## ABSTRACT

Traditional curing methods for spices are laborious and consume a lot of time. So, it was found that there is a need to standardize the parameters of curing for vanilla. In this study, an attempt was made to standardize the curing procedure for vanilla. Vanilla curing consists of four steps viz: killing, sweating, slow drying and conditioning. Sweating and drying was carried out in aconvective dryer at 45 °C, 50 °C, 55 °C and 60 °C instead of sun drying in the traditional (Bourbon) method. The quality of cured beanswas also analysed for vanillin content. The vanillin content for the five samples were 1.07%, 1.59%, 1.79% and 1.63% respectively for treatments at 45 °C, 50 °C, 55 °C and 60 °C. The sample which was sun dried had vanillin content of 1.75%. Moiosture content of the four convectively dried samples were 14%, 20%, 22% and 19% respectively. The sun dried sample had moisture content of 25%. Microbial infestation by fungus of *Aspergillus* sp. and *Fusarium* sp. was also identfied in one of the samples. Shelf life studies on cured vanilla under Modified Atmospheric Packaging was also studied with different gauge (125, 250, 400) polypropylene bags. The extract of vanilla was recovered by the cold extraction process and was found to have a vanillin content of 1.05% by weight.

#### INTRODUCTION

Vanilla, member of the orchid family is known as 'orchid of commerce' since it is a high valued crop. The fully grown pods of the orchid, *Vanilla planifolia* is the valued product. The beans are processed to extract the flavour, which is widely used in foods confectionary, pharmaceuticals and beverages.

Indonesia provides about 50% of the world supply and the rest from Madagascar, Mexico, Tonga as well as Comoro and Reunion. In India, Karnataka occupies the largest area of vanilla cultivation with 1,465 hectares followed by Kerala (812 hectares) and Tamil Nadu (268 hectares). Mainly three countries dominate vanilla imports from India viz. The United States of America, United Kingdom and France (Anon 2004 a). The export of vanilla from India in the year 2003-04 was estimated to about Rs. 3606.35 lakh (@ 13870.58 Rs/Kg). The quality standards demanded for export to the international markets are high. But processing technologies for vanilla are still primitive in India.

The vanilla beans are harvested when they are just ripe. Free vanillin is not present in green beans. This is developed as a result of enzymatic activity during the curing process. Curing is the process of alternate sweating and drying the beans until they lose moisture to a predetermined level. The beans of the vanilla need to be cured for better quality. The moisture content of beans after curing, conditioning and storage conditions affect the quality of beans. The quality may also vary with the place and condition in which the plant grows, where they are processed etc.

The traditional curing methods involves killing, sweating, slow drying and conditioning (Sudarshan, 2002). The general practice followed by the farmers does not have any restrictions with respect to the parameters followed in the curing process. Hence there is a need to standardize the parameters, such as temperature, duration, moisture content and relative humidity etc. This can help the produce to compete in local as well as the international market.

The vanilla beans cured by the traditional methods are tied in to bundles and wrapped in bee wax paper. These wrapped bundles are then stored in wooden boxes. This is generally termed as the conditioning process, done for three to four months. The stored vanilla should be preserved to retain its aroma. Till date no standard conditions for storage or packaging material has been identified for the cured vanilla beans. In this context various methods for storage can be tried out for enhancing the shelf life of cured vanilla beans. Modified atmospheric packaging (MAP) could be an ideal storage method to extend the shelf life and quality of the beans with minimum cost. Modified atmospheric packaging is defined as the packaging of perishable product in an atmosphere, which has been modified, so that its composition is other than that of air. There is commodity generated or Passive MAP and gas flushed or active MAP. In the passive MAP a matching micro atmosphere related to respiratory characteristics of the product is obtained (Kader *et al.*, 1989). This type of a storage system was found to be much suitable for the storage of spices. In the gas flushed MAP, there is use of selected concentrations of gases like CO<sub>2</sub> and N<sub>2</sub>. The active MAP is used generally for perishable produces like fruits and vegetables (Labuza *et al.*, 1989). Availability of packaging films has also added extensively to MAP technology. A large number of packaging materials or polymeric films with varying perm abilities have become an aid to the MAP technology. (Ooraikul, B. and Stiles, M. E., 1995). During storage the cured vanilla beans are attacked by various micro-flora and microorganisms, which cause deterioration to its quality as it affects the appearance and flavour characteristics. Fungus and mites are the general infection causing organisms.

Presently vanilla in the form of raw or cured beans has found market in India. Value addition and the production of by products from vanilla could be a boost to the income of vanilla growers. Vanilla can be used to produce a large number of by products like vanilla extract, vanilla powder, vanilla tincture and vanilla absolute. This could add to the employment opportunities to many rural youth and thus become a supplement to the economy.

With this background, an attempt has been made at K.C.A.E.T, Tavanur with the following objectives

1. To study the parameters for standardization of vanilla curing

- 2. To study effect of Modified Atmospheric Packaging on cured vanilla
- 3. To analyse quality of stored cured vanilla.
- 4. To produce vanilla extract.

# **REVIEW OF LITERATURE**

This chapter refers to some of the works done in the past years and some general information regarding the subject.

#### 2.1 Harvesting

The beans or pods are ready for harvest 6-9 months after flowering. The pods may be 12-25 cm long (Pruthi, 2000). The stage of harvest is important in the quality of the processed beans. The beans are harvested when they are just ripe and at this time the blossom end of the fruit turns yellow. If picked early, inferior beans will be produced. If picked too late, the beans will split at blossom end (Sudharsan, 2002).

#### 2.2 Post harvest technology

The primary quality requirement is the aroma and flavour characteristics of cured vanilla beans. The commercial top quality beans are required to have a black or brown colour, soft and flexible to touch, they need to be fleshy, supple and oily in appearance. They should not bear any scars or blemishes. The moisture content should be about 30-40%. The low quality beans are usually hard, dry, thin, brown or reddish brown with poor aroma. It has to be kept in airtight boxes to avoid loss of aroma. Small crystals of vanillin are formed on the surface of beans during storage and it is regarded as an indicator of good quality. Vanillin content is up to 3.5% in superior quality beans. (Menon, 2002)

#### 2.2.1 Curing methods

Fresh vanilla beans do not have any flavour or aroma, as vanillin and other chemical substances responsible for imparting the flavour are not present in free form at the time of harvest. During curing, free vanillin is developed in the beans as a result of the series of enzymatic actions on several glycosides. Simultaneously various aldehydes, aromatic esters, prochatechic acid, benzoic acid, vanillic acid anisic alcohol is also formed. All these components together give the fragrance of natural vanilla. Many curing processes have been developed in various vanilla growing countries to meet the quality requirements of the vanilla market. Curing is characterized by four phases (Theodose, 1973).

#### 2.2.1.1 Killing

This process stops further vegetative development in the fresh bean and initiates the onset of enzymatic reaction, which is responsible for the production of aroma and flavour. Killing is indicated by the development of a brown coloration in the bean.

#### 2.2.1.2 Sweating

This involves raising the temperature of killed beans to promote the desired enzymatic reaction and to provoke a first, fairly rapid, drying to prevent harmful fermentation. During this operation the beans acquire a deep brown coloration and become quite supple, and the development of aroma is perceptible

## 2.2.1.3. Slow drying

The third stage entails slow drying at ambient temperatures usually in the shade, until the beans have reached about one third of their original weight.

#### 2.2.1.4 Conditioning

The beans are stored in closed boxes for a period of three months or longer to permit the full development of desired aroma and flavour.

The various methods of treating vanilla pods are: -

#### 2.2.2 Indonesian process

In the process, the curing is done much more quickly with beans cured over a smoky fire.

#### 2.2.3 Bourbon process

In bourbon process (Purseglove *et al.*, 1988) bamboo baskets with beans are immersed in hot water (63-65°C) for 3 minutes. After rapidly draining the water when the beans are still hot, they are kept in wooden boxes lined with blankets. They are then spread in the sun on dark colour cotton covers for 3-4 hours and later rolled up to retain the heat and stored in wooden

boxes. The process repeated for 6-8 days, during which beans loose some weight and become supple. Later the beans are dried by spreading them out in wooden trays under shade in an airy location. The duration of drying varies according to the size of the beans and usually lasts for 15-20 days. Properly dried beans are kept in closed containers where the fragrance is fully developed. Finally they are graded according to size and kept in iron boxes lined with paraffin wax paper. Properly cured vanilla beans contain about 2.5% vanillin.

#### 2.2.4 Guiana Process

In Guiana process (Anon., 2004) the pods are collected and dried in sun till they shrivel. Later they are wiped and rubbed with olive oil. The ends are tied up to prevent splitting and then bundled.

#### 2.2.5 Mexican process

In this two forms of curing are employed. They are sun wilting and oven wilting procedures (Anon, 2004).

#### 2.2.5.1 Sun wilting

Fresh beans are set aside a few days in a store and during this time, the beans shrivel. The beans are then killed by exposing them to sun for a period of about five hours on the day after sorting. Fresh beans are spread out on dark blanket resting on wooden racks. The beans become hot by afternoon and are then covered by edges of blanket. In mid to late afternoon before beans begin to cool, the thick ends are laid towards the centre of blanket and rolled up. They are then immediately taken indoor and placed in blanket lined mahagony boxes to undergo first sweating. Blankets are placed over the boxes to prevent heat loss. The beans acquire a dark brown colour indicating good killing. Then the beans are subjected to alternate sun drying and sweating.

In first stage of sun drying or sweating there is fairly rapid drying in which sun drying are given virtually every day and several overnight sweating until they become supple. This takes about 6 days. Sorting in to lots is done at this stage. In second phase, sun drying is not carried out daily. Then after 20-30 days after killing, the bean becomes supple and resemble closely the final product. Then it is subjected to slow drying in indoors. After the slow drying, the beans are

conditioned. They are straightened by drawing them through finger. This is useful in spreading oil to give the bean characteristic lustre. The beans are tied into bundles and wrapped in wax paper and placed in metal conditioning boxes. Conditioning is done for three months.

#### 2.2.5.2 Oven wilting

In this procedure, specially constructed brick or cement room, known as a colorifico, which serves as autoclave is used. The room measures approximately 4×4×4 metres and incorporates a wood fired heater, which is stoked from outside. The beans to be cured are piled up and then rolled in blankets and covered with matting to form mallata. The mallatas are moistened and placed in the calorifico. In about 12 hours, temperature inside calorifico reaches 60°C. A temperature of 70°C is attained and is maintained for 8 hours. The mallatas are removed after 36 hours in the calorifico. Matting is quickly stripped from mallatas and blanket wrapped beans are placed in sweating boxes. After 24 hours, the beans are removed and inspected. They are then subjected to repeating sunning and sweating.

## Research highlights

Abdulla (1997) conducted studies on drying of vanilla pods using a green house effect solar dryer, and found, at RH of 34 % and temperature range of 50 to 60 <sup>o</sup>C time needed for drying vanilla pod from moisture content 80.9 %(wb) initial to 37.8 % (wb) was 51.3 hours or seven days as compare to 12 to 15 days in sun drying.

Ansaldi *et al.* (1990) developed a method of killing in which the beans are frozen by dipping in liquid Nitrogen or by holding the beans for a few hours in a freezer ( $0^{\circ}$ C to –  $80^{\circ}$ C).

Arana (1943); Theodose (1973) concluded that the stated purpose of various killing methods is to bring out the cessation of vegetative life of the vanilla bean and allow contact between enzymes and substrates.

Arana (1944) compared traditional sun-drying/sweating procedures with an electric oven set at 45 °C in which the humidity was kept high. Oven sweating and drying was found to have

advantages in that the incidence of mould was less, a shorter time was required and the procedure was less labour-intensive.

Arana (1944) and Jones and Vincente (1949c) showed that the common practice of harvesting green beans does not flavour the production of cured vanilla with a fine aroma and flavour or a high vanillin content. The best results are obtained with beans harvested at the blossom-end yellow phase.

Balls and Arana (1941) conducted the sweating of vanilla beans by holding them at high humidity and high temperature (45 to 65<sup>o</sup>C) for 7 to 10 days. They concluded that the purpose of sweating is to retain enough moisture to allow enzymes to catalyse various hydrolytic and oxidative processes.

Corell (1953); Bouriquet (1954) developed a system of seven grades for export of whole beans, in descending order of quality as: Extra, Superior, Good Superior, Good, Medium Good, Medium and Ordinary. This was based on the moisture content, colour, general appearance and aroma quality.

Dignum *et al.* (2001) conducted vanilla curing under laboratory conditions in which the cured vanilla beans were analysed for enzyme activity and aroma. The activity of the enzyme was highest in green beans. They concluded that the normal scalding leads to inactivation of non-specific glucosidase while the prolonged scalding also inactivates the specific glucosidase.

Dignum *et al.* (2001 a) proved that the storage of frozen beans must be carried out at -70°C or below to preserve the viability of enzymes that are involved in the curing process.

Havkin-Frenkel *et al.* (2003) conducted studies on the botany of vanilla beans which revealed that flavour precursors are found in the bean interior while the enzymes which catalyse the release of the flavour precursors to the flavour compounds are localized mostly in the outer fruits wall region.

Manjusha, M. *et al.*,(2006) Conducted study on Vanilla beans cured by Bourbon method, Hot water killing followed by high temperature convective drying, and hot water killing followed by low temperature convective drying. The moisture kinetics in each curing stage was also studied.

Theodose (1973) reported a curing method in which beans are not chopped until after killing by scalding and an initial sweating. The killed beans are then sliced into 2-3 cm in lengths and are subjected to hot-air drying at 65°C in a tunnel drier. Then they are sweated in boxes for 24hrs at 50°C, for 12 days. The moisture content of the product obtained was found to be 20-25%.

#### 2.3 Storage studies

#### 2.3.1 Modified Atmospheric Packaging

The shelf life of perishable goods is increased by the reduction in the atmospheric oxygen, which results in growth of microorganisms. These microorganisms cause a change in colour, flavour, texture and thus deterioration in quality. Atmospheric composition of air is 78%  $N_2$ , 21%  $O_2$ , and less than 0.1% CO<sub>2</sub>. Modification of atmosphere within the package by reducing oxygen and increasing levels of CO<sub>2</sub> or  $N_2$  has been shown to extend shelf life of perishable foods

MAP has been found advantageous in the storage of food products. This method helps in greatly increasing the shelf life of the product stored. It improves the presentation of the product. This means that the packaging allows a clear view of the product. The packaging is hygienic. It is also stackable. The product is completely sealed and free from odour. There is no need of chemical preservatives. By using MAP there is a reduction in the storage costs due to better utilization of space and labour.

Apart from its advantages the MAP also has some major disadvantages. The capital cost involved in gas packing is high. The cost involved in the gases and packaging material is also

unaffordable. The pack volume increases which may result in the increment of transportation cost. Another major disadvantage is that the benefit of MAP is lost once the pack is opened. MAP can be classified into different systems.

#### 2.3.1.1 Active modified atmospheric packaging.

The active MAP can be established by withdrawing air from package system with vacumization and by back flushing with selected gas mixture the advantages of active modification of micro atmosphere is the rapid establishment of desired gas mixtures. Adsorbents and absorbents may be included in the package system to reduce O<sub>2</sub> and CO<sub>2</sub>, ethylene and vapour. This kind of packaging is generally used in case of highly perishable goods like minimally processed vegetables and fruits (Labuza *et al.*, 1989).

#### 2.3.1.2 Passive modified atmospheric packaging.

The passive atmospheric packaging is also called the commodity generated modified atmosphere. In this there is matching of the commodity respiratory characteristics with gas permeabilities of the packaging system so that a suitable equilibrium micro atmosphere can be evolved (Kader *et al*,1989). This is through the consumption of  $O_2$  and evolution of  $CO_2$  in respiration process. This system of packaging can be adopted for some keepable commodities. This could be tried out for spices also.

#### 2.3.1.3 Vacuum packaging

It is the earliest form of modified atmospheric packaging developed commercially used for products such as meat cuts, cheeses and ground coffee. It is not suitable for bakery products since it causes deformation of the product (Parry,R.T. 1999). The process involves packaging the product in film of low oxygen permeability and sealing after evacuating the air. Under good vacuum conditions the oxygen level is reduced to less than 1%. Due to barrier properties of films used, entry of oxygen from outside is restricted.

## 2.3.2 Factors affecting MAP.

In processed food products like bakery products the main factors resulting in spoilage are characterised into two. They are the intrinsic and the extrinsic factors.

#### Intrinsic factors

These are the internal physical properties of the product which include the acidity, moisture content, water activity, nutrient content, occurrence of anti microbial compounds and the oxidation reduction potential

#### Extrinsic factors (environmental)

These are the environmental factors like the relative humidity, the gaseous components and temperature.

As water activity increases chances of spoilage due to bacteria, yeast and moulds increases. pH of food is also important in controlling microbial spoilage. A low pH favours growth of yeast and mould while neutral and alkaline pH foods are spoiled by bacteria (Parry, R.T. 1999).

MAP was done on the processed food by increasing  $CO_2$  content inside packaging. The air inside was replaced with inert gases such as  $N_2$  also or a mixture of  $CO_2$  and  $N_2$  was used.  $CO_2$  is used as bacteriostatic agent and  $N_2$  prevents collapse of package after  $CO_2$  is absorbed into product (Ooraikul, B. and Stiles M. E., 1995).

MAP controls biochemical and degradation process and slows oxidation. It reduces growth of mould and bacteria. It increases shelf life of product by reducing moisture loss.

### **Research highlights.**

Gerson et al (1988) reported that micro perforated film for over wrapping whole fruits and vegetables has been used to provide relatively high air flow into the micro atmosphere. This was to ensure that adequate O2 is available for respiration even under temperature abuse conditions.

Paine, F.A. (1987) concluded that with the advance of engineered plastic films with wide range of permeabilities, beneficial modified atmosphere can be attained with fresh fruits and vegetables in flexible films.

Rolle and Chism (1987) pointed out that tissue injury due to physical impact brings about stress ethylene production, increased respiration rates and initiates deteriorative enzyme reaction. Therefore for MAP, fruits and vegetables must have no fissures in the epidermal tissue and if present, only minor bruises. Hand picked cultivars are generally preferred for MAP.

Shewfelt, R. L. (1987) studied that for prolonging shelf life of fresh commodities under MAP, high quality standards must be established for the input produce. Colour, flavour and tissue integrity are some quality factors that need to be considered. These are governed by the cultivar, growing conditions and maturity at harvest.

## 2.3.3. Packaging materials commonly used in MAP

Plastics are usually the materials used in packaging. It is used mainly because they are less bulky and have excellent barrier properties to moisture, odour, oxygen and gases so a desired shelf life can be obtained. They do not promote bacterial growth (Athalye, 1992). The major plastics used are polyethylene, polystyrene, BOPP, LDPE, LLDPE etc. There are different gauges for the plastic films available in the market. They are as follows: -

Microns(µ )	Millimetres	Gauge
25	0.03	100
40	0.04	160
50	0.05	200

Table 2.1 Thickness of plastic
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Microns(µ )	Millimetres	Gauge
60	0.06	240
75	0.07	300
100	0.10	400
125	0.125	500
175	0.175	700
250	0.25	1000

 $100 \text{ Gauge} = 25 \mu = 0.025 \text{ mm}$ 

Polypropylene, polystyrene, Biaxially oriented polypropylene has excellent aroma retaining properties and thus they can be used for packaging spices.

## 2.4 Microbial infestation on stored products

There may be attack of bacteria and fungus like organisms on the stored processed material. The load of micro organisms on their material can be calculated by standard procedures. Studies on microbial load on stored cured vanilla have not been done but studies on processed pepper are available

A study done by Bimi *et al.*, (2004) gave the following findings. Pepper was sun dried unhygienic practices followed by the farmers like drying in cow dung plastered soil or mats, add microbes and extraneous matter on the berries

#### 2.4.1 Shelf life of spices

Extension of shelf life in fresh or processed state requires a decrease in the metabolism and inhibition of both microbial growth and oxidation. Spice flavour is usually concentrated in the resin canals or epidermal glands. Effects of storage conditions are of concern with view of retension of flavour contributing oils.(Subbulskshmi *et al.*, 2002). Post harvest water loss and storage quality of pepper has been studied by Iowands et al (1994). Water loss rate

was found to be lower at 20°C. The coluor development depended on the package used. Peter, K. V (1997) has emphasized clean spices as an integral part of food security research attainment. Spices are amenable biologically and are arich source of viable bacteria. Out of this some may be pathogenic and toxigenic.

#### 2.5 Spoilage of vanilla.

#### 2.5.1 Moulds

Vanilla beans are quite susceptible to infection by *Penicilium sp.* and *Aspergillus sp.* moulds and this generally occurs during the conditioning and subsequent storage periods (Bouriquet, 1964). In appearance, there are two types of moulds: one is white at first and turns green later, while the other is black and grows very fast. Infection always begins at the stem end of the beans and if left uncontrolled, the whole bean becomes wrinkled, dry, and acquires a disagreeable odour. It is virtually impossible to eliminate the odour once the mould takes hold and this considerably reduces the market value. Mould infection most frequently occurs with beans, which have been harvested before they mature.

Development of the mould is also encouraged if beans have not been killed properly as they do not dry uniformly, and if the beans have excessive moisture content on conditioning. Sweating and drying the beans in the sun also leads to a higher incidence of moulds than when an oven is used. The other contributors are dirty blankets and a general lack of cleanliness and ventilation in the curing room. Apart from ensuring that the curing is carried out properly, other preventive measures include regularly sterilizing equipment in boiling water blankets in aseptic solutions. If mould attack is severe, it may be necessary to paint and disinfect the curing room with 1:1000 solution of formaldehyde (Childers and Cibes, 1948). Another form of transmitting moulds is by packers who place the binding strings in their mouths. Beans should be examined weekly during the conditioning and storage to permit removal and treatment of mouldy ones. If mould infection is identified and treated immediately it can be checked. A common treatment for the beans suffering from initial attack by moulds is to clean the bean with a cotton swab soaked in 95% alcohol. With cases of slight infection in Mexico, it is customary to wipe them with oily secretion of any mature beans and the moulds have taken a good hold, the beans are immersed in hot water for an hour. If mould attack is severe, it is usual to cut or distract the infected portion. The remaining portion is called the cut.

# 2.5.2 Infestation

Vanilla beans are prone to attack by mites of the *Tyrophagus* species, which imparts a disagreeable odour to the beans. The mites appear during conditioning, shipment or subsequent storage and may be detected by the small holes, which they make in the beans. In case of limited infestation, alcohol treatment or sunning may be effective. Fumigation of the equipment and the conditioning room with flowers of sulphur may also help.

# 2.5.3 Cresoted vanilla

Some vanilla may develop cresote like aroma, which is impossible to eliminate once it is formed. It appears at a quite early stage of the sweating process. The principal cause is believed to be the improper storage methods of fresh beans before killing. Preparation of vanilla products the quality of vanilla extract is dependent upon a number of factors that include:

- 1. Careful handling and storage of the beans prior to extraction.
- 2. Appropriate blending of the beans and their selection.
- 3. The degree of comminution of the beans
- 4. The method and condition of extraction
- 5. Proper ageing of the extract to allow full development of flavour.

# 2.6. Extraction processes

Extraction is the process by which the vanillin content of the cured vanilla beans is recovered. The vanilla extract is used in a number of culinary, medicine and perfumery. Extraction is done in a number of ways out of which extraction by maceration and cold extraction is widely used

## 2.6.1 Extraction by maceration

This is the traditional process of vanilla extract and involves placing of the chopped beans in a vessel where they are allowed to steep in menustruum up to 1 year. Wooden barrels and 50% ethanol content menustruum was used. This process produced a vanilla extract of good flavour but of a non standard quality. Modern macerators are air tight vessels, made of stainless steel, tin or lined glass, which permit slow agitation by stirring, rocking or tumbling. This can give a good quality product in a time of 1-3 months.

#### 2.6.2 Extraction by percolation

The equipment for this has a stainless steel vessel fitted with a series of perforated trays to hold the chopped beans. The menustruum is sprayed on to the top of the tray and is allowed to percolate down. Some vessels are fitted with a hot water jacket so that extraction can be done at a slightly elevated temperature of 38-49°C. Merory (1968) described an operation to prepare two-fold vanillin extract with 20g per 100ml with 35% ethanol content. Initial menustruum had 98% ethanol water and glycerine. The menustruum was loaded in the extractor and was circulated twice daily for 8-10 days. The extractor is then drained for the second extraction; warm water at 60°C was poured into the extractor. The temperature drops in subsequent days and ethanol content becomes 30%. Third extract is prepared by repeating the same process. Then the beans are washed and the extracts are mixed together to get an extract of 35% ethanol concentration. Then it is aged for 30 days to 90 days to get a modification in the aroma and flavour.

# 2.7.0 OTHER PRODUCTS OF VANILLA

## 2.7.1 Vanilla Flavouring

This is similar to vanilla extract but contains less than 35% ethyl alcohol by volume (Pruthi, 2000).

#### 2.7.2 Vanilla Tincture

This is prepared by maceration from one part of vanilla beans by weight to ten parts of aqueous alcohol by volume and contains added sugar. It differs from vanilla extract in having an ethyl alcohol content of at least 38% (Felter and Lloyd, 1898).

## 2.7.3 Vanilla Oleoresin

Oleoresin is the solid or semisolid residue obtained by the solvent extraction of vanilla followed by complete removal of the solvent by distillation under vacuum. Extraction is carried out either in a percolated vessel or in a sealed vessel. The prepared solvents are 50% ethanol and 50% aqueous iso-propanol (Purseglove et al., 1988).

## 2.7.4 Vanilla Powder

It is a mixture of vanilla oleoresin with sugar, food starch or gum acacia (Pruthi, 2000).

## 2.7.5 Vanilla Absolute

This is prepared by direct alcohol extraction of vanilla beans followed by solvent stripping or by alcohol washing of an oleoresin prepared by extraction with a hydrocarbon solvent. This is most concentrated form of the vanilla aroma, being 7to 13 times stronger than good quality vanilla beans (Purseglove et al., 1988).

#### **MATERIALS AND METHODS**

This chapter deals with the methods of curing procedure adopted in the experiment. A complete description of the equipments is also given in this chapter.

#### 3.1 Raw Material

Fully matured, fresh vanilla beans (variety *Vanilla planifolia*) purchased from Vettilappara, Malappuram district were used for curing, under different treatments, maturity was justified by observing the yellow colour at tips of the beans

## 3.2 Experimental Set up

The experimental set up for curing vanilla beans mainly includes the following components viz; water, black blanket, white cloth, convective drier, wooden box, wooden shelf, thermometer and hygrometer.

#### **3.2.1 Water**

Hot water was used for killing the vanilla beans.

## 3.2.2 Black blanket

Ten black woollen blankets each having dimensions 135 x 80 cm was used for the sweating of vanilla beans

## 3.2.3 White cloth

White mill cloth of size 115 x 100 cm was used for sweating of the beans. This was cut into twelve equal parts each of dimensions

## 3.2.4 Convective Drier

The convective drier available in Product Analysis Laboratory was used in convective drying. Three trays each having dimensions 60 x 60 cm were used.

## 3.2.5 Wooden box

A wooden box made of teak having dimensions 152 x 60 x 60 cm was used for sweating and conditioning of beans

## 3.2.6 Thermometer

A mercury thermometer with LC of 1<sup>o</sup>C was used to measure the temperature at various stages of curing.

## 3.2.7 Wooden shelf

A wooden shelf of dimensions 122 x 30 x 182 cm was used for slow drying of the beans. It consisted of five racks with each rack made of nylon mesh (opening size- 3mm & diameter of wire - 0.75 mm), enclosed within a wooden frame of dimensions 122 x 30 cm.

## 3.2.8 Hygrometer

A digital hygrometer was used to determine the RH at various stages of curing.

## 3.2.9 Vernier caliper

A vernier caliper of LC 0.02 mm was used to measure the thickness of the vanilla beans.

#### **3.3 Preparation of the sample.**

Fresh bean were washed soaked and sorted on the basis of lengths into different grades. Two grades of sample were obtained which are B and C grade based on lengths. B grade samples had a length greater than 15 cm and C grade with lengths less than 15cm.the samples were marked respectively. Each of the samples of grade B and grade C were divided into 5 samples. They were marked as B 45, B 50, B 55, B 60, B BB and C 45, C 50, C 55, C 60, C BB respectively. B 45, B 50, B 55, B 60 corresponds to treatments of drying at 45 °C, 50 °C, 55 °C, 60 °C and B BB treated by Bourbon method. Similarly C 45, C 50, C 55, C 60 corresponds to treatments at 45 °C, 50 °C, 55 °C, 60 °C and C BB treated by Bourbon method.

# **3.4 Determination of initial moisture content of the beans**

Three samples of the vanilla beans approximately weighing 10g each were taken. These were sliced and put in moisture cans labelled accordingly. Initial weights of the cans were noted. The cans were placed in the oven for drying for 24 hrs at 105 °C. Drying process was continued till constant weight is achieved. The dry weights of the samples were taken. And moisture content both in dry basis and wet basis was found out

#### 3.5 Curing

#### 3.5.1 Traditional process of curing.

Curing of the beans were performed under the traditional method or the Bourbon method In this the beans were killed in hot water. The curing was done by drying in the sun for 8 days. Slow drying was also performed on the beans. Then the cured beans were conditioned and packed in appropriate storage material.

Killing: It is done with hot water at 65°C. The duration is 2-3 minutes. Excess water was wiped off using muslin cloth. Killing process is shown in Plate 1.

Sun drying: Temperature of killed beans were raised by wrapping in black woollen blankets lined with white cloth and stored in air tight wooden box for 24 hrs. This was then followed by exposing to the sun for 1- 1.5 hrs during the day on a raised platform erected about 75-100cm above the ground. This process was repeated for 10

#### **Plate 1: Sorting of fresh beans**

## Plate 2: Killing using hot Water

days. This treatment was done for the samples for traditional curing namely, B BB and C BB. Sun drying shown in Plate 3

## 3.5.2. Improved method.

In this method the killing is proceeded in the same way as in the case of Bourbon method. but the subsequent sweating and drying process is done by using convective dryer, instead of sun drying.

## 3.5.3. Alternate Sweating and Drying

Convective drying was done on the samples. Drying was done for the temperatures of 45 °C, 50 °C, 55 °C, 60 °C. As discussed earlier, B and C grade samples were separated into four parts for the drying. Drying was done for 1hr at 12hrs interval to reduce the moisture content of the sample to the required level. After each drying the beans were immediately wrapped in the black woollen blankets lined with the white cloth. This process is termed as the sweating. This

process of sweating was conducted on each of the sample after each drying till the required condition is obtained. Drying shown in Plate 4 and sweating in Plate 5.

## 3.5.4 Slow drying

This was done on perforated trays. The air should have a relative humidity of 80%. This RH was maintained by hanging wet cloth in the slow drying room and by keeping trays of water beneath the racks. The beans were checked regularly for mould growth and turned upside down for uniform drying. Their dimensions were measured every 12 hrs. The duration of this was 10 days. Slow drying shown in Plate 6.

## Plate 3: Sun drying for bourbon process

**Plate 4: Convective drying** 

# Plate 5: Sweating in wooden box after drying

Plate 6: Slow drying in racks

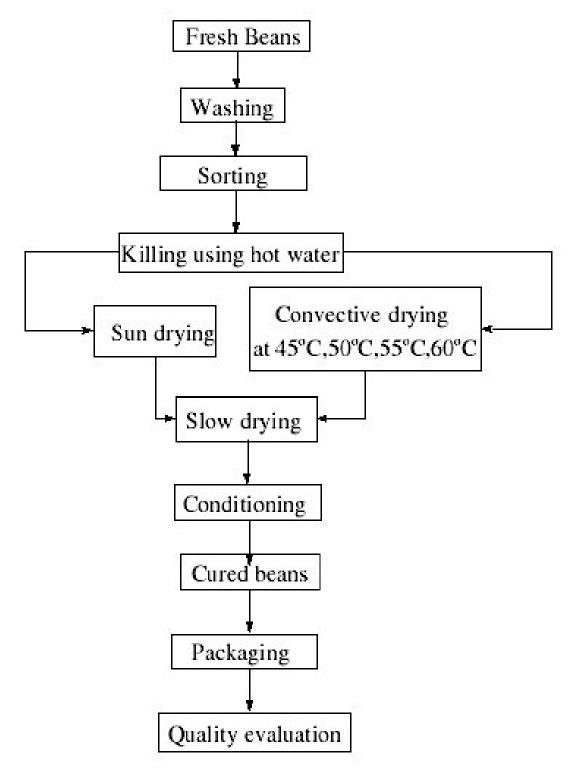


Fig 3.1 Flow chart showing vanilla curing process

3.5.5 Conditioning

The beans were bundled and tied at both ends using threads. To avoid infection from the threads these were washed and sterilised in hot water at 100 °C. They were packed and stored in the airtight boxes to allow full development of aroma and flavour. Duration was 30 days.

## 3.6 Measurement of thickness of the sample

Among each of the sample three beans were labelled and their thickness was recorded regularly every 24 hours using a vernier calliper of least count of 0.02

## Packaging of

## vanilla

3.7

In order to study the packaging qualities and shelf life of cured vanilla, the vanilla cured by the above processes were put in to bags made of polypropylene. Three different gauges of polypropylene bags were used. They are 125, 250, and 400 gauges. Each of the ten samples of the cured vanilla were divided to three and packed in the three different plastic materials. The packets were sealed using the sealing machine available in the Product Analysis lab. The storage study was conducted for three months. This was done to study the aroma retention and contamination prevention qualities of material used. Packed samples shown in Plate 7.

# 3.8

### **Microbial**

## infection studies.

Previously cured samples of vanilla and cured samples collected progressive farmers were found infested with fungus. The infested samples were used for identification. The infested part showing fungal growth, seen as a white patch were separated and made into bits. These bits were then cultured in Potato Dextrose Agar media (PDA). 250g of potatoes were sliced and cooked in 500 ml of water till extract is obtained, keep adding water and make the extract to 500 ml. In another 500 ml of water mix the dextrose (15g) and agar (15g) and heated till agar melts. These two are then mixed and sterilized in an autoclave at 15-pound pressure for twenty minutes at 120°C. The media is poured into sterile petri dishes under sterile conditions. Eight petri dishes were taken out of which 4 of the media had antibiotic streptomycin added to it to prevent bacterial contamination.

## 3.8.2 Inoculation of fungus

The pieces of samples were surface sterilized with 0.1% mercury chloride solution. Forceps are sterilized by showing it to a flame of a Bunsen burner. The samples are taken using forceps and washed thoroughly in mercuric chloride solution and then washed in sterilized water to remove the traces of mercuric chloride completely. Then three to four bits of sample are put into the petri dishes and were scaled up completely and kept aside for the fungus to grow. The fungus was seen to grow after four to five days. It was then taken for secondary culture to get an isolated sample. It was then stained and the fungus was identified.

## **3.8.3** Serial dilution method for the preparation of fungal slides

In this method the sample with the fungal growth was isolated and cut to samples weighing 1g. This was then put into 100 ml of sterile water in a conical flask. The flask is placed in a shaker for twenty to thirty minutes. Out of this 1ml was pippetted out and added to 99 ml of sterile water again if contamination is high. In case of vanilla sample, the contamination was less. The solution is poured into the media and is incubated at room temperature. Fungal colonies start developing on the third day. It is observed under the microscope. The spores are isolated and placed in slides and put under the microscope and fungus is identified by the kind of spore or the mycelium. The slides are usually mounted with lacto phenol solution also called cotton blue.

## 3.9 Preparation of vanilla extract by cold percolation process.

Vanilla extract is a value added product of vanilla. It can be prepared by the cold percolation process. This process uses a menustruum, which is allowed to percolate through cut vanilla beans taken in an extraction column. The menustruum contains ethanol, distilled water and glycerine in the proportion of 21: 7: 3.

#### **3.9.1** Preparation of the menustruum for extraction.

It was studied from Purseglove (1988), that 150 g of cut vanilla beans may require 465 ml of the solvent. The solvent was prepared from 315 ml ethanol, 105 ml distilled water and 45 ml of glycerine. Pour the measured quantities into a round bottom flask and mix thoroughly.

#### 3.9.2 Extraction procedure

In the cold percolation process, 150g of the cured vanilla beans are cut into small pieces and filled into an extraction column. The solvent or the menustruum consisting of 98% ethanol, distilled water and glycerine in measured quantity is percolated through it slowly with the help of a dripping mechanism. The solvent is allowed to percolate through the media twice a day. Percolation process is done for seven to eight days. This gives the first extract of vanilla. The extract so prepared is collected in a container sealed and kept for three weeks for ageing. set up for extraction shown in Plate 8

Plate 7: Vanilla packed in polypropylene bags

**Plate 8: Set up for extraction** 

## **RESULTS AND DISCUSSION**

This chapter enunciates the various experiments conducted to standardize the curing process and the various parameters involved. The chapter also discusses in detail the packaging and storage of the cured vanilla beans along with the quality aspects of the stored beans. The production of 'first extract' of vanilla has also been discussed.

## 4.1 Standardization of vanilla curing

Curing was under taken in vanilla beans using the hot water killing method. This facilitated the enzymatic process that transforms glucovanillin into vanillin and development of flavour and aroma. The beans were wrapped in blankets and kept for 12 hours. It resulted in change of the colour of bean to greenish brown. This indicates completeness of process. Subsequently the physical properties of the beans were changed which cause a reduction in the initial weight of the beans. The result obtained is discussed under the following heads.

# 4.1.1 Measurement of physical properties

Vanilla beans procured from the local market were used for the experiments. The test samples were prepared according to the sorting procedure discussed in 3.3. The dimension of the fresh beans and the initial weight of each sample were measured by standard methods and results are tabulated. Length and thickness of ten beans of each grade were measured and the average values are given in table 4.1

Sampl e	Avg. Length (cm)	Avg. Thickness (cm)	Moisture (wb,%)	Colour
В	16.12	10.95	86.1	Green
С	12.90	11.10	89.3	Green

Table. 4.1 Physical properties of fresh beans

The average of the wet basis moisture content of three samples were taken to get the moisture content of the fresh beans and it was estimated to be 88.16%.

# 4.2 Variations in the physical properties of the beans during curing

Stag e of Curi ng	Ti me	Temperature (°C)	Relative Humidity (%)
Killi ng	2.5 – 3.5 min	65	-
Swe atin g	8 – 10 days	45 - 65	65
Slow dryi ng	15 days	28	90 – 92
Con ditio ning	3 months	28-30	55 - 65

## **Table 4.2. Conditions of Curing**

Alternate sweating and drying was done as explained under 3.5. The changes in the physical properties of the beans viz, the moisture content, thickness etc have been discussed under the following heads.

# 4.2.1 Moisture Content variation during sweating and drying

The variation in moisture content for different treatments of the samples of grades B and C are given below.

## 4.2.1.1. Drying kinetics for B grade vanilla beans

The figure 4.1 describes the variation in the moisture contents in dry basis for the grade B sample.

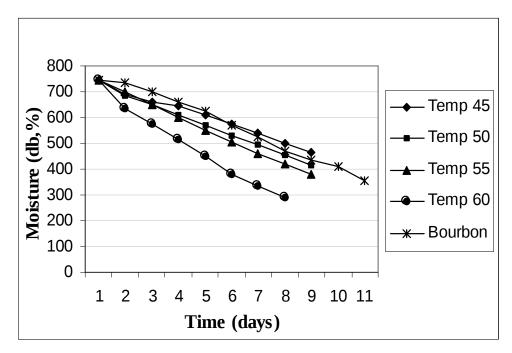


Fig 4.1 MC Vs Time during alternate sweating and drying for grade B

The grade B beans were dried in convective drier at four different temperatures; 45 °C, 50 °C, 55 °C and 60 °C. The samples had initial weights of 668.8, 660.4, 732.6, and 677grams each. The sample for bourbon treatment had an initial weight of 649.4 g. The highest reduction in moisture content was found in case of the sample dried at 60 °C (60.71%) which was followed by 55 °C(48.71%), 50 °C(44.21%), 45 °C(37.6%). The sun drying showed a reduction of 52.29% (fig 4.1 & Appendix I).

The linear relationship between moisture content and drying time for the different methods are as follows:

For convective drying:

 $45 \,^{\circ}\text{C:}$  - y = -33.178x + 768.46 (R<sup>2</sup> = 0.992) ------(4.1)

50°C: -	y = -40x + 772.96	(R <sup>2</sup> = 0.9977)	(4.2)
55°C:-	y = -46.059x + 787.55	(R <sup>2</sup> = 0.9985)	(4.3)
60°C: -	y = -62.948x + 774.39	(R <sup>2</sup> = 0.98610)	(4.4)

For Bourbon method:

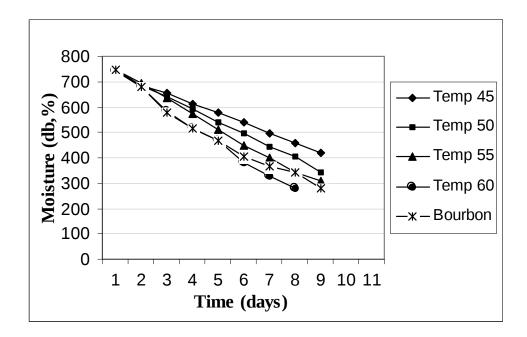
y = -41.092x + 813.03 (R<sup>2</sup> = 0.9908) ------(4.5)

Where, y = moisture content (db) of the beans in %

x = time for sweating in days

# 4.2.1.2 Drying kinetics for C grade vanilla beans

The variation in moisture content for the grade C beans has been discussed with respect to the duration of drying process. This is shown in figure 4.2. The grade C was given similar treatment as in case of the grade B.



#### Fig 4.2 MC Vs Time during alternate sweating and drying of grade C

The initial weights of the samples were 1011.6, 920.6, 927.9, 819.4 and 898.5g respectively for treatment under 45 °C, 50 °C, 55 °C, 60 °C and sun drying. The variations in the moisture content were found to be almost same for sample treated at 60 °C and the one under sun drying. The percentage decrease in moisture content was 62.51% and 62.49% respectively. The reduction in MC was more at 60 °C followed by 55 °C(58.4%), 50 °C(53.9%) and 45 °C(43.8%). The increased reduction in MC at higher temperature is due to the higher drying rate at elevated temperatures (Fig 4.2 & Appendix II)

The linear relationship between moisture content and drying time for the different methods are as follows:

For convective drying:

45 ° C: -	y = -39.878x + 776.14	(R <sup>2</sup> = 0.998)	(4.6)
50°C: -	y = -49.037x + 788.23	(R <sup>2</sup> = 0.999)	(4.7)
55°C: -	y = -56.407x + 799.53	(R <sup>2</sup> = 0.997)	(4.8)
60°C: -	y = -56.407x + 799.53	(R <sup>2</sup> = 0.997)	(4.9)

For Bourbon method:

y = -56.795x + 769.77 (R<sup>2</sup> = 0.9748) ------(4.10)

Where, y = moisture content (db) of the beans in %

x = time for sweating in days

## 4.2.2 Variation in thickness of the samples.

Previous study on vanilla curing revealed the fact that the variation in length and breadth of vanilla beans are insignificant. Therefore in the present study variation in thickness was observed.

## 4.2.2.1 Variation in thickness during curing for grade B

The percentage reductions in the thickness of the samples under various treatments are as shown in figure 4.3

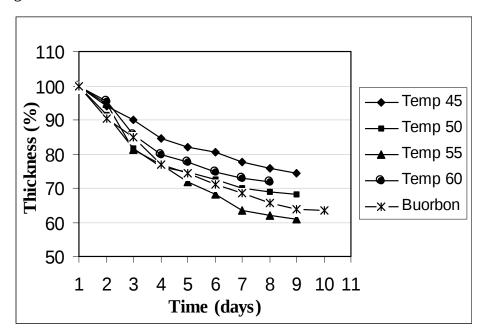


Fig 4.3 Thickness Vs Time during curing, for grade B

The average initial thickness were found to be 11, 11.7, 12.7, 11.7, 11.3 mm for treatment under 45 °C, 50 °C, 55 °C, 60 °C and sun drying. The maximum thickness reduction was observed for sample treated at 55 °C( 39.9%). (Fig 4.3 & Appendix III)

The linear relationship between percentage reduction in thickness and dryingtime for the different methods are as follows:

For convective drying  $45 \,^{\circ}\text{C:} - y = -3.0727x + 99.667$  (R<sup>2</sup> = 0.9438) ------(4.11)  $50 \,^{\circ}\text{C:} - y = -3.7042x + 96.744$  (R<sup>2</sup> = 0.8536) ------(4.12)

55°C:-	y = -4.9621x + 100.33	(R <sup>2</sup> = 0.9223)	(4.13)
60°C:-	y = -4.1222x + 100.78	(R <sup>2</sup> = 0.9057)	(4.14)

For Bourbon method:

y = -3.8659x + 97.209 (R<sup>2</sup> = 0.9101) ------(4.15)

Where, y = percentage reduction in thickness of the beans

x = time for sweating in days

# 4.2.2.2 Variation in the thickness of C grade beans during sweating and drying

The grade C beans was studied for its thickness variation during sweating and drying. It was found that the change was similar to that as for the grade B.

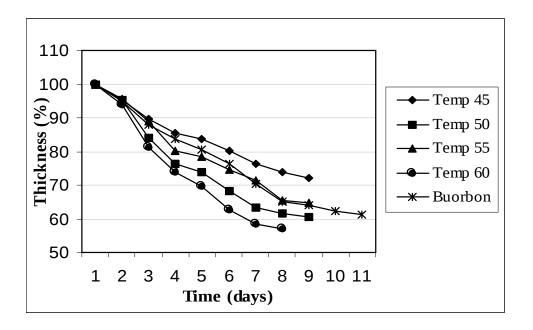


Fig 4.4 Thickness Vs Time during alternate sweating and drying for grade C

The average thickness of the samples were initially recorded as 11,10.17, 10.4, 11.2 and 9.9 respectively for 45 °C, 50 °C, 55 °C, 60 °C and sun drying. The maximum percentage reduction in thickness was found to be 43%. This variation was seen in the sample dried at 55 °C. (fig 4.4 & Appendix IV)

The linear relationship between percentage reduction in thickness and drying time for the different methods are as follows:

For convective drying:

(4.16)	(R <sup>2</sup> = 0.9771)	y = -3.4595x + 101.35	45 ° C: -
(4.17)	(R <sup>2</sup> = 0.9404)	y = -5.1202x + 101.47	50°C: -
(4.18)	(R <sup>2</sup> = 0.9703)	y = -4.5501x + 102.72	55°C:-
(4.19)	(R <sup>2</sup> = 0.9595)	y = -6.4029x + 103.4	60°C: -

For Bourbon method:

$$y = -4.0404x + 101.15$$
 (R<sup>2</sup> = 0.9684) ------(4.20)

where, y = percentage reduction in thickness of the beans

x = Time for sweating in days

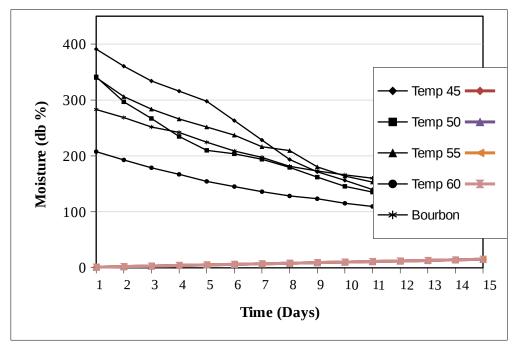
# 4.2.3 Variation in moisture content during slow drying

The variation of MC for B and C grade vanilla beans are shown in fig 4.5 and 4.6.

# 4.2.3.1 Moisture kinetics of B grade beans during slow drying

Convective drying was done until the required reduction in moisture content was

obtained. The beans were then taken to the next treatment, slow drying. The relative humidity in the drying room was maintained at 80%. This was achieved by placing moistened clothes near the drying racks. The weights of the samples were taken and recorded at 12 hours duration. The figure 4.5 shows the change in moisture content (db %) of the samples.



# Fig 4.5 MC Vs Time during slow drying, for grade B

The initial weight of the samples for slow drying was 388.8, 345.1, 381.7, 246.6 and 294.5g each. The percentage reduction in the moisture content of each sample was calculated. It was found that the grade B dried at50 °C showed the highest reduction in the percentage moisture content. The value was 69.7%. This was followed by 45 °C(67.4%), 55 °C(63.3%) and 60 °C(58%). The lowest percentage reduction was 50.32% and was shown by the sample which was sun dried. (Fig 4.5 & Appendix V)

The linear relationship between moisture content and drying time for the different methods are as follows:

For convective drying:

45 ° C: -	y = -20.36x + 388.03	(R <sup>2</sup> = 0.9384)	(4.21)
50°C:-	y = -15.734x + 313.41	$(R^2 = 0.9389)$	(4.22)

55°C: - 
$$y = -15.357x + 331.91$$
 (R<sup>2</sup> = 0.972) ------(4.23)  
60°C: -  $y = -8.3399x + 202.33$  (R<sup>2</sup> = 0.9692) ------(4.24)

For Bourbon method:

$$y = -10.355x + 279.31$$
 (R<sup>2</sup> = 0.9546) ------(4.25)

Where, y = moisture content (db) of the beans in %

x = time for sweating in days

# 4.2.3.2 Moisture kinetics of C grade beans during slow drying

Slow drying was conducted on the grade C for seven days. The moisture content dry basis Vs time duration for slow drying was plotted and is shown in figure 4.6

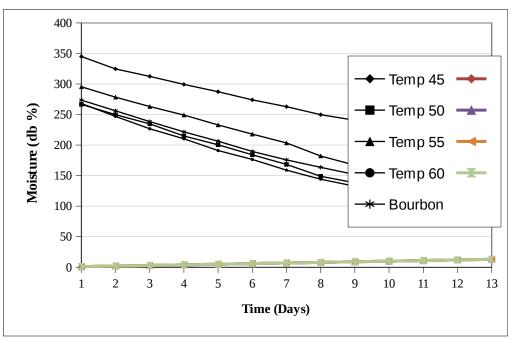


Fig 4.6 MC Vs Time during slow drying, for grade C

The samples before slow drying had weights of 533, 397.6, 434.5, 357.2 and 395.5g for the treatments at 45 °C, 50 °C, 55 °C, <sup>2</sup>60 °C and sun drying respectively. The percentage reduction

in the dry basis moisture content was analysed from the graph. It was found that for the convective dried samples the highest percentage reduction was observed for the sample dried at 50 °C (63%). The lowest reduction was for sample dried at 45 °C, which was 51.79%. The sun dried sample showed the reduction of 62.38%. (Fig 4.6 & Appendix VI)

The linear relationship between moisture content and drying time for the different methods are as follows:

For convective drying:

(4.26)	(R <sup>2</sup> = 0.9937)	y = -13.358x + 354.88	45 ° C: -
(4.27)	(R <sup>2</sup> = 0.9714)	y = -13.326x + 269.43	50°C: -
(4.28)	(R <sup>2</sup> = 0.9964)	y = -15.471x + 309.63	55°C: -
(4.29)	(R <sup>2</sup> = 0.9732)	y = -14.074x + 267.88	60°C: -

For Bourbon method:

y = -13.442x + 277.36 (R<sup>2</sup> = 0.9859) ------(4.30)

where, y = moisture content(db) of the beans in %

x = time for sweating in days

## 4.3 Analysis of vanillin content.

The samples after slow drying were conditioned in closed wooden boxes. The beans were packed in appropriate packaging material. It was stored for three months. To asses the quality of curing, the vanillin content of the five samples under the various treatments was found out. Each sample weighing 100 g each was sent to the Spices Board, Cochin. The vanillin content and moisture content of the sample were found out and are as given below:

The highest vanillin content was derived for the sample dried at 55 °C and it was found to be 1.79%. The moisture content of that sample during the testing was found to be 22% (wb). It was also seen that the sample, which was treated under the traditional bourbon method, showed a comparable value of 1.75%. It also had wet basis moisture content of 25%. The sample of vanilla treated at 60 °C showed a vanillin content of 1.63%. The sample had wet basis moisture content of 19% during the testing. The vanillin content for the sample that was dried at 50 °C was 1.59%. The sample had moisture content of 20%. The sample that was dried at 45 °C gave a vanillin content of 1.07% and the moisture content was 14% wet basis. This treatment resulted in the lowest vanillin content of all the treatments. A close look on to the values of the vanillin content and the moisture content shows that there is some correlation between the moisture content and derived vanillin content of the cured beans. It was seen that the values of higher vanillin was obtained for the beans, which had moisture content in the range of 20-25% (Appendix VII). The fig 4.7 explains the relation of the temperature with the vanillin content of the samples, which have undergone various treatments.

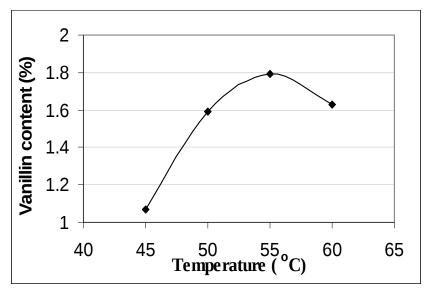


Fig 4.7 Treatment temperature Vs. vanillin content.

The graph when plotted showed a parabolic curve, which could be defined by the following equation:

 $y = -0.0068x^2 + 0.7516x - 18.984$ 

The plot had an  $R^2$  value as follows

 $R^2 = 0.9997$ 

It could be analysed from the graph that as the temperature of the treatment increased, the vanillin content obtained also increased. The lowest vanillin content at 45 °C could be due to the lower MC and fungal attack. Similarly the reduction in vanillin content at 60 °C could be due to loss of vanillin content at high temperature drying. But it could be seen that after a particular temperature the production of vanillin inside the beans reduced. This could be due to the unfavourable reactions that may be taking place due to the excessive temperatures. It was also observed that the convective drying at 45 °C deteriorated the quality of the sample due to the insufficient drying. This shows that temperature 45 °C, was low for the complete development of the vanillin content.

Sl No	Treatment	Temperature (° C)	Moisture content (%wb)	Vanillin content ( %)
1	Convec drying	45	14	1.07
2	Convec drying	50	20	1.59
3	Convec drying	55	22	1.79
4	Convec drying	60	19	1.63
5	Bourbon method	Sun drying	25	1.75

Table 4.3 Treatments given to vanilla

#### 4.4 Storage studies of cured vanilla beans

The vanilla beans were slow dried and were made to the required moisture content after which it was kept for storage. The study was undertaken to see the applicability of MAP for cured vanilla beans.

The cured beans of each treatment were split into three sets. These were then placed in each of the packages with thickness of 125, 250 and 400 gauges. The material for packaging was polypropylene. The packets had dimensions of 31×18 cm for 125 gauge, 18.5×20 cm for 250 gauge and 30×22.5cm for 400 gauge. The time available for the storage study was only three months. It has been found that the time was inadequate to attain a good result.

The storage practice followed was commodity generated MAP. In this system the micro environment was modified with passage of time, the concentration of the gases changes with the metabolic activities of the product stored.

Not much of microbial load was identified on the samples under study. The sample of the vanilla beans cured at 45°C and packed in the 250-gauge pack showed mould growth. It was seen around the thread that was used to tie the ends of the cured beans. Thus it could be concluded that the packaging material was impermeable to the infection causing organisms. It could also be said from the study that the packaging material has the capability to restrain the entry of oxygen gas. The presence of oxygen is important for the survival of the fungus. Thus by restricting the entry of  $O_2$  a control could be established on the fungal infestation. The fungus was seen on the sample as white tuft. Another sample of vanilla that was treated at 45°C and packed in the 125-gauge material was found to be infected with mites.

The packaging material was found to be efficient in retaining aroma. It was studied that higher the gauge, more was the aroma retention capability of the packaging material.

Microbial study of samples available in the Product Analysis lab was also studied. Some of these samples were fungus affected due to improper storage. The samples were under storage for over a year. The samples were kept packed in bee wax paper. The samples also had mite infection. The fungal infections on the beans were seen as a black cottony growth. Fungal colonies were also seen around the cotton threads, which were used to tie the ends of the beans. They were seen as a white growth.

Gauge	Fungal /mite infestation	Treatment given
125	Mite infection	45 °C convec drying
250	Penicilium and Fusarium	45 °C convec drying

 Table 4.4 Infection in sample

Gauge	Fungal /mite infestation	Treatment given
400	Nil	-

## 4.5 Identification of fungal infestation.

The samples infected with fungus and mites were taken to RARS (Regional Agricultural Research Station), Pattambi. The experiment for identification was conducted at the pathology department laboratory. The samples for identification were prepared as discussed in the chapter three. The slides prepared were kept aside for development of the fungal colony .the development of the colony was visible by the third day.

The slides were place under the electron microscope to get a good view of the growth. The one year old sample of vanilla was analysed for its fungal growth. It was found infested with fungi namely *Fusarium* sp. and *Apergillus* species. The sample which was treated at 45°C was detected to have two kinds of fungal infection namely *Pencillium* sp. and *Fusarium* species

The fungus can be easily identified by their conidiophores or by the spores that they produce. *Fusarium* sp. was identified by observation of the sickle shaped spores that they produce. *Aspergillus*'s was identified by the round spores. They were found as a disintegrated mass in the slide prepared. The fungal colony of *Penicillium* sp. was identified by their branched conidiophores.

## 4.6 Extraction of cured vanilla beans

The vanilla extract is one of the by-products of vanilla, which can be prepared easily. For the preparation of vanilla extract, the apparatus was set up at the Product analysis laboratory . 150 gm of the one year old cured vanilla was used for the extraction. The beans were observed to have a change in the quality as they had become harder. Much of the aroma of the beans were also lost due to the improper storage. The solvent was allowed to percolate slowly through the cut vanilla beans inside the extraction column. The vanillin compounds leached out along with the solvent and got collected in the conical flask placed below the column. The same solvent was used for the repeated percolation. This procedure was continued for seven days. The extract termed as the first extract of vanilla was produced. It had a golden brown colour. The extract had a syrupy texture. The extract produced had a strong odour of vanillin accompanied by odour of ethyl alcohol. The extract was then collected into a bottle. It was aged for three weeks. The ageing process was done to allow the complete development of aroma and flavour in the extract.

## 4.7 Evaluation of vanillin content.

50 ml of the extract produced was sent to the Spices Board, Cochin. the quality of extracted vanilla was analysed for its vanillin content. The test results showed that the extract had a vanillin content of 1.05% by weight. This was much less compared to the vanillin content of the cured sample. This could be accounted to the loss of the flavouring compound, vanillin during storage. The compounds that are responsible for the aroma and flavour are highly unstable and evaporate off easily if not properly preserved. The samples were seen to be infested with fungus, which could be a reason for the low vanillin content.

#### SUMMARY AND CONCLUSION

Vanilla beans are one of the most expensive spices traded in the global market. The fresh vanilla beans do not have any flavour or aroma because vanillin and other chemical substances responsible for it are not present in the free form at the time of harvest. During the process of curing, free vanillin is developed in the beans as a result of a series of enzymatic actions on several glycosides.

Traditional curing method (Bourbon method) is time consuming and laborious. This puts off the farmers from curing beans. With this in view, an attempt was made in KCAET, Tavanur to standardize the curing techniques and to study the possibility of producing value added products from cured vanilla beans

The beans for the experiment were procured from the market. They were sorted into different grades based on the lengths. Two grades of beans were obtained. Grade B (15-20 cm), and C (<15 cm) were separated out. Each set was then divided into Five samples and subjected to five different curing techniques-

Method I: Hot water killing followed by convective drying at 45 °C. Method II: Hot water killing followed by convective drying at 50 °C Method III: Hot water killing followed by convective drying at 55 °C Method IV: Hot water killing followed by convective drying at 60 °C Method V: Hot water killing followed by sun drying (Bourbon process)

Each method consists of four stages viz; killing, sweating, slow drying and conditioning. The moisture kinetics at the alternate drying and sweating stage and at slow drying stage was monitored. The study of moisture kinetics for the conditioning stage could not be done since it was subjected to MAP at that stage. The thickness of the sample during the drying stage was also analysed. It was seen that there was no marked change in thickness during slow drying.

At the time of sweating and drying, maximum reduction in moisture content (60.71%) was seen in B grade beans cured at 60 °C treatments. Grade C beans dried at 60 °C showed a percentage reduction in moisture content of 62.51% followed by the Bourbon treatment with 62.49%. The reduction in moisture content was lowest for the 45 °C treatments in both the Grades of vanilla.

During slow drying B grade beans dried at 50 °C showed the highest reduction in moisture content. The sun dried sample showed the lowest reduction (50.32%). Similarly for the Grade C, highest reduction in percentage moisture content, for convective dried sample was shown for the sample dried at 55 °C.

The maximum variation in thickness for B grade beans was seen to have a value of 39.9%. This was for the sample treated at 55 °C. C grade beans also had the highest reduction in percentage thickness for the sample dried at 55 °C (43%).

The samples were then packed into polypropylene bags. The bags used for the experiment were of three gauges, 125, 250 and 400. This was to study the shelf life of vanilla beans under modified atmospheric packaging. The type of the MAP system adopted in the experiment was called the commodity generated or passive MAP. The packaging was very effective in preventing fugal attack. Though there was infestation seen in the sample of vanilla treated at 45 °C, it was due to the improper sterilized thread, which was used to tie the ends of beans. The higher gauge plastic (400 gauge) was found more effective in aroma retention.

The treatment at 45°C was found to have fungal growth around the thread used for tying up the ends. This sample was analysed at the pathology laboratory of RARS, Pattambi. The fungal growth was identified to be that of *Penicilluim* species and *Fusarium* species.

Vanillin extract was prepared by the cold extraction process, by using an extraction column. The solvent used, consisted of ethyl alcohol, distilled water and glycerine. The vanillin content of the extract thus produced 1.07% by weight.

After curing, the vanillin content and moisture content of the beans were tested at the Quality Evaluation Laboratory, Spices Board, Cochin. The vanillin content was found to be 1.07%, 1.59%, 1.79% and 1.63% for convective drying at 45°C, 50 °C, 55 °C, 60°C and Bourbon method respectively. The maximum moisture content was determined to be 25% in the case of beans cured by Bourbon method, followed by convective dried sample at 55 °C (22%), 50 °C (20%), 60 °C (19%) and 45 °C (14%).

# **SUGGESTIONS**

- Conditioning could be done at different temperatures and humidity
- MAP could be studied using different packaging materials like polyethylene, poly styrene etc.
- > Active MAP could be tried on the beans during conditioning.
- Threads made from natural fibres could be used instead of the synthetically produced threads.
- Possibilities of producing other value added products viz: vanilla tincture vanilla absolute etc.