition

O₂ - CO₂ Gas analyzer: O₂ - CO₂ Gas analyser (Make: PBI Dansensor, UK, Model: CheckMate II) is automatic and easy to use (Fig. 1). When the syringe is introduced into the material package, the built-in pump automatically starts sampling the gas, ensuring an easy and accurate operation. The gas analysis result is shown in the built-in LCD display and also stored in the memory. The results can then be exported to an external computer via the USB data connection or saved on a memory stick, or printed on the built-in printer.

The O₂ - CO₂ Analyzer, Check Mate II, consists of a Zirconia (Zr) sensor. The Zr sensor operates like a solid-state battery which produces a small voltage or electromotive force (EMF) in the presence of oxygen. This EMF is directly related to the oxygen concentration of the gas passed
into the sensor, which is then shown in the LCD display. The sensor is remarkably sturdy and stable, the readings are highly repeatable and the sensor is very fast. The Zr sensor will not get saturated in high oxygen concentrations and have a measuring range from 0-100% with a high resolution, speed and accuracy.

The CO₂ sensor is a self-contained non-dispersive IR sensor complete with IR source and dual-wavelength filter. There are no moving parts and the sensor is very sturdy. The CO₂ sensor range is 0-100 per cent. The sensor was calibrated before the actual experimentation.

2.2 Respiration Study of Betel Leaves

The respiration rates of the betel leaves were measured at ambient (28°C) temperature. The experimental chambers, for respiration study, consist of ordinary glass jars of 2000 ml capacity with airtight plastic lids. Teflon tape was wrapped around the mouth of the jars to seal the lids for airtightness. A hole of 5 mm diameter was drilled on each of the lid and a septum (silicon rubber) was fixed airtight using glue. The septum facilitated the insertion of the needle of the gas analyzer for measurement of gas concentration, inside the chamber.

Betel leaves were placed inside the experimental chambers and the lids were closed airtight. One set of samples were kept at ambient condition and the betel leaves allowed to respire inside the experimental chamber for known period and the O₂ and CO₂ gas concentrations inside the chamber were measured using a O₂-CO₂ gas analyzer (Make: PBI Dansensor, UK; Model: Checkmate II).

2.3 Storage Condition

Ambient temperature (28°C).

2.4 Confirmatory Studies

Packaging and storage studies on the selected treatments were carried out under ambient and refrigerated conditions. One best observed treatment among the above treatments and similar one best performed of method were selected and were further studied under different temperature condition to enhance the shelf life of betel leaves.

2.5 Pre-treatment of Betel Leaves for Storage in Diffusion Channel Chambers

The two varieties of betel leaves (Madras and Kolkata) were procured from the Bengaluru market. Fresh and disease-free betel leaves of uniform size and color were selected. The leaves were thoroughly washed with potable water and then dipped in 1 per cent Citric acid solution for 1 minute to remove the surface contaminating micro organisms if any. Leaves were then wiped with blotting paper to dry the surface moisture.

2.6 Diffusion Channel Storage

The diffusion channel system is based on the principle of diffusion of gases through a channel. Diffusion channel is a channel or a tube of a specific diameter and length connecting a closed chamber to the atmosphere. Fick's first law of diffusion states that a species of gas diffuses in the direction of decreasing mole fraction of the same gas, just as heat flows by conduction in the direction of decreasing temperature [7].

![Fig. 1. O₂-CO₂ analyzer](image)
The leaves are stored in a closed chamber which is connected to ambient air through a diffusion channel (or tube). During the respiration of the leaves, $O_2$ is consumed and $CO_2$ is liberated resulting in the change of concentration of $O_2$ and $CO_2$ inside the chamber. The concentration of $O_2$ decreases and that of $CO_2$ increases concerning ambient air at rates depending on the respiration rate of the produce. This creates concentration gradients through the channels. The steady-state concentration levels of gases depend upon the type and mass of the commodity stored, respiration rate and the rate of diffusion of the gases. The rate of diffusion of gases depends upon the length and cross-sectional area of the channel and the difference in concentration of $O_2$ and $CO_2$ between the chamber and the ambient air.

Betel leaves were stored in PET (Polyethylene terephthalate) jars having diffusion channels. The PET jars were neatly washed with water and left to dry. Cotton balls were wetted with alcohol and used to wipe the inside of the jars to remove surface contaminating microorganisms. About 25 leaves were placed in each PET jars along with 100 g of silica gel in perforated polythene covers to absorb the moisture released during storage. Teflon tape was wrapped around the mouth of the jar so that the screwed lid of the jar is made airtight.

**Table 1. Details of the treatments of diffusion channel storage system for storage studies of two varieties of betel leaves**

| Diffusion treatment | Diameter of diffusion channel (mm) | Length of diffusion channel (cm) |
|---------------------|-----------------------------------|----------------------------------|
| $T_1$               | 5                                 | 5                                |
| $T_2$               | 7                                 | 7.5                              |
| $T_3$               | 9                                 | 10                               |

**Fig. 2. Experimental storage chambers (PET Jars) of 2 varieties of betel leaves with diffusion channels**
The diffusion channels were basically glass tubes of different inner diameters (5, 7 and 9 mm) and lengths (5, 7.5 and 10 cm) that were fixed vertically on the jar lids through a hole of corresponding diffusion channel diameter drilled through each lid. The tubes were left to protrude outside through the jar lid for exchange of gases with the atmosphere. Septum was firmly fixed on one side of the lid with araldite to facilitate sampling of the gases inside the jars for analyzing O₂ and CO₂ concentrations. The PET jar so designed was airtight and had the visibility to assess the physical condition of the product during storage. The advantages of this design were ease of construction, perfect air tightness and durability.

Gas concentrations were measured inside the storage chambers using O₂-CO₂ analyzer once a day to know the O₂ and CO₂ concentrations. The physical condition of leaves was visually observed and the physiological loss of weight (PLW) was also measured during the storage period.

2.7 Experimental Studies on Diffusion Channel Storage of Betel Leaves

The experiments were carried out with 3 treatments, replicated thrice and sample size was approximately 25 leaves per treatment per replication. The treatment (T₁, T₂ and T₃) details are presented in Table 1.

The storage studies of the above treatments were carried out at ambient (28°C) temperature (Fig. 2). At ambient temperature storage, the leaves were kept in open condition was used as control treatment.

3. RESULTS AND DISCUSSION

3.1 Diffusion Channel Storage of the Two Varieties of Betel Leaves

The results of studies on storage of betel leaves at ambient temperature in different diffusion channels are:

3.2 Effect of Diffusion Channels on Headspace O₂ and CO₂ Concentration at Ambient Temperature Storage for the Two Varieties of Betel Leaves

The headspace gas concentration in terms of O₂ in the storage containers (PET jars) with different diffusion channels during the 14 days of ambient storage of Madras leaves are presented in Table 2 and Fig. 3. For diffusion channel of size T₁-5 mm X 5 cm, T₂-7 mm X 7.5 cm and T₃-9 mm X 10 cm the O₂ concentration decreased to 14.27, 15.37 and 13.50 per cent, respectively on the 14th day of storage up to which satisfactory quality of Madras betel leaves was maintained. At the beginning of the storage period in ambient condition (28°C), the rate of O₂ depletion was gradual and finally flattened at the end of storage. The above results are by the findings of Rayaguru [8].

Fig. 3. Concentration of O₂ in the head space of storage chambers for different diffusion channels during storage of Madras leaves at ambient temperature (28°C)
Table 2. Concentration of O₂ in the head space in storage chambers for different diffusion channels during storage of Madras leaves at ambient temperature (28°C)

| Diffusion Treatments | Dia. (mm) | Length (cm) | Concentration of O₂ (%) | Days of storage |
|----------------------|-----------|-------------|-------------------------|----------------|
|                      |           |             | 0th | 1st | 3rd | 6th | 9th | 12th | 14th |
| T₁                   | 5         | 5           | 21.10 | 18.90 | 17.60 | 16.60 | 15.95 | 14.84 | 14.27 |
| T₂                   | 7         | 7.5         | 21.10 | 19.80 | 18.60 | 17.30 | 16.50 | 15.92 | 15.37 |
| T₃                   | 9         | 10          | 21.10 | 19.20 | 18.50 | 17.10 | 16.03 | 14.10 | 13.50 |

Fig. 4. Concentration of O₂ in the head space of storage chambers for different diffusion channels during storage of Kolkata leaves at ambient temperature (28°C)

Fig. 5. Concentration of CO₂ in the head space of storage chambers for different diffusion channels during storage of Madras leaves at ambient temperature (28°C)

Table 3. Concentration of O₂ in the head space in storage chambers for different diffusion channels during storage of Kolkata leaves at ambient temperature (28°C)

| Diffusion Treatments | Dia. (mm) | Length (cm) | Concentration of O₂ (%) | Days of storage |
|----------------------|-----------|-------------|-------------------------|----------------|
|                      |           |             | 0th | 1st | 3rd | 6th | 9th | 12th | 15th | 18th |
| T₁                   | 5         | 5           | 21.10 | 19.63 | 18.90 | 17.87 | 17.21 | 16.80 | 15.76 | 13.65 |
| T₂                   | 7         | 7.5         | 21.10 | 19.45 | 17.87 | 16.54 | 15.95 | 14.98 | 13.56 | 12.00 |
| T₃                   | 9         | 10          | 21.10 | 18.78 | 17.65 | 16.54 | 15.11 | 14.11 | -     | -     |
Table 4. Concentration of CO\textsubscript{2} in the head space in storage chambers for different diffusion channels during storage of Madras leaves at ambient temperature (28°C)

| Diffusion Treatments | Dia. (mm) | Length (cm) | Concentration of CO\textsubscript{2} (%) |
|----------------------|-----------|-------------|-----------------------------------------|
|                       |           |             | Days of storage 0\textsuperscript{th} 1\textsuperscript{st} 3\textsuperscript{rd} 6\textsuperscript{th} 9\textsuperscript{th} 12\textsuperscript{th} 14\textsuperscript{th} |
| T\textsubscript{1}    | 5         | 5           | 0.03 0.60 1.40 2.50 3.40 4.40 4.80 |
| T\textsubscript{2}    | 7         | 7.5         | 0.03 0.37 1.30 2.20 3.10 4.43 4.80 |
| T\textsubscript{3}    | 9         | 10          | 0.03 0.50 0.90 1.70 2.50 3.40 3.60 |

Table 5. Concentration of CO\textsubscript{2} in the head space in storage chambers for different diffusion channels during storage of Kolkata leaves at ambient temperature (28°C)

| Diffusion Treatments | Dia. (mm) | Length (cm) | Concentration of CO\textsubscript{2} (%) |
|----------------------|-----------|-------------|-----------------------------------------|
|                       |           |             | Days of storage 0\textsuperscript{th} 1\textsuperscript{st} 3\textsuperscript{rd} 6\textsuperscript{th} 9\textsuperscript{th} 12\textsuperscript{th} 15\textsuperscript{th} 18\textsuperscript{th} |
| T\textsubscript{1}    | 5         | 5           | 0.03 0.27 0.85 1.45 2.21 2.94 3.87 4.87 |
| T\textsubscript{2}    | 7         | 7.5         | 0.03 0.30 1.20 2.34 3.67 5.10 6.50 7.87 |
| T\textsubscript{3}    | 9         | 10          | 0.03 0.43 0.98 2.54 3.76 4.87 - - |

Fig. 6. Concentration of CO\textsubscript{2} in the head space of Storage chambers for different diffusion channels during storage of Kolkata leaves at ambient temperature (28°C)

The headspace gas concentration in terms of O\textsubscript{2} in the storage containers (PET jars) with different diffusion channels during 18th days of ambient storage of Kolkata leaves are presented in Table 3 and Fig. 4. For diffusion channel of size and T\textsubscript{1} - 5 mm X 5 cm and T\textsubscript{2} - 7 mm X 7.5 cm the O\textsubscript{2} concentration decreased to 13.65 and 12.00 per cent respectively. For diffusion channel of size T\textsubscript{3} – 9 mm X 10 cm, the O\textsubscript{2} level decreased to 14.11 per cent on the 12\textsuperscript{th} day of storage up to which satisfactory quality of Kolkata betel leaves could to be maintained. At the beginning of the storage period, the rate of O\textsubscript{2} depletion was relatively slow which increased more rapidly towards the end of the storage period. From the study, it is clear that different varieties of betel leaves will have different rates of respiration for a particular size of diffusion channel and temperature. In other words varietal variation occurs concerning respiration rate under identical condition.

The concentration of CO\textsubscript{2} in the storage chambers of Madras leaves with different sizes of diffusion channels are presented in Table 4 and Fig. 5. There was a continuous increase of CO\textsubscript{2} gas with a duration of storage in all the
treatments. The rate of CO₂ builds up steadily increased with the progress of storage period reaching high CO₂ concentration of 4.80 per cent in T₁ and T₂ the treatments. For the diffusion channel T₃ the peak CO₂ levels inside the storage container were 3.60 per cent after 14 days of storage. These results are in agreement with the findings of Tynsong [9], who reported that the rate of CO₂ increases with the progress of storage period.

The concentration of CO₂ in the storage chambers of Kolkata leaves with different size diffusion channels is presented in Table 5 and Fig. 6, during ambient storage. There was a steady increase of this gas with the duration of storage in all the treatments. The rate of CO₂ increase was initially slow for about 3 days and then it increased with progress in storage period reaching high CO₂ concentration in all the treatments. For the diffusion channel T₂, the peak CO₂ levels inside the storage containers were maximum with 7.87 per cent followed by T₁ with 4.87 per cent. In the T₁ diffusion channel the CO₂ concentration was 4.87 per cent on the 12th day of storage up to which the quality of Kolkata betel leaves was satisfactory.

The above results are in accordance with the findings of the earlier investigators [10,11,12]. The above result indicates that respiration rate is a function of temperature for a particular variety of commodity and varies with temperature for any defined circumstances, higher storage temperature accelerated the ageing as the respiration rate increases with temperature. The reasons for deterioration may be due to the low level of O₂ concentration and higher levels of CO₂ concentration, as reported. From the experiments, it is observed that the concentration of O₂ decreased and CO₂ increased with an increase in the storage period.

4. CONCLUSION

Different rates of respiration for a particular size of diffusion channel and temperature, with different varieties of betel leaves, will have different rates of respiration. Respiration rate is a function of temperature for a particular variety of commodity and varies with temperature for any defined circumstances. Hence, higher storage temperature leads to ageing as the respiration rate increases with temperature. Therefore, any change in temperature will affect the rate of respiration and the equilibrium conditions within the package.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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