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A study on effect of storage of betel leaves at refrigeration temperature

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Abstract

A research study was conducted for studying the storage of Madras and Kolkatta varieties of betel leaves under refrigeration temperature. The storage study for the two varieties of betel leaves at refrigeration temperature (4 °C) under different diffusion storage systems was observed for different treatments. In the treatment T_2 for the Madras leaves, the concentration O_2 reduced to 17.00 per cent and CO_2 concentration increased to 2.40 per cent on the 18th day of storage at 4 °C. For Kolkatta leaves it was recorded that in the treatment T_2, O_2 concentration decreased to 13.89 per cent and CO_2 concentration increased from 0.03 to 4.23 per cent on the 21th day of storage at 4 °C. It was clear that at any given time of storage, the concentration of CO_2 in betel leaf samples was less at 4 °C compared to any other ambient temperature (28 °C) which could be attributed to the lower rate of respiration. Any change in temperature affected the rate of respiration and the equilibrium conditions within the package. Hence, it is clear that different varieties of betel leaves will have different rates of respiration for a particular size of diffusion channel and temperature.

Keywords: Storage, betel leaves, refrigeration temperature

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 (Fonseca *et al.*, 2005) [3]. 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 (Guha *et al*. 2004) [5].

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 Maharashtra are kallipatti, kapoori and bangla (ramtek) while important betelvine varieties cultivated in Orissa are godi bangla, nova cuttak, sanchi and birkoli. 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 (Lee and Kader, 2000) [6].

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 (Rayaguru *et al*. 2007) [7].

Betel leaves consumption is mainly in the 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.

Hence, improvements in the existing packaging and storage systems are to be explored for preserving the nutritional and medicinal qualities of the leaves. Therefore, there is a need to study the existing storage methods and exploring new methods of storage of betel leaves.

Material and Methods

Measurement of gas composition

O_2 - CO_2 Gas analyzer

O_2 - CO_2 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 O_2 - CO_2 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 electro motive 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 CO_2 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 CO_2 sensor range is 0-100 per cent. The sensor was calibrated before the actual experimentation.

Fig 1: O_2-CO_2 Analyzer

Respiration study of betel leaves

The respiration rates of the betel leaves were measured at refrigeration (4 °C) temperature. The experimental chambers, for respiration study, consist of ordinary glass jars of 2000ml capacity with airtight plastic lids. Teflon tape was wrapped around the mouth of the jars to seal the lids for air tightness. 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 inside the refrigerator maintained at 4 °C and the betel leaves allowed to respire inside the experimental chamber for known period and the O_2 and CO_2 gas concentrations inside the chamber were measured using a O_2-CO_2 gas analyzer (Make: PBI Dansensor, UK; Model: Checkmate II).

Storage condition

Refrigeration temperature (4 ^{0}C)

Pre-Treatment of betel leaves for storage in diffusion channel chambers

The two varieties of betel leaves (Madras and kolkatta) 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.

Diffusion channel storage

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 (Bird, 1960) [1].

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 with respect to 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 micro-organisms. 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.

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 atmosphere. Septum was firmly fixed on one side of the lid with araldite to facilitate sampling of the gases inside the jars for analyzing O_2 and CO_2 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 O_2-CO_2 analyzer once a day to know the O_2 and CO_2 concentrations. The physical condition of leaves was visually observed and the physiological loss of weight (PLW) was also measured during the storage period.

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 (T_1, T_2 and T_3) details are presented in Table 1.

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

The storage studies of the above treatments was carried out at refrigeration temperature of 4 ^{0}C (Fig 2). At refrigeration temperature storage, the leaves packaged in perforated polyethylene bags were used as control treatment for low temperature storage.

T_1 T_2 T_3

Fig 2: Experimental storage chambers (PET Jars) of 2 varieties of betel leaves with diffusion channels

Results and Discussion

Diffusion channel storage of the two varieties of betel leaves

The results of studies on storage of betel leaves at refrigeration temperature in different diffusion channels are presented below.

Effect of diffusion channels on headspace O_2 and CO_2 concentration at storage temperature storage for the two varieties of betel leaves

At 4 °C storage temperature, the changes in the head space gas concentration in terms of O_2 for Madras betel leaves in storage containers (PET jars) with different size diffusion channels during 18 days of storage are presented in Table 2 and Fig 3. The results indicated that the level of O_2 inside the storage chambers progressively decreased during the 18 days of storage.

Decrease in O_2 concentration was minimum in diffusion channel T_3 with 15.00 per cent followed by T_2 with 17.00 per cent. In diffusion channel T_1, O_2 concentration was high with 17.60 per cent.

At 4 °C storage temperature, the changes in the head space gas concentration in terms of O_2 in storage containers (PET jars) with different diffusion channels during 21 days of storage of Kolkatta leaves are presented in Table 3 and Fig. 4. The results indicate that the level of O_2 inside the storage chambers progressively decreased as storage period advanced. For diffusion channel T_2, the O_2 concentration decreased to 13.89 per cent followed by T_3 with 16 per cent and in T_1 O_2 concentration was 17.43 per cent.

The CO_2 concentration in different size diffusion channel storage chambers of Madras betel leaves at 4 °C are presented in Table 4 and Fig 5. The CO_2 build up was highest in T_3 with 3.20 per cent, followed by T_2 with 2.40 per cent and CO_2 build up was minimum in T_1 with 2.10 per cent on the 18th day of storage upto which there was satisfactory quality of betel leaves.

For Kolkatta betel leaves the CO_2 build up at 4 °C refrigeration storage in different diffusion channel chambers are presented in Table 5 and Fig. 6. The CO_2 concentration was highest in T_2 with 4.23 per cent followed by T_3 diffusion channel with 3.81 per cent and least build of CO_2 was in T_1 diffusion chamber with 3.76 per cent on the 21st day of storage. 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 with respect to respiration rate under identical condition. The above results are in accordance with the findings of the earlier investigators (Gonzalez, *et al.*, 1990; Yantarasri, *et al.*, 1995; Escalona, 2003) [4, 8, 2].

It was clear that at any given time of storage, the concentration of CO_2 in betel leaf samples was less at 4 °C compared to ambient temperature which could be attributed to the lower rate of respiration. Any change in temperature affected the rate of respiration and the equilibrium conditions within the package.

Conclusion

Betel leaves are used as a stimulant, an antiseptic and a breath freshener. Betel leaves consumption is mainly in the fresh form. However, limited shelf life is a great detrimant 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. Hence, improvements in the existing packaging and storage systems are to be explored for preserving the nutritional and medicinal qualities of the leaves. It was clear that at any given time of storage, the concentration of CO_2 in betel leaf samples was less at 4 °C compared to ambient temperature which could be attributed to the lower rate of respiration. Any change in temperature affected the rate of respiration and the equilibrium conditions within the package.

Authors' Contributions

This research work was carried out in collaboration among all authors. The work is based on author Srujana Shrunkala, 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), Bengaluru. India. All authors read and approved the final manuscript.

Table 2: Concentration of O_2 in the head space in storage chambers for different diffusion channels during storage of Madras leaves at refrigeration temperature (4 ^{0}C)

Diffusion Treatments	Dia. (mm)	Length (cm)	Concentration of O_2 (%)							
			Days of storage							
			0th	1st	3rd	6th	9th	12th	15th	18th
T_1	5	5	21.10	19.87	19.70	19.40	19.10	18.60	18.10	17.60
T_2	7	7.5	21.10	19.77	19.50	19.20	19.00	18.50	17.90	17.00
T_3	9	10	21.10	19.80	19.30	18.30	17.50	16.50	15.80	15.00

Table 3. Concentration of O_2 in the head space in storage chambers for different diffusion channels during storage of Kolkatta leaves at refrigeration temperature (4 ^{0}C)

Diffusion Treatments	Dia. (mm)	Length (cm)	Concentration of O_2 (%)								
			Days of storage								
			0th	1st	3rd	6th	9th	12th	15th	18th	21th
T_1	5	5	21.10	20.00	19.76	19.55	19.38	19.16	18.90	18.54	17.43
T_2	7	7.5	21.10	19.00	18.45	17.83	17.00	16.00	15.32	14.65	13.89
T_3	9	10	21.10	20.00	19.80	19.30	18.67	18.00	17.21	16.76	16.00

Table 4: Concentration of CO_2 in the head space in storage chambers for different diffusion channels during storage of Madras leaves at refrigeration temperature (4 ^{0}C)

Diffusion Treatments	Dia. (mm)	Length (cm)	Concentration of CO_2 (%)							
			Days of storage							
			0th	1st	3rd	6th	9th	12th	15th	18th
T_1	5	5	0.03	0.20	0.40	0.70	1.00	1.30	1.60	2.10
T_2	7	7.5	0.03	0.07	0.40	0.80	1.10	1.50	1.80	2.40
T_3	9	10	0.03	0.13	0.27	0.80	1.30	1.80	2.50	3.20

Table 5: Concentration of CO_2 in the head space in storage chambers for different diffusion channels during storage of Kolkatta leaves at refrigeration temperature (4 ^{0}C)

Diffusion Treatments	Dia. (mm)	Length (cm)	Concentration of CO_2 (%)								
			Days of storage								
			0th	1st	3rd	6th	9th	12th	15th	18th	21th
T_1	5	5	0.03	0.00	0.19	0.65	1.12	1.64	2.34	2.98	3.76
T_2	7	7.5	0.03	0.13	0.54	1.10	1.76	2.45	3.10	3.76	4.23
T_3	9	10	0.03	0.30	0.64	1.10	1.54	2.10	2.76	3.24	3.81

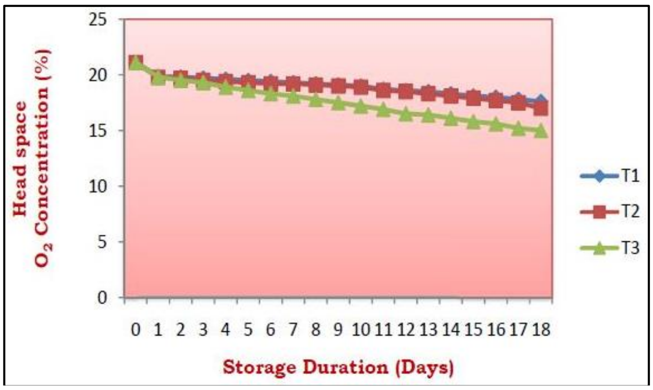

Fig 3: Concentration of O_2 in the head space of storage chambers for different diffusion channels during storage of Madras leaves at refrigeration temperature (4 °C)

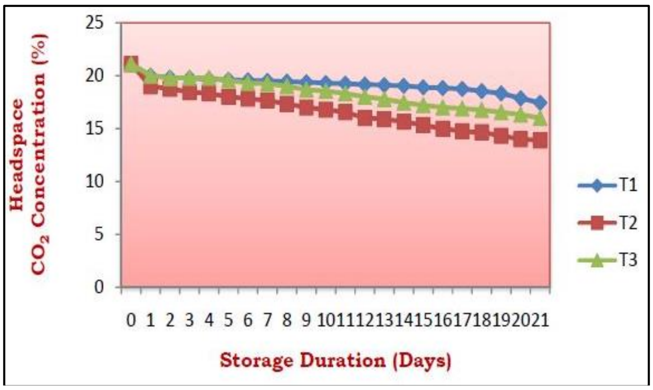

Fig 4: Concentration of O_2 in the head space of storage chambers for different diffusion channels during storage of Kolkatta leaves at refrigeration temperature (4 °C)

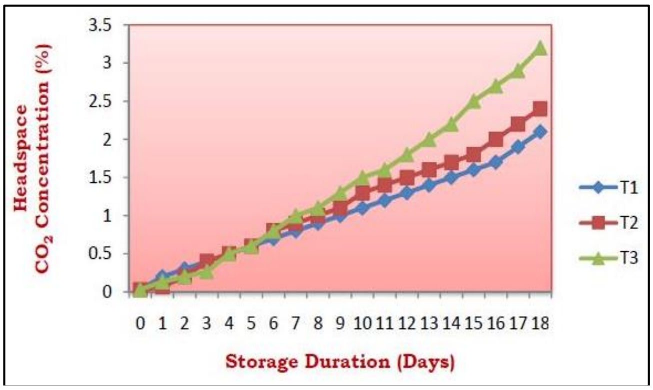

Fig 5: Concentration of CO_2 in the head space of storage chambers for different diffusion channels during storage of Madras leaves at refrigeration temperature (4 °C)

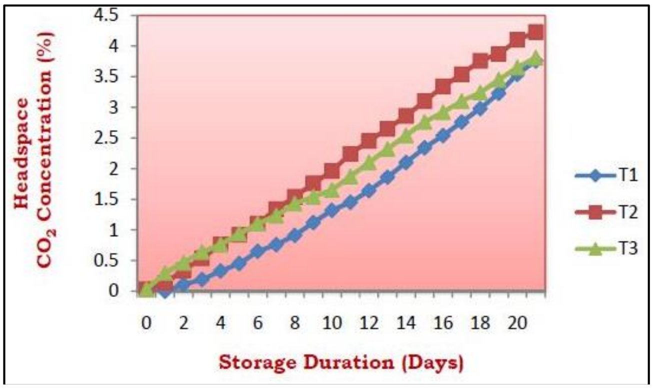

Fig 6: Concentration of CO_2 in the head space of Storage chambers for different diffusion channels during storage of Kolkatta leaves at refrigeration temperature (4 °C)

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