FABRICATION AND TESTING OF VANILLA EXTRACTOR

BY

DIVYA.E

NITHYA.N.S

PARVATHI.S

PROJECT REPORT

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DECLARATION

We hereby declare that this project entitled "Fabrication and Testing of Vanilla Extractor" is a bonafide record of project work done by us during the course of project and that the report has not previously formed the basis for the award to us of any degree, diploma, associateship, fellowship or other similar title of any other university or society.

Divya.E,

Nithya, N.S,

Parvathi.S.

Place: Tavanur

Date: 10-01-2008

CERTIFICATE

Certified that this project report, entitled, "Fabrication and Testing of Vanilla Extractor" is a record of project work done jointly by Divya.E, Nithya,N.S and Parvathi.S under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to them.

Er.Prince.M.V,
Asst. Professor ,
Department of PHT & AP,
KCAET, Tavanur

Place: Tavanur

Date: 10-1-2008

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DEDICATED TO OUR BELOVED

ACHAN AND AMMA

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

SYMBOLS AND ABBREVIATIONS

%	**percentage**
^{o}C	**degree Celsius**
^{o}F	**degree Fahrenheit**
µm	**micrometer**
µg	**microgram**
cm	**centimetre**
et al.	**and other people**
Fig.	**Figure**
gm	**gram**
ha	**hectares**
hrs	**hours**
Hz	**hertz**
kg	**Kilogram**
m	**metre**
mg	**milligram**
min	**minute**
ml	**millilitre**
mm	**millimetre**
nm	**nanometre**
PHT&AP	**Post Harvest Technology and Agricultural Processing**
W	**watt**

INTRODUCTION

CHAPTER I
INTRODUCTION

Vanilla is the world's most expensive spice next to saffron and cardamom. It belongs to orchidaceous family, largest family of flowering plants. Vanilla is a native of the Atlantic coast from Mexico to Brazil. The Spanish adopted the word as "Vaina", which developed into the diminutive form , "vainilla", meaning "Little sheath". The Spanish coined this diminutive name of the plant because its pods resemble sheaths.

The flavorings come from the seed pod, or the bean of the vanilla plant. The prepared beans are dark brown, slender, pleated and about 20 cm (8 inch) long. The bean is tough and pliable. The vanilla bean contains the active ingredient "Vanillin" that produces the characteristic fragrances and is produced during the process of induced fermentation.

Vanillin (4-hydroxy 3 methoxybenzaldehyde), is the most widely used food flavoring, and, in its natural form, one of the most wanted spices. Till recently, the bulk of the vanillin produced was used as a flavoring agent in different foods. And the remaining quantity was used in deodorants, perfumes, odour fixatives and as a masking agent in pharmaceuticals and vitamin preparation. Vanillin can also prevent foaming in lubricating oils; it can be used as a brightener in Zinc- planting baths: it can also be used as an aid for the oxidation of linseed oil and as solublizing agent for riboflavin (Vitamin B2) as vanillin is an antioxidant. Vanillin was first isolated from vanilla by *Gobley* (1858). It was artificially produced by *Tiemann* and *Haarmann* in 1874, from the glucoside coniferin, which occur in the sapwood of certain conifers.

According to the International Trade Centre (ITC), Geneva Report (1982), about 1,600 to 3000 tones of vanilla, valued at US $45 – 55 million, enter the world trade each year. During 1980, the total world imports amounted to about 1565 tones valued at US$54.00 million. United States is the largest single importer, followed by France, importing about 400 tones a year, Canada 100 tones, Japan 60 tones Saudi Arabia 20 tones.

This continuous demand for Vanilla has led to an unpredictable rise in price which has tempted many countries to grow this crop. The countries include Tongo, Mexico, Fiji. Brazil, Costa Rica, Srilanka, some French islands and India. Indian production of vanilla is about 92 tones from 2500 hectares in 2002 and increased to 200 tones from 3000 hectare in the year 2003, and the average vanillin content is estimated to be 3.5 percentage.(*http://dfid-agriculture-consultation.nri.org*).The major areas contributing to vanillin production in India are Karnataka (1465 ha), Kerala (812 ha), and Tamil Nadu (268). Karnataka is the leading producer in India.

Consumers all over the world, especially in the advanced countries prefer to use natural products in food material. Also some advanced countries insist on the use of natural flavours in food products. Technology has been developed now to develop synthetic vanillin from lignin, a byproduct of the sulphite paper pulping process, at a very low cost. The natural vanilla therefore has to surrender its market to synthetic vanillin, which accounts for 90-95% of vanilla flavors. At present synthetic vanillin is sold at US$ 25 per kg while the international price of natural vanilla beans is us$ 72 per kg. While the consumer prefers natural vanillin because of its well rounded flavor compared to the rough and incomplete aroma and taste of synthetic vanillin. Hence there is a race between natural vanilla and synthetic vanillin in the consumer's market. From the research of the synthesis of vanillin so far, it can be stated that it is rather impossible to make a product having the same quality as that of natural vanilla. Hence there is a definite future for natural vanillin industry, even though vanilla beans are very costly. What is required for the growth of natural vanillin industry is to modernize the agro techniques in the production and processing of vanilla beans, and make the extraction process simple and cost effective ,besides , bringing down the cost of production by increasing the productivity.

Although extraction with solvents constitutes the most efficient method for the recovery of vanillin from vanilla, it is relatively the most advantageous and since minimum heat treatment is involved, the quality is found to be maximum. The existing method of vanilla extraction in any form is tedious and involves sophisticated machineries and instruments which needs to be imported also. The capital investment involved is so high that small scale farmers or industries cannot afford to go for such extraction methods. Due

to these, farmers sell the processed beans to the exporter through the middle men and the importing countries do the extraction and return the extracted vanillin to the needies and to the local markets on their label. This means that the profit which could have been added to the product due to value addition is simply being snatched away by the importing countries. It is therefore imperative that simple, cost effective methods of extraction of vanilla in the standard forms prescribed by the international agencies be developed indigenously. Keeping this in view, an attempt was under taken at Kelappaji College of Agricultural Engineering and Technology, Tavanur to extract vanillin with the following objectives.

1. **Development of a prototype vanilla extractor for the production of vanilla extract.**
2. **Performance evaluation of the vanilla extractor.**

Review OF LITERATURE

CHAPTER II
REVIEW OF LITERATURE

This chapter includes review of the part works related to the topic under study.

2.1. CLIMATE

Vanilla thrives well from sea level up to an elevation of about 1066.8m in hot , moist, tropical climates with well distributed and adequate rainfall. Arid conditions and violent winds are detrimental to the plant growth. *(Pruthi,* 2001*).*

***Correll*(1953),opinioned that regularity of ideal climate is a very important aspect to be taken care of in the cultivation of vanilla, since temperature, humidity and various other ecological factors are considered to affect the quality of vanilla especially aroma and other parameters.**

***Purseglove et al* in 1988 suggested that the optimum temperature required for vanilla is 21- 33°C, with an average around 27°C. The ideal relative humidity is around 80%.**

***Anon* (1998), studied the moisture and vanillin content of cured vanilla at Quality Laboratory, Spices Board, Kochi and concluded that in high ranges, vanilla curing in raised platforms yielded more vanillin.**

2.2. SOIL

Soils rich in organic matter with adequate but not excessive drainage are suitable for vanilla cultivation. It is adaptable to a wide range of soil types provided there is plenty of humus in the soil. (*Pruthi*, 2001).

For successful cultivation of vanilla, soil factors such as texture and pH appear to be more important than soil fertility (*childers etal,*1959)

***Ridley etal* (1912) opinioned that a stiff clay soil which dries rapidly and water logged ground is not at all suited for the growth of the plant.**

2.3. HARVESTING

Though vanilla begins to yield from the 4th year onwards, the maximum yield is obtained only in the 7 th year. Plucking or harvesting in the bunch should be avoided. In the harvesting of vanilla, individual plucking is done by twisting the bean in the upward direction. Completely matured beans will be obtained easily when twisted and others remain intact to the vine. (*Purseglove*, 1988).

A good vanillery is said to yield about 500- 800 kg of cured beans per ha/ annum during a crop life of about 7 years. (*Lionnet*, 1958-9).

***Lionnet*, (1959), and *Purseglove etal* (1988), suggested that although the pods reach their greatest size 6 weeks after pollination, it is 8-9month before they are ripe for picking.**

***Lancher*(1989), studied the effect of harvesting date on vanillin content of pods of *vanilla Tahitensis* in French Polynesia. It was found that vanillin content reached maximum when the pods were tending to turn brown.**

2.4. COMPOSITION

The proximate composition of the whole vanilla bean is shown in Table 2.1

Table 2.1 Proximate composition of vanilla bean

Composition	Amount in percentage
Moisture	**25.85- 30.93%**
protein	**2.56- 4.87%**
Fatty oil	**4.68-6.74%**
Volatile oil	**Upto 0.64%**
Carbohydrate	**7.1-9.1%**
Fiber	**15.27-19.6%**
Ash	**4.5-4.7%**
Vanillin	**1.48-2.9%**
Resins	**1.5-2.6%**
Calcium	**19.7 mg%**
Potassium	**16.2 mg%**
Sodium	**6.7 mg%**
Phosphorous	**9.5 mg%**

Source: *J.S. Pruthi*, 2001

2.5. POST HARVEST TECHNOLOGY

Free vanillin is not present in the green beans when they are harvested and the characteristic vanilla aroma due to vanillin will also be absent. This is developed as a result of an enzyme action at the time of the curing of the beans. Curing is the process of alternately sweating and drying the beans until they lose the moisture. The following curing process gives a satisfactory product. (*Pruthi*, 2001).

The beans after harvest are allowed to shrivel for 3-4 days. They are then immersed in hot water at 60ºC for about one minute and spread out on woolen blankets for sun drying. When the beans become too hot to hold in the hand, the blanket is folded over them and kept for the rest of the day. At night the beans are kept in the sweating boxes

lined with the blanket. The next day the beans are again put in the blanket and dried in the sun. This process is repeated for about 8-10 days depending on the weather. By this time, the beans lose a major portion of their moisture and attain a dark chocolate brown color. The vanilla aroma may also have developed. The beans are then sorted out, according to their length, tied into bundles and kept in air tight container. (*Pruthi*, 2001)

***Bouriquet* (1954) and *Morison* and *Smith* (1964) reported about the chemical analysis of vanilla beans.**

***Lionnet* (1959),found out that curing of vanilla pods is an extremely important stage in production, since during curing they under go all the enzymatic reactions responsible for the characteristic flavor of vanilla.(*Correll* (1953).**

***Theodose* (1972), reported that a number of procedures have been evolved for curing of vanilla, but they are all characterized by four phases-killing/wilting, sweating, slow drying and conditioning**

Purseglove (1988) found out that the top quality cured vanilla beans are long , fleshy, very dark brown to black in color, oily in appearance, strongly aromatic, free from scars and moisture content is 30-35%.

2.6. VANILLA EXTRACT

This is a hydro-alcoholic solution containing the extracted aroma and flavor principles of vanilla beans and may also contain added sugar and glycerin. Glycerin and sugar are frequently included in the formulation as they lend smoothness and viscosity to the extract and help to fix the aromatic constituents, there by extending the shelf life. (*Purseglove*, 1988).

The initial extraction solvent is known as menstruum. It is usually aqueous alcohol.

Merory **(1968) has described an operation to prepare 379 litres of two fold vanilla extract with 35% ethanol content.**

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Business Daily from THE HINDU group of publications published on May 02, 2007 that Natural vanilla extract manufactured using the cold process technology under the brand name `Nature's Nurture ' has been launched at Kottayam. The company has technical collaboration with a US-based vanilla extraction company under which home products could be exported to the US. Natural vanilla extract produced with this technology gives both fragrance and the taste of natural vanilla whereas the vanilla extract produced through carbon dioxide extraction gives only the smell. In US and Europe more than 95 per cent of vanilla extract is manufactured based on this technology. This technology is introduced for the first time in India, claims Mr John P. John, CEO, Tharakan & Company, Kottayam. The success of this product will be a boon to the struggling vanilla farmers in the country, he added.

2.6.1. Types of Pure Vanilla Extracts

2.6.1.1. ***Mexican vanilla extract***

It is made from ***Vanilla planifolia*** **(sometimes called fragrans) plant stock indigenous to Mexico. It is a very smooth, creamy, spicy vanilla. It's especially good in desserts made without heat or with a short cooking time. Dark chocolate, cream desserts, alcoholic and non-alcoholic beverages, ethnic foods, poultry or meat, all benefit from Mexican vanilla. The Mexican vanilla which was once sought after, being the original and of the finest quality has almost disappeared from the international trade at present due to heavy decline in production.**
(*Theodose*, 1973).

Richard D. Thompson **and** ***Terry J. Hoffmann*** **(1988) developed a high-performance liquid chromatographic procedure for the isolation and quantification of coumarin from vanilla-based liquid flavorings of Mexican origin. Forty products representing fourteen**

different Mexican brands were assayed for coumarin, vanillin, and ethyl vanillin by the proposed method. The procedure has been adapted to the analysis of other products including domestic vanilla extracts and imitation vanilla flavorings for vanillin, ethyl vanillin, 4-hydroxybenz-aldehyde and piperonal. Chromatographic retention data for thirty-seven compounds associated with vanillin and vanilla products employing two mobile phase systems are presented.

Arturo Longares-Patrón **and** ***M.P. Cañizares-Macias*** **(2006) reported that new method for a quick extraction of vanillin and p-hydroxybenzaldheyde (PHB) of vanilla beans from vanilla fragans is proposed. Samples were irradiated with microwaves energy to accelerate the extraction process and photometric monitoring was performed at 348 and 329 nm (vanillin and PHB, respectively). The simultaneous determination of vanillin and PHB from extracts was performed using the Vierordt's method, which showed a precision, expressed as relative standard deviation, smaller 2.5% for both analytes. Conditions such as microwaves irradiation power, number of irradiation and non-irradiation cycles, irradiation time and ethanol concentration were optimized by means of multivariate screening that showed that irradiation power and number of irradiation cycles is the most significant condition in the vanilla extraction process. The focused microwave-assisted extraction (FMAE) was applied to commercial (dried vanilla beans from fresh green vanilla beans), lyophilised and dried (commercial vanilla dried at 135 °C in oven) vanilla beans samples. The results showed that the extraction of vanillin and PHB in the commercial vanilla samples were higher than in dried and lyophilised samples. With the proposed FMAE a decrease in the extraction time of 62 times and an increase in the vanillin and PHB concentrations between 40 and 50% with respect to the official Mexican extraction method, were obtained.**

2.6.1.2. ***Bourbon vanilla extract***

It is a generic term for Vanilla planifolia, the vanilla most of us are familiar with as it's the most commonly used variety in extracts. Vanilla planifolia stock originated in Mexico, vanilla's birthplace, but cuttings were taken to other tropical countries beginning in the 1700s. In the 1800s, the French developed large plantations on Reunion, known then

as the Ile de Bourbon, which is how the name Bourbon came into being. Although vanilla extract is high in alcohol content, it is not made from Bourbon whiskey.

Eric Feyertag **and** ***Robert Hutchins*** **(1981) reported that Separation of a fragrant 5-piperidone compound, containing three methyl groups and also of methylbenzoate from bourbon vanilla bean extract was achieved by liquid chromatography and by gas-liquid partition chromatography, respectively.**

Bourbon and Mexican vanillas have the familiar natural vanillin flavor that we associate with vanilla ice cream and other vanilla-flavored desserts and beverages. Bourbon vanilla can be used baked goods, ice cream and anything where a traditional vanilla flavor is desired.

2.6.1.3. ***Indonesian vanilla extract***

Depending on how Indonesian vanilla is cured and dried, it can be much like Bourbon vanilla, or it can have very distinctive differences. Some growers harvest their beans too early and use a short-term curing process that gives the vanilla a more woody, phenolic flavor. As the early harvest keeps the beans from fully developing their flavor profile, it can be harsher and not as flavored. It's important to note that not all Indonesian vanilla is harvested early; premium grade Indonesian vanilla is excellent.

Frequently Indonesian vanilla is blended with Bourbon vanilla to create a signature flavor. Indonesian vanilla tends to hold up well in high heat, so anything slow-baked or exposed to high heat (i.e. cookies), benefits from Indonesian vanilla. Indonesian vanilla is also quite good with chocolate as its flavor overides the sweetness of chocolate and gives it a beneficial flavor-boost. Chocolate's popularity is due, in part, from the sparkle it receives from other flavors as it tends to be somewhat dull on its own. ***(www.wikipedia.org*****)**

2.6.1.4 ***Tahitian vanilla (Vanilla tahitensis) extract***

It comes from *planifolia* stock that was taken to Tahiti. Somehow it mutated, possibly in the wild. It is now classified as a separate species as it's considerably different in appearance and flavor from Bourbon vanilla. It is similar, however, to *Vanilla Pompona*, a variety of vanilla rarely used commercially, but one that has religious and cultural significance with the Totonacas of Mexico, the first cultivators of vanilla. They consider *Pompona* the queen of vanilla, and she is always planted in a prominent place wherever they grow vanilla.

Tahitian vanilla is sweeter and fruitier and has less natural vanillin than Bourbon and Mexican vanilla. Instead, it contains heliotropin (anis aldehyde), which is unique to its species. This gives it a more cherry-like, licorice, or raisiny taste. It has a very floral fragrance, the bean is fatter and moister than Bourbon vanilla, and contains fewer seeds inside its pod. Tahitian is especially nice in fruit desserts, as well as in sauces for poultry and seafood. Since vanilla is a very labour intensive agricultural product, it is expensive. Tahitian vanilla has always been more expensive than Mexican and Bourbon vanillas. This is especially true now as it is less readily available. (*www.vanilla.com)*

2.6.2. Fold of Vanilla

A fold is the relative measure of the strength of vanilla extract. Single fold vanilla is typically what the consumer buys at the market. For food processing, two, three or four fold vanillas are typically used. A single fold vanilla contains the extractive matter of 13.35 ounces of vanilla beans, containing less than 25% moisture, in one gallon of 35% aqueous ethyl alcohol. Two fold uses 26.7 ounces of vanilla beans, contains twice as much extractive matter and is twice as strong. Three fold and four fold are just three or four time the content of one fold. This is a standard of identity set by the FDA for Pure Vanilla *(www.springerlink.com).*

2.6.3. Quality of Vanilla Extract

Quality of vanilla extract (*Purseglove*, 1988) is dependent upon the number of factors that includes:

- **Careful handling and storage of the beans prior to extraction.**
- **Appropriate blending of the beans and their selection**
- **Degree of comminution of the beans**
- **The method and condition of the extract.**
- **Proper ageing of the extract to allow full development of the flavor.**

2.7. METHOD OF EXTRACTION

The extraction consists of the following steps (*Purseglove*, 1988) :

2.7.1. Communition of Bean

The beans are washed with the portion of the men strum to dissolve any surface vanillin, prior to slicing. The beans are sliced into short length of about 1-2 cm. During cutting operation also a little of menstruum is allowed to flow over the beans to prevent overheating and evaporation losses.

1. **Extraction by Maceration**

This is a traditional method of preparing vanilla extract. The chopped beans are placed in the vessel where they are allowed to steep in menstruum for up to 1 year. Earlier wooden barrels and 50% ethanol were used. This provides superior quality product; but has low output. Modern macerators are airtight vessels, constructed from stainless steel, which permits slow agitation by stirring, racking or tumbling to a menstruum of 60% ethanol. (*Purseglove*, 1988)

2. **Extraction by percolation**

The equipment consists of a stainless steel vessel fitted with a series of perforated trays to hold the chopped beans. The menstruum is sprayed on to the top tray, percolates down from tray to tray, collects in the base of the vessel and is recirculated by pump. (Purseglove, 1988)

Merory **(1956) has recommended three consecutive extractions, each for at least 5 days, using a menstruum with 60% ethanol for the first extraction, 30-35% for the second extraction and 15% for the third. In order to permit full development of flavor, extract should be aged. After that, the extract should be filtered or centrifuged.**

2.8 VANILLA PRODUCTS

2.8.1. Natural Vanillin

Natural vanillin is one of the over two hundred organic components that make up the flavor and aroma of vanilla, and it's the one we most associate with vanilla. Vanilla beans sometimes have pure vanillin crystals that develop on the bean's surface. The crystals give off an iridescent sparkle in sunlight and are quite edible.(***Gobley*****, 1858).**

Tiemann **(1885) reported the isolation of two heterosides, which he named as glucovanillin as the precursor of vanillin.**

Lecompte **(1913) postulated that the vanillin precursor present in beans was coniferoside, which under the action of an oxide would split to glucovanillin which inturn woud yield vanillin.**

Swami **(1947) suggested that vanillin is produced in glandular hairs.**

Childers etal **(1959) observed that vanillin crystals formed during curing appear mostly at the blossom end of the green vanilla pods.**

Merory **(1968) showed that the organoleptic properties of cured vanilla beans are determined by the volatile constituents of which vanillin is the most important one. The resins, the nonvolatile fractions have no aroma but have a pleasant taste and they fix the volatile constituents in the solvent extract.**

Klimes **and** ***Lamparsky*** **(1976) have identified more than 170 volatile aromatic compounds in madagascar beans, of which vanillin is the most abundant.**

Ranadive etal **(1983) conducted research on vanillin biosynthesis in vanilla beans and found out that when protected against protolysis, beta- glucosidase activity expressed as 75.2% in green outer tissue, 32.3% in the placental tissue and 11.1% in the glandular cells.**

Guavino **and** ***Brown*** **(1985) have developed a liquid chromatographic method that can be efficiently used for the qualitative analysis of vanillin.**

L. Sagrero-Nieves **and** ***S. J. Schwartz*** **(1988) reported that Vanilla, vanillic acid, and 4-hydroxybenzaldehyde (HBA) content of Vanilla planifolia were measured from beans harvested from August to December, 1986. The moisture content of the beans decreased from 87.6 to 81.4%. Vanillic acid remained constant, and both vanillin and HBA increased from 0.20 and 0.05 to 11.3 and 1.03 mg/g dry weight, respectively. The higher phenolic content could be attributed to fermentation on the vine; however, no correlation was observed between the extract color (i.e., green vs brown) and the vanillin content.**

Ulf Butehorn **and** ***Ute Pyell*** **(1996) reported that as a general example of the potential use of micellar elektrokinetic chromatography (MEKC) in food analysis, a rapid method for the determination of vanillin and related compounds and possible synthetic additives to vanilla flavourings by MEKC is described as a screening method for quality control. Under optimized conditions, baseline separation of nine vanilla constituents and three possible adulterants is possible within 9 min. The method was applied to additives to bakery products and flavoured beverages. The influence of organic solvents in the sample solution on peak shapes and migration times was investigated. Detection limits and precision of the**

method are given. It was shown that the use of an internal standard substantially increases the precision of the method. The linear calibration range covers two orders of magnitude with the use of an internal standard.

***Anon*(1998),found out that the chief constituent of vanilla is the aromatic, crystalline substance,vanillin,which is the aldehyde of methyl-protocathuic acid.Good beans contain vanillin from 2 to 2.72%. Other constituents are Vanillic acid, resin (4%), fat (11%), sugar (10%) etc.**

***Pu-Fan etal* (1998) conducted an experiment in China in which the content and variation of four compounds (vanillin, vanillic acid, parahydroxybenzoic acid and parahydroxybenzaldehyde) in *V. Planifolia* pods during curing, using an enzymatic treatment were determined using HPLC. Pods were treated with beta glucosidase. The contents of the above four compounds increased upon the treatment with the enzyme.**

***Paul R. Haddadb* and *Tomislav Sostarica*(2003) developed a mixed micellar electrokinetic capillary chromatography (MECC) method for the qualitative and quantitative determination of key components, including vanillin, 4-hydroxybenzaldehyde, 4-hydroxybenzoic acid, vanillin acid and 3-methoxybenzaldehyde, that contribute to vanilla flavour was investigated. The micellar phase consisted of sodium dodecyl sulfate (SDS) and sodium cholate (SC). The percent relative standard deviation (R.S.D.%) for migration time was <1 over six runs. The R.S.D.% for peak areas ranged between 0.85–1.96% over six runs. Peak efficiencies were excellent with theoretical plate numbers typically in the range of 130,000–200,000 per column (52 cm effective length). The limits of detection (LOD) were between 5–10 μg/ml. The quantitative data was verified by high performance liquid chromatography (HPLC) and gas chromatography (GC). The mixed MECC method was successfully applied to a number of natural vanilla extracts, nature identical extracts and synthetic flavourings.**

***Mark J. W. Dignuma, Rob van der Heijdenb, Josef Kerlerc, Chris Winkelc* and *Rob Verpoorte* (2004) reported that natural vanilla is extracted from the fruits of Vanilla planifolia. In the overall vanilla aroma, minor compounds p-cresol, creosol, guaiacol and 2-**

phenylethanol have a high impact. This is shown by the GC-Olfactometry analysis of cured vanilla beans. The presence of β-D-glucosides of these compounds was investigated, in order to determine if these compounds are derived from glucosides or if they are formed during the curing process via different pathways. Glucosides of vanillin, vanillic acid, p-hydroxy benzaldehyde, vanillyl alcohol, p-cresol, creosol and bis[4-(β--glucopyranosyloxy)-benzyl]-2-isopropyltartrate and bis[4-(β--glucopyranosyloxy)-benzyl]-2-(2-butyl)tartrate have been identified in a green bean extract. The kinetics of the β-glucosidase activity from green vanilla beans towards eight glucosides naturally occurring in vanilla and towards p-nitrophenol were investigated. For glucosides of p-nitrophenol, vanillin and ferulic acid the enzyme had a Km of about 5 mM. For other glucosides (vanillic acid, guaiacol and creosol) the Km-values were higher (>20 mM). The Vmax was between 5 and 10 IU mg−1 protein for all glucosides tested. Glucosides of 2-phenylethanol and p-cresol were not hydrolysed. β-Glucosidase does not have a high substrate specificity for the naturally occurring glucosides compared to the synthetic p-nitrophenol glucoside (Km 3.3 mM, Vmax 11.5 IU mg−1 protein).

Anuj Sharma, Subash Chandra Verma, Nisha Saxena, Neetu Chadda, Narendra Pratap Singh, Arun Kumar Sinha **(2005) have done Microwave- and ultrasound-assisted extraction of vanillin and its quantification by high-performance liquid chromatography in Vanilla planifolia.Microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE) and conventional extraction of vanillin and its quantification by HPLC in pods of Vanilla planifolia is described. A range of nonpolar to polar solvents were used for the extraction of vanillin employing MAE, UAE and conventional methods. Various extraction parameters such as nature of the solvent, solvent volume, time of irradiation, microwave and ultrasound energy inputs were optimized. HPLC was performed on RP ODS column (4.6 mm ID×250 mm, 5 m, Waters), a photodiode array detector (Waters 2996) using gradient solvent system of ACN and ortho-phosphoric acid in water (0.001: 99.999 v/v) at 25°C. Regression equation revealed a linear relationship (r 2 >0.9998) between the mass of vanillin injected and the peak areas. The detection limit (S/N = 3) and limit of quantification (S/N = 10) were 0.65 and 1.2 g/g, respectively. Recovery was achieved in the range 98.5-99.6% for vanillin. Maximum yield of vanilla extract (29.81, 29.068 and 14.31% by conventional extraction, MAE and UAE, respectively) was found in a mixture of**

ethanol/water (40 : 60 v/v). Dehydrated ethanolic extract showed the highest amount of vanillin (1.8, 1.25 and 0.99% by MAE, conventional extraction and UAE, respectively).

***Krzysztof N. Waliszewski etal*(2006) have studied the prehydration and enzymatic treatment of vanilla beans on the kinetics of vanillin extraction by three cellulolytic enzyme products. Vanilla beans are hydrated in water with 5 % ethanol upto 72 hr and reducing sugar and glucose were measured. Prehydration condition (5% ethanol at 48 hr) were used for the study of effect of pH and temperature on the kinetics of vanillin liberation.**

***F. Bettazzia, I. Palchettia, S. Sisallib* and *M. Mascinia*(2006) developed a disposable electrochemical sensor for the detection of vanillin in vanilla extracts and in commercial products. An analytical procedure based on square-wave voltammetry (SWV) was optimised and a detection limit of 0.4 µm for vanillin was found. A relative standard deviation of 2% was calculated for a vanillin concentration of 100 µm. The method was applied to the determination of vanillin in natural concentrated vanilla extracts and in final products such as yoghurt and compote. The obtained results were compared with those provided by a reference method based on HPLC. The electrochemical behavior of other compounds (vanillic acid, p-hydroxybenzaldehyde, p-hydroxybenzoic acid, etc.), generally present in natural oleoresins, were also studied, to check for interferences with respect to the vanillin voltammetric signal..**

***Violeta T. Pardiob* and *Sandy L. Ovandoa* (2006) describe a simple and rapid HPLC technique for vanillin determination in alcohol vanilla extract. Vanillin was separated on a Nucleosil C18 column by using water and methanol (40:60) as the mobile phase and retention time was only 2.2 min. The measurements were made using a photodiode array detector of the most adequate maximum wavelength absorbance at 231 nm. This method has been successfully applied for the determination of vanillin in some commercial extracts.**

***A. Pérez-Silvaa, d, E. Odouxa, P. Brata, F. Ribeyrea, G. Rodriguez-Jimenesb, V. Robles-Olverab, M.A. García-Alvaradob and Z. Günatac*(2006) reported that volatile compounds from cured vanilla beans were extracted using organic solvents. Sensory**

analysis showed that the aromatic extract obtained with a pentane/ether (1/1 v/v) solvent mixture provided the extract most representative of vanilla bean flavour. Sixty-five volatiles were identified in a pentane/ether extract by GC–MS analysis. Aromatic acids, aliphatic acids and phenolic compounds were the major volatiles. By GC–O analysis of the pentane/ether extract, 26 odour-active compounds were detected. The compounds guaiacol, 4-methylguaiacol, acetovanillone and vanillyl alcohol, found at much lower concentrations in vanilla beans than vanillin, proved to be as intense as vanillin.

Claudia Valdez-Floresa **and** ***M.P. Cañizares-Macias*** **(2007) developed a continuous flow method coupling the dilution of extracts, obtained by application of ultrasound, and thus vanillin may be detected directly . The flow method allowed the quantification of vanillin in a range between 200 mg l−1 and 2000 mg l−1 with a repeatability and reproducibility of 3.79% and 3.03%, respectively, for a standard of 1200 mg l−1. The extraction conditions such as irradiation power, irradiation time, non-irradiation time and number of cycles are some of the most significant conditions in the vanilla extraction by ultrasound assisted extraction (USAE). The obtained results were compared with other conventional extraction methods: Soxhlet and maceration in accordance with the Mexican official method. The results showed that with the application of the USAE the extraction efficiency was increased between 19% and 72% in comparison with Sox let and maceration, respectively.Besides, the extraction time decreased between 83% and 98%.**

Lowri S. de Jager, a, Gracia A. Perfettia, and Gregory W. Diachenkoa**, (2007) developed a LC–MS method for the determination of coumarin, vanillin, and ethyl vanillin in vanilla products. Samples were analyzed using LC–electrospray ionization (ESI)–MS in the positive ionization mode. Limits of detection for the method ranged from 0.051 to 0.073 µg mL−1. Using the optimized method, 24 vanilla products were analyzed. All samples tested negative for coumarin. Concentrations ranged from 0.38 to 8.59 mg mL−1 () for vanillin and 0.33 to 2.27 mg mL−1 () for ethyl vanillin. The measured concentrations are compared to values calculated using UV monitoring and to results reported in a similar survey in 1988. Analytical results, method precision, and accuracy data are presented.**

***Reuz-Tevan etal*(2007) extracted glucovanillin from greenpods of V.Planifolia and simultaneously transformed to vanillin by a combination of enzymatic activities involving cell wall degradation and glucovanillin hydrolysis. The reaction is best carried out with 45.5% aqueous ethanol solution during 8 hr at 70˚C in a two step enzymatic reactor. The amount of extracted vanillin is 3.13 times higher than that obtained with soxhlet apparatus.**

2.8.2. Vanilla Oleoresin

Vanilla oleoresin is a semi-solid concentrate obtained by removing the solvent from the vanilla extract. A solution of isopropanol is frequently used instead of ethanol for the preparation. Some flavor and aroma is lost during removal of the solvent, but it does contain essential oils. Vanilla oleoresin is used in non-food products. Unfortunately, it isn't always stable in candle and soap making, which is too bad, as it's considerably less expensive than Vanilla Absolute.(*Purseglove,*1988)

2.8.3. Vanilla Absolute

Vanilla absolute is the most concentrated form of vanilla. It is often used to in perfumes and other aroma-based products. Because it's so expensive, most candles, soaps, and other scented specialty merchandise, are made from synthetic vanillin. Vanilla Absolute is used in very high-end products in small quantities, often mixed with other fragrances in perfumes.(*www.vanilla.com*)

2.9. USES OF VANILLA

Most vanilla used in the food industry is in dairy products, followed by beverages, baked goods and confections. However, vanilla is often used as a background note or flavor enhancer to round out the flavor profiles of many food products. The type of vanilla used depends on the product, the ingredients in the base formulation, and the desired flavor profile.

2.9.1. Flavor Enhancer

The merging of vanilla and chocolate has been a successful one, dating back to the 1500s. Vanilla softens or rounds out harsh, bitter notes in most chocolate applications such as ice creams, cakes and syrups. In confections such as chocolate bars, powdered vanillin is used most often. Vanilla is often used to enhance fruit flavors in many dairy and beverage applications. It rounds out many fruit flavors and takes off some of the tart edges. It is generally used as a background note in a variety of sweet and fruit flavors to round out the flavor profile. Vanilla enhances the sweetness perception of foods, especially in bakery products.(www.joyofbaking.com)

2.9.2. Dairy Products

Vanilla is the most popular flavoring for ice cream. The type, or "category," of vanilla used determines how ice cream is labeled.

Category 1: Natural vanilla extract: Two-fold vanilla is commonly used. Ice cream products must be labeled as "vanilla ice cream."

Category 2: Vanilla-vanillin extract: This is considered natural and artificial (N&A), where the natural component is the characterizing flavor. Ice cream products must be labeled as "vanilla flavored ice cream."

Category 3: Natural and artificial vanilla flavors or artificial vanilla flavors, where the artificial component predominates. Ice cream products must be labeled "artificially flavored vanilla ice cream."

***Elke Anklam, Silvia Gaglione* and *Anne Muller* (1997) investigated the stability of vanillin in dairy products by means of high-performance liquid chromatography. While vanillin was oxidized to vanillic acid in fresh and pasteurized milk, it was stable in all other milk products investigated (UHT milk, pasteurized and UHT cream, yoghurt, curd and**

butter). The oxidation of vanillin to vanillic acid was shown to be pH-dependent. The relatively thermolabile enzyme xanthine oxidase was shown to be partly responsible for the formation of vanillic acid. However, peroxidase, which is also present in dairy products, did not lead to vanillic acid. Oxidation to the dimeric product, divanillin, occurred in the presence of peroxidase and hydrogen peroxide. The oxidation rate of synthetic vanillin to vanillic acid was very similar to that of vanillin from natural vanilla extracts or vanilla capsules. Both were shown to be stable in dairy products for subvention, such as butter and cream.

2.9.3. Bakery Products

Pure vanilla extract is generally not used for baking because the aromatic components of extracts begin to volatilize at about 280° to 300°F, a temperature that is readily attained in cookie baking. Cakes rarely exceed 210°F internally, so an extract or blend of extracts may be used successfully, but a stronger extract such as a two-fold may be more effective. Vanilla-vanillin extracts and artificial flavors are generally recommended for baking applications. Natural and/or artificial flavors give food product designers the added benefit of blending vanilla with various flavor notes such as buttery, nutty and brown sugar.(*www.culinarycafe.com)*

2.9.4. Beverages

Vanilla is an important flavor component in colas, in addition to the complex of spice and citrus notes. A recent publication listed vanilla as well as 25 other flavor notes responsible for a cola flavor. Cream sodas, root beer, and some fruit beverages also may contain vanilla. Vanillin or vanilla flavors are used in many alcoholic beverages, such as whiskeys, cordials and cocktails, to round out and smooth the harsh edges of the alcohol. In whiskey products, vanillin is one of the chemicals extracted from the oak barrels in which the products age. (*www.vanilla.com*)

MATERIALS AND METHODS

CHAPTER III
MATERIALS AND METHODS

The procedure for standardization of parameters for extraction, the details of the components of the vanilla extractor and the test procedure for evaluation of the performance of the extractor are described in this chapter.

3.1. DETERMINATION OF MOISTURE CONTENT OF CURED VANILLA BEANS

3.1.1. Materials Used:

a. **Cured Vanilla beans**
b. **Moisture analyzer**

3.1.2. Methodology:

Cured vanilla beans of about 5gm was taken in a sample pan and kept inside the sample chamber of the moisture analyzer. The sample was kept in the chamber for about 30 minutes. When a beep sound was heard, the moisture content was directly read from the display screen.

3.2. STANDARDIZATION OF VARIOUS PARAMETERS FOR EXTRACTION

3.2.1. Selection of Solvent for Extraction

A thorough review of the Literature revealed that ethanol is the most suited solvent for extraction of vanilla. Therefore ethanol and water (being a universal solvent) were analyzed for their suitability for use as solvent. Five gram of chopped cured vanilla bean was dipped in 50ml of 99.9% ethanol for 5days. Similarly, 5 gm of chopped cured vanilla was dipped in 50ml of pure water for 5 days. Then the physical appearance and vanillin

content of both the samples were analyzed. The vanillin content was analyzed using a UV spectrophotometer.

3.2.2. Standardization of Concentration of Ethanol for Extraction

***Merory (*1956) has recommended three consecutive extractions; each for atleast five days, using a menstruum with a 60% concentration for the first extraction, 30 % for the second and 15% for the third extraction. This extract will have a residual alcohol content of 35% in the extract which is the recommended percentage as per standards.**

Standardization of the concentration of ethanol as solvent for extraction was carried out in three stages. In the first stage,10 gm of vanilla beans each were dipped in 60%,30% and15% ethanol separately in three standard flasks for five days. Afterwards the amount of extract obtained and percentage vanillin were found.

In the second stage, dual combination of these concentrations such as (60%, 30%), (30%,15%) and (60%,15%) were prepared in which 10 gm of cured vanilla beans were dipped in 1st concentration for 5 days in a standard flask and then the beans were subjected to dipping in second concentration of each of these dual combination of ethanol concentration, after removing the previous extract for 5 days. The extract obtained after the two treatments of concentrations of each dual combinations were mixed. The amount of extract and vanillin content of the three dual combinations were determined.

The third stage constitutes the combination of the three concentration in which 10 gm of cured vanilla beans were dipped in 60% ethanol for 5 days in a standard flask, then the beans were subjected to dipping in 30% ethanol after removing the previous extract, for 5 days and then with 15% ethanol for another 5 days after removing the extract of the previous treatment. The extract obtained after the treatments with 60%, 30% and15% ethanol were mixed and the amount and the vanillin content of the extract were determined.

3.2.3. Standardization of Method of Extraction

There are two main methods of vanilla extraction.i.e.percolation and dipping. Percolation is a step by step process in which the menstruum is circulated over the chopped cured vanilla beans for a specified period of time which facilitates the complete extraction of all the major aromatic constituents from the cell sap of the bean. Thus the percolation method yields the maximum amount of quality vanillin. The dipping method on the other hand is a traditional method of preparing vanilla extract and it involves placing the chopped beans in a vessel where they are allowed to steep in the menstruum.

To compare the effectiveness of both the extraction methods, a laboratory set up was arranged in the product analysis lab.

3.2.3.1. Laboratory Set-Up for Vanilla Extraction

3.2.3.1.1. *Materials used:*

a. **Burette 50 ml**
b. **Holding glass tube with adjusting flow knob**
c. **Collecting flask**
d. **Circulating tube**

3.2.3.1.2. *Methodology:*

Fifteen grams of cured chopped vanilla beans were placed in the holding tube, which was held straight by a stand. The ethanol concentration selected was the combination of (60%, 30%, and 15%) each for 5 days. Initially, ethanol of concentration 60% was filled in the burette and by adjusting its knob and connecting a passage tube to the holding tube; the ethanol was made to fall over the chopped beans drop by drop. The holding tube also consist of a knob which can be adjusted to collect the percolated solution into the collecting flask which was kept under holding tube .The circulation of the collected

extract was carried out twice a day over the same chopped beans. The extract was collected after 5 days. Similarly 30% and 15% ethanol was circulated over the beans each for 5 days. Entire set-up was made air tight. The three extracts collected were mixed and stored in air tight black bottle. The laboratory set up for the extraction is shown in plate 3.1.

Plate 3.1: Laboratory set- up for vanilla extraction

3.2.4. Standardization of Set of Concentration of Ethanol

In order to further verify the suitability of combination of ethanol concentrations, the upper and lower levels of concentration of 60%,30%and15% ethanol were also tested so as to get a 35% ethanol concentration in the final extract mix, which is the recommended limit for safe consumption.(*Purseglove,* 1988).The set of concentration so selected were (70%,25%,10%) and (50%,40%,15%) besides (60%,30%,25%).The experiments were conducted in the same way as for (60%,30%,15%)ethanol as explained in section 3.2.3.1.

3.2.5. Standardization of Days for Extraction

Standardization of days was done with the (60%,30%,15%) ethanol concentration on 10gm chopped cured vanilla beans by percolation method as explained in section 3.2.3.1 for 4days,5 days, and also for 6 days. The extracts were then collected and the amount of extract and vanillin content were determined.

3.3 DEVELOPMENT OF THE VANILLIA EXTRACTOR

3.3. 1.General Layout and Details of the Equipment

The equipment consists of the following components:

A. **Extraction cylinder**
B. **Hot water jacket**
C. **Cup assembly**
D. **Frame assembly**

The controls and other accessories include:

a. **Heater (1000 W)**
b. **Thermostat**
c. **Pump (CF self priming, 50 Hz, 0.5 hp, 0.37 KW)**
d. **Power supply unit that supply electric power to the heater, thermostat and pump.**

The elevation, plan, and side view of the fabricated extractor is shown in fig3.1, 3.2 and 3.3 and plates 3.2, 3.4 and 3.5

3.3.1.1. ***Extraction cylinder:***

The extraction cylinder is the major component of the extraction unit. This is made of 22 gauge stainless steel sheet bent to form a cylindrical container with a diameter of 128mm and length 800mm. The extraction cylinder is connected to the inlet of a 0.5 hp self priming centrifugal pump for circulation of the solvent though a T joint. The T joint is connected to the 1/2″ diameter stainless steel drain pipe which would facilitate the discharge of the extract, whenever needed, through the drain pipe and at the same time help in recirculation of menstruum. A thermostat, provided on the extraction unit would sense the temperature of the extract and would cut-off electric supply to the water heater when the preset temperature is reached. This will ensure a constant temperature in the water bath based on the temperature of the extract.

3.3.1.2 ***Hot water jacket:***

The extraction cylinder is jacketed for hot water using 22gauge stainless steel sheet, with a length and diameter of 900mm and 196mm respectively. The bottom portion of the water jacket forms a water tank of 100mm height (fig3.1).A 1000W immersion water heater is provided inside the water tank. The water jacket is provided with an inlet port and a drain pipe.

3.3.1. 3 ***Cup assembly:***

The cup assembly consists of five stainless steel cups of 110mm diameter and 50mm height.Four millimeter diameter holes are drilled at the bottom of each cup for easy draining of solvent. The cups are arranged as an assembly one above the other at a distance of 100mm on a 6mm diameter MS rod (plate3.6).The cup assembly can be lifted or lowered into the extraction cylinder using a wire and pulley attachment. The assembly can be lifted and held at any position in the extraction cylinder, for the loading or unloading of the chopped vanilla by a sprocket and stopper attachment. The top end of the cup assembly is

connected to the lifting wire through a shower nozzle and a SS closing lid. The shower nozzle is connected to the outlet of a centrifugal 0.5hp self priming pump though a 12.25mm diameter flexible pipe (plate 3.3)

3.3.1. 4 *Frame assembly:*

The frame assembly supports the entire unit satisfactorily without any vibration.This is made of M.S angle iron of size of 23mm×23mm×4mm.

3.4. EXPERIMENTAL PROCEDURE FOR TESTING THE VANILLA EXTRACTOR

The extraction of vanilla for the production of vanilla extract using the fabricated vanilla extractor is carried out as per the flow diagram as shown in fig 3.4.Cured chopped vanilla beans weighing 450 gm are placed uniformly in each of the perforated cups. Ethanol is used as the solvent for the extraction and is sprayed through the shower nozzle from the top of the cup assembly. The temperature of ethanol is maintained at 45ºC as at this temperature the enzyme activity in vanilla is at its peak and is recommended for spice extraction. The ethanol in the extraction cylinder receives heat from hot water jacket. The thermostat provided in the extraction unit senses the temperature of the ethanol and cuts-off the electric supply to the immersion water heater when the ethanol temperature of 45ºC is reached. Thus a constant warm temperature of 45ºC is maintained for ethanol. This warm ethanol from the extraction tank is pumped by the centrifugal pump and sprayed from the top of the cup assembly. The ethanol so sprayed percolates though the bed of vanilla kept in the cup assembly as it descends and in the process vanillin from the bean sap is extracted. The ethanol after each circulation is collected in the tank and is recirculated. The pumping, circulation and percolation is carried out for three different concentration .i.e.60%, 30% and 15% ethanol. Each concentration is percolated for two hours in a day for 5 days in the descending order. Finally the three sets of extract are collected and mixed so that the over all concentration of ethanol would be 35% which is considered optimum as per standards. This mixture is kept for ageing and finally the vanilla extract is obtained.

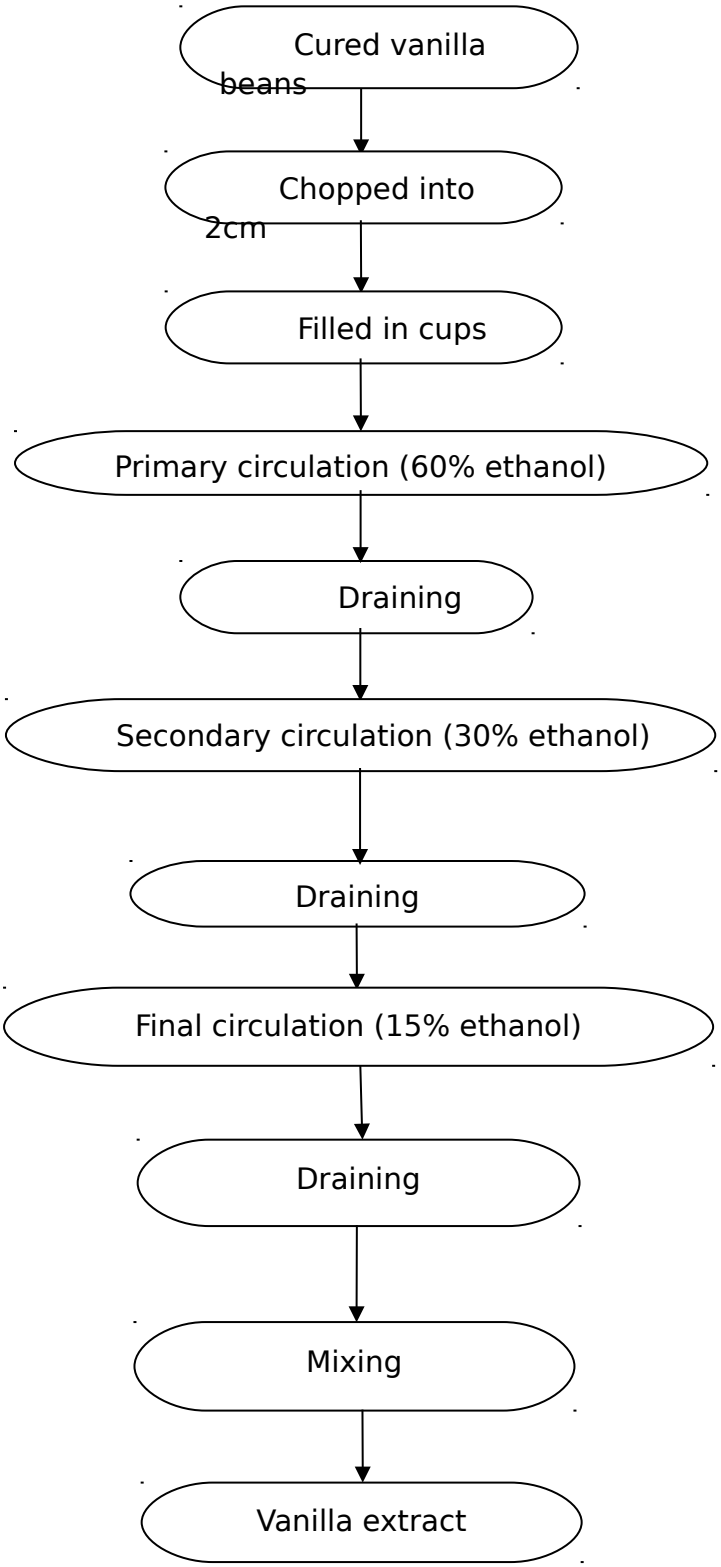

Fig3.4 Flow diagram of the preparation of vanilla extract

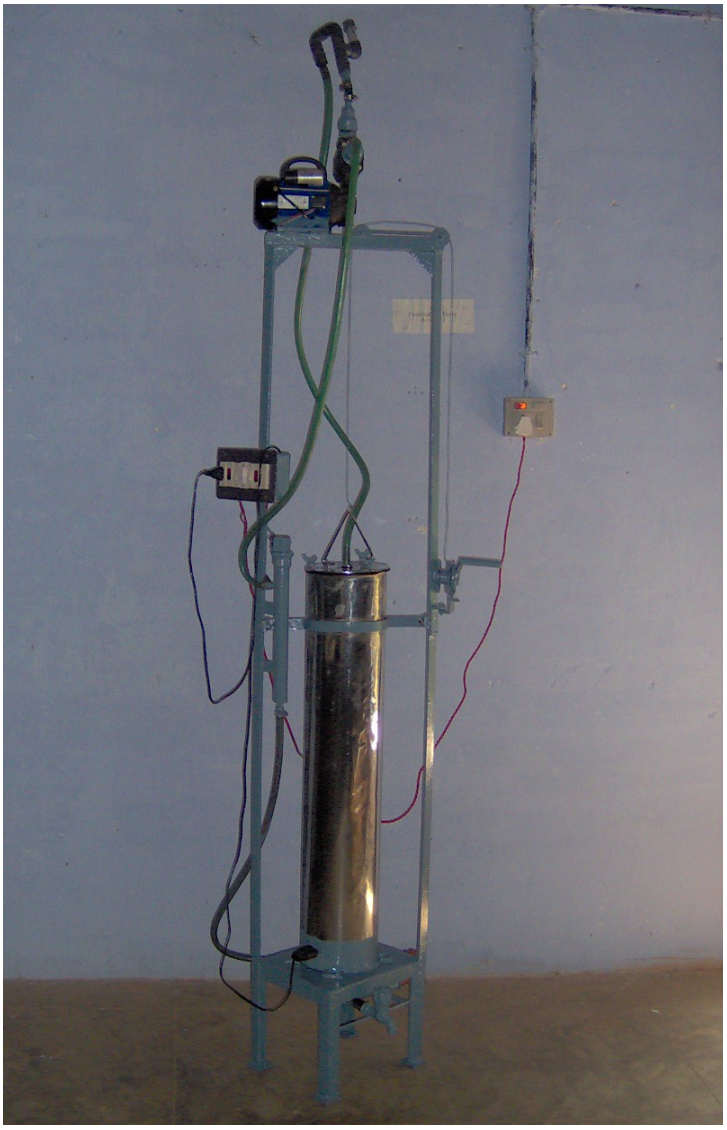

Plate3.2. Elevation of the fabricated vanilla extractor

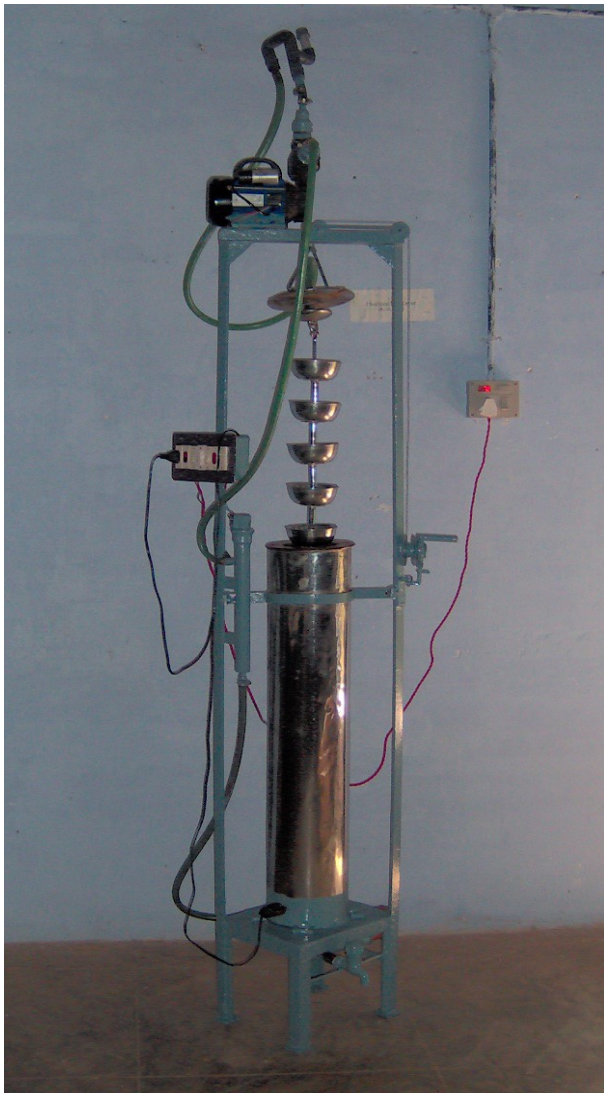

Plate.3.3. Elevation of the fabricated vanilla extractor along with cup assembly

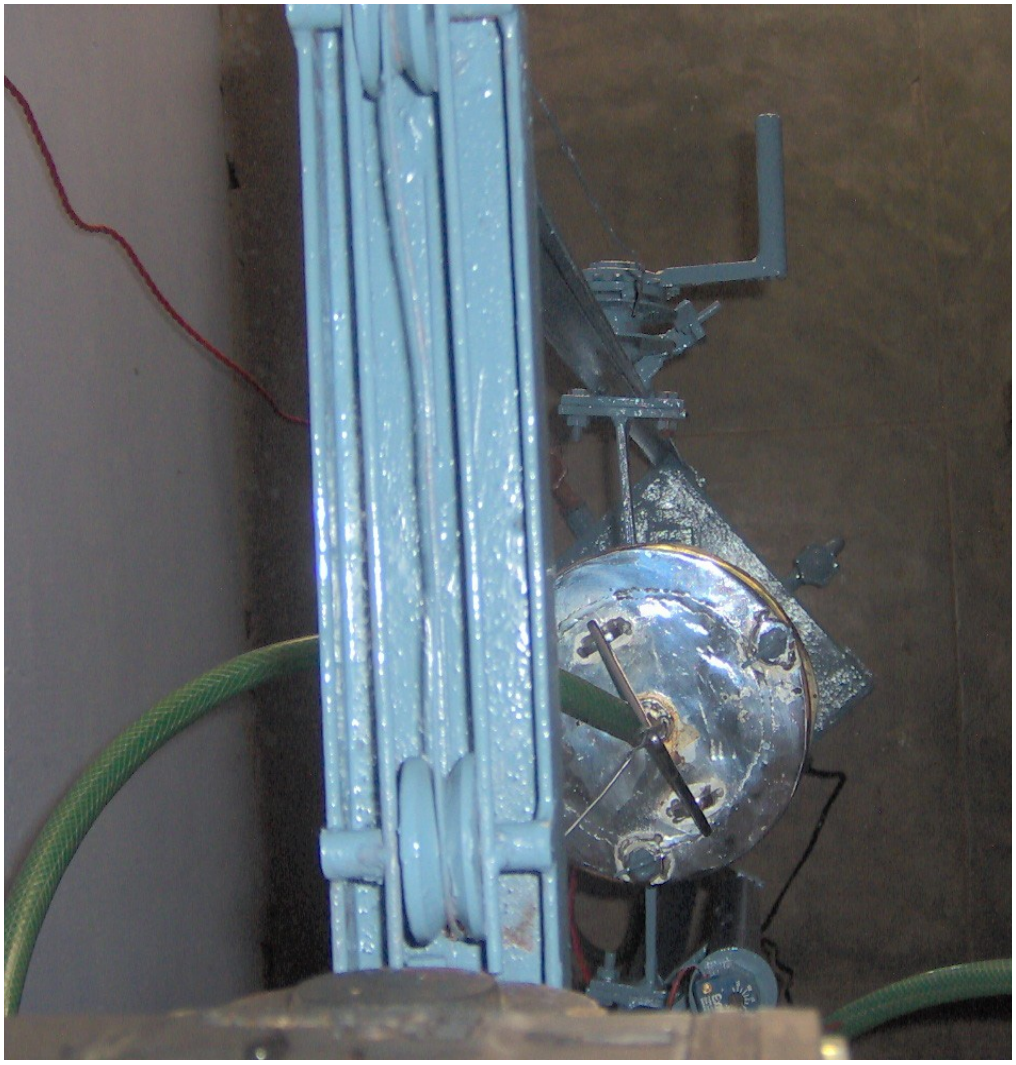

Plate 3.4. Plan of the vanilla extractor

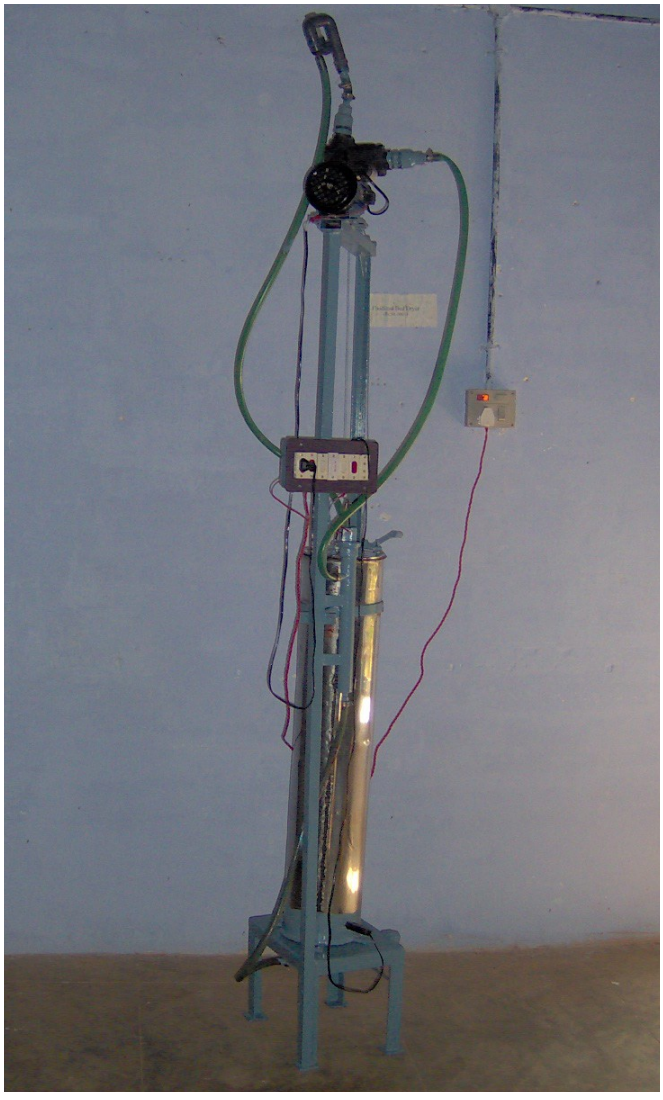

Plate 3.5. Side view of the vanilla extractor

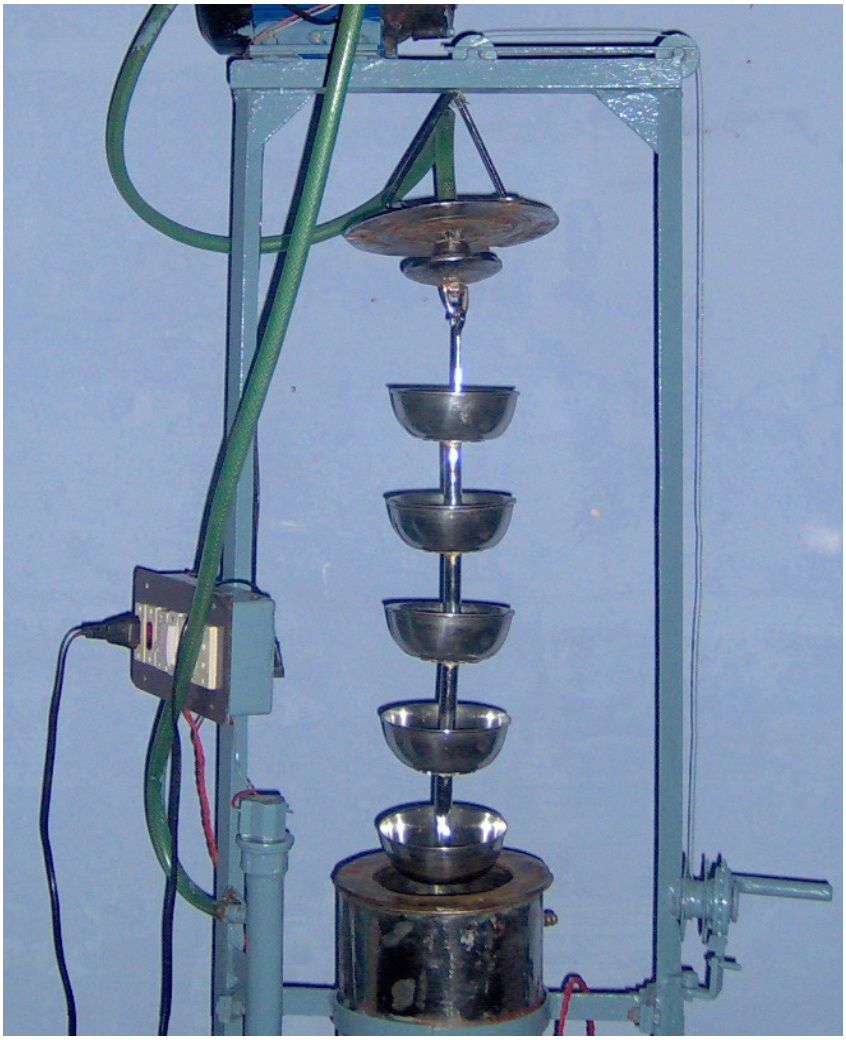

Plate 3.6. Cup assembly with wire and pulley arrangement

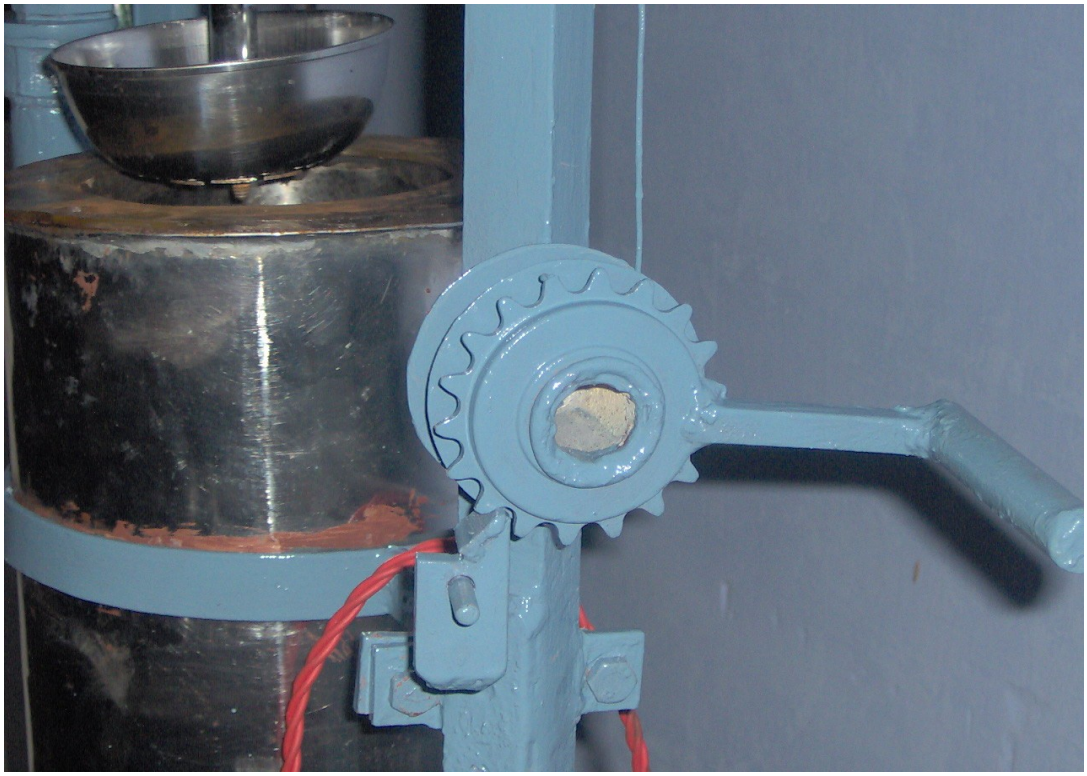

Plate 3.7. Sprocket and stopper arrangement

3.5. ESTIMATION OF VANILLIN CONTENT IN THE EXTRACT

3.5.1 Reagents Used:

1. Ethanol
2. Distilled water
3. NaOH pellets

3.5.2. Preparation of Working Standard without NaOH

Hundred milligram vanillin powder obtained from analysis lab of Department of Plantation crops and Spices, College of Horticulture, Vellayanikara was dissolved in 5 ml of 99.9% ethanol and diluted to 100 ml distilled water. Transferred 2, 4, 6, 8, and 10 ml each to 100ml volumetric flask. Made up the volume to 100 ml with the distilled water. Pipetted out 5 ml each of the working standards to 100 ml volumetric flask made up to the volume and mixed thoroughly.

3.5.3. Preparation of NaOH Solution

NaOH pellet of about 0.4gm was taken and dissolved in 50ml of distilled water and thoroughly stirred for about five minutes. Then it was made up to 100ml.

3.5.4. Preparation of Working Standard with NaOH

Another set of pure vanillin solution of 2, 4, 6, 8, 10ml was taken in separate volumetric flasks of 100 ml. Made up the volume with distilled water. 5 ml of each of the solution was pipetted out to 100 ml volumetric flask and to it 2 ml 0.1 N NaOH was added. Volume was made up to 100 ml. From this, samples were taken in the quartz cell of the UV spectrophotometer and the absorbance read at 348 nm. The standard graph was plotted using difference in values obtained with and without NaOH.

3.5.5. Preparation of Test Sample with Vanilla Extract

Vanilla extract of about 5 ml was taken in a 100ml standard flask and it was made up to 100ml using distilled water. This was kept for 15 minutes. From that two 5ml samples were pipetted out into two separate standard flasks. NaOH solution of about 2ml from the prepared sample of NaOH was pipetted out into one sample and the other was kept blank. Then both the samples were made up to 100ml.From each of these two samples, about 5ml was taken for testing.

3.5.6. Testing the Sample using Ultraviolet Spectrophotometer

Ultraviolet spectrophotometer is initially kept for warming for 15minutes.Then the wavelength is set for 348nm which comes under the ultraviolet region. When the extract without NaOH has to be analyzed, water was taken as blank sample in quartz cell and absorbance was set zero. The extract without NaOH was then kept in the quartz cell and absorbance was read. Similarly, when the extract with NaOH has to be analyzed, the prepared working sample with NaOH was taken as blank sample in the quartz cell and absorbance was set zero. The extract sample with NaOH was then kept in quartz cell and absorbance was noted.

3.5.7. Calculation of Reading obtained from Ultraviolet Spectrophotometer

3.5.7.1. *Preparation of standard graph*

A standard graph was plotted with X-axis and Y-axis as concentration of vanillin in mg/l and optical density respectively.

Optical density = /absorbance of sample with NaOH--- absorbance of sample without NaOH/

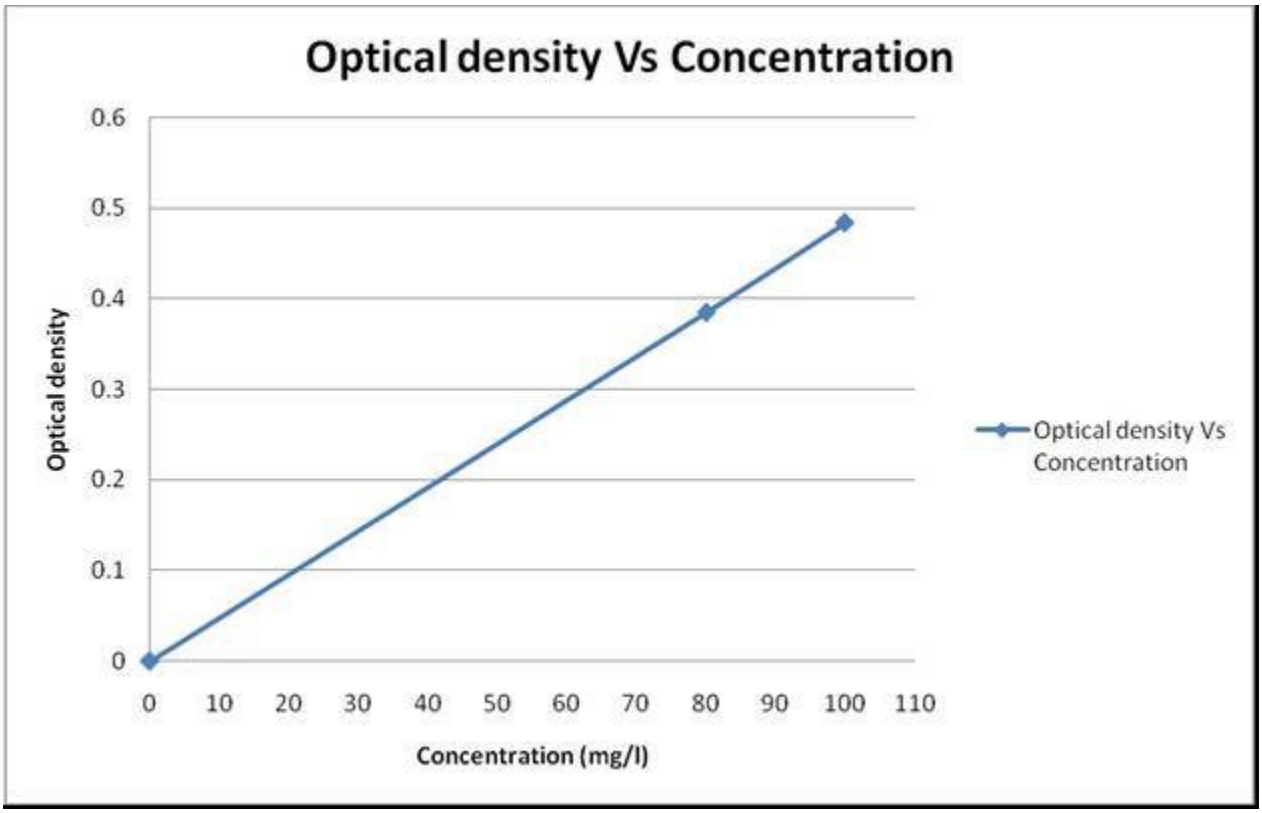

Fig.3.5.Standard graph for vanillin estimation

3.5.7.2. ***Calculation of vanillin content***

The vanillin content in percentage can be obtained using the formula,

$$\text{Percentage vanillin content} = 100 \times \frac{(P^* \times C \times V \times E)}{(P \times v \times W)}$$

where:

P*--- Optical density of test sample
P---- Optical density of standard solution
C---- Concentration of standard solution (mg/l)
V---- Total volume of sample prepared (ml)
v--- Volume of sample taken (ml)
E---- Volume of extract obtained (l)
W--- Mass of vanilla beans taken for extraction (mg)

3.6 ESTIMATION OF EXTRACTION EFFICIENCY

Twenty five samples of cured vanilla beans processed in the Drying Lab of the Department of PHT&AP,each of 10 gm were analyzed for their vanillin content at the Analytical Lab of the Spices Board,Cochin.As this vanilla was used as the raw material for the production of extract, the average value of the vanillin content could be taken as the vanillin content of the cured vanilla beans.

The extraction efficiency was calculated using the formula

Extraction efficiency (%) =

$$\frac{\text{Vanillin content of the extract obtained from vanilla extractor}}{\text{Vanillin content of the cured vanilla beans used as the raw material}} \times 100$$

3.7 ESTIMATION OF EVAPORATIVE LOSS OF THE EXTRACTOR

The percentage evaporative loss from the equipment is calculated by the formula

$$\% \text{ Evaporative loss} = \frac{(S-E)}{(S)} \times 100$$

where:

S -- Amount of solvent used for extraction

E -- Amount of vanilla extract obtained

RESULTs AND DISCUSSION

CHAPTER IV
RESULTS AND DISCUSSION

Results and discussion on the experiments carried out for standardization of various parameters leading towards the development of the Vanilla extractor and testing of the extractor are dealt in this chapter.

4.1 TEST SAMPLE

Cured Vanilla beans available in the PHT & AP department of Kelappaji College of Agricultural Engineering and Technology, Tavanur was used for the experiments. The initial moisture content of the vanilla beans was estimated by the standard method explained in chapter 3 and the results are tabulated in the table 4.1

4.1.1 Moisture Content of Vanilla Beans

Moisture content of beans during conditioning has profound influence on aroma and flavor quality. Fresh green beans have a moisture content of about 80%. The major moisture loss in curing process occurs during the sweating/drying stages. Also moisture reduction occurs during the first three months of conditioning afterwards the moisture loss becomes negligible. The moisture content of top grade beans should be high, i.e. 30-40% where as moisture content of less than 10% is considered as low grade.

Therefore, it may be inferred that the cured vanilla used for extraction is of low grade as the moisture content is in the range 5.07 – 5.078 giving an average value of 5.08% in wet basis.

Table 4.1: Moisture content of the cured vanilla beans

Weight of sample (gm)	Moisture content (%wb)	Average moisture content (%wb)
5	5.07	5.08
5	5.1	
5	5.078	

4.2 STANDARDIZATION OF VARIOUS PARAMETERS FOR THE EXPERIMENT

4.2.1 Selection of the Solvent for Production of Vanilla Extract

The results of the experiment conducted for the selection of the solvent for extraction were presented in Table 4.2.From the results, it may be revealed that water is not found to be suitable solvent for vanilla extraction as the vanillin content from the beans was found to be only 0.7% where as it was 1.3% when ethanol was used as solvent. Also, it was found that the cut vanilla beans which were dipped in water for a specified time got swelled by absorbing water and the samples treated with ethanol for the same specified time was found to be shrunken in appearance. This indicated that there was diffusion of the contents from the vanilla beans into the solvent in the latter case whereas in the former case water is penetrating into the beans, which resulted in fermentation, and occurrence of bad odor. Therefore, it was concluded that water alone could not be used as a solvent for efficient extraction. So, ethanol may be used as the solvent for production extract. The swelled and shrunken appearances of vanilla beans treated with water and ethanol are shown in plate no 4.1.

4.2.2 Standardization of the Concentration of Ethanol for Extraction

For standardization of the concentration of ethanol as solvent for extraction, a series of set of concentrations were made, that is single set, two set and three set concentrations as explained in ChapterIII. The results of the experiments are shown in Table 4.3. Among the single set of concentrations, three concentrations, that is, 60%, 30% and 15% ethanol were tested. Among these three, the highest vanillin content of 1.5% was found for 15% ethanol. The three two sets combinations of concentrations, i.e. (60%, 30%), (30%, 15%) and (60%, 15%), shows no significant difference in vanillin content. The combination of all the three concentrations i.e. 60%, 30% and 15% gave maximum percentage of vanillin.i.e.1.62%. This may be due to the fact that when the beans are treated with 60% ethanol several glycosides are removed, then when the bean comes in contact with the 30% ethanol various aldehydes, aromatic esters, protocatechic acids, benzoic acids, and vanillic acid are removed, finally 15% ethanol completely extracts the vanillin from the bean resulting in a very strong aromatic extract.

Table 4.2: Percentage vanillin obtained when water and ethanol was used as solvent

Solvent used	Weight of vanilla beans(gm)	Amount of solvent (ml)	Amount extract obtained (ml)	Vanillin (%)
Water	5	50	38	0.4
Ethanol (99.9%)	5	50	49	1.3

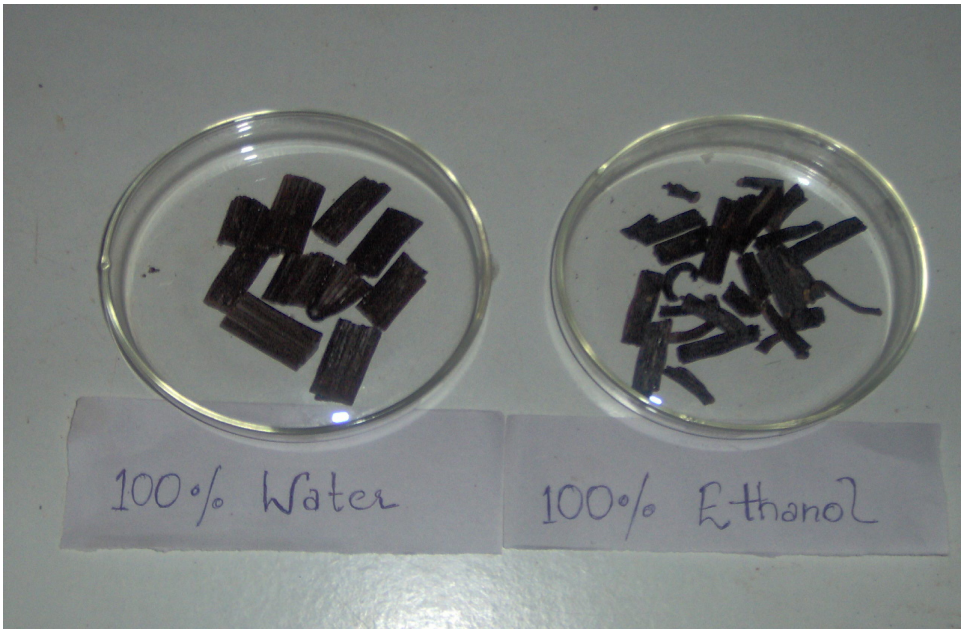

Plate 4.1: Vanilla samples treated with water and ethanol

Table 4.3: Standardization of concentration of ethanol for extraction

Weight of chopped beans (gm)	Ethanol (%)	Water (%)	Ethanol (ml)	Water (ml)	Amount extract obtained (ml)	Vanillin (%)
10	60	40	60	40	88	1.4
10	30	70	30	70	87	1.2
10	15	85	15	85	86	1.5
10	(60, 30)	(40,70)	(30, 15)	(20,35)	87.5	1.25
10	(30,15)	(70,85)	(15,7.5)	(35,42.5)	86.5	1.247
10	(60,15)	(40,85)	(30,7.5)	(20,42.5)	87	1.26
10	(60,30,15)	(40,70,85)	(20,10,5)	(13.3,23.3,28.4)	88	1.62

4.2.3 Standardization of Method of Extraction

Results of the experiments conducted to establish the best method of extraction out of the two general methods such as Percolation and Dipping are shown in Table 4.4.The standardized set of concentrations that is (60%,30%,15%) of ethanol was employed. Dipping method yielded low vanillin as indicated in the above result. This is due to incomplete and inefficient extraction of vanillin from the cell sap of the beans as compared to the percolation method. This established that percolation method is best and efficient method for production of vanilla extract.

4.2.4 Standardization of the Set of Concentrations of Ethanol

Results of the experiments presented in Table4.3 revealed that the set of (60%, 30%, 15%) ethanol is the best suited combination of solvent for efficient production of vanilla extract. However, to further substantiate and verify the findings, tests were carried out using two more sets of concentrations as shown in the table 4.5, each sets summing up to 35% ethanol concentration, which is considered optimum and safe for use.

4.2.5 Standardization of Number of Days for Extraction

Table 4.6 presents the results of the trials conducted for optimization of the number of days of percolation for complete extraction of vanilla. It may be seen that there is no significant variation in the percentage of vanillin obtained for 5 days and 6 days treatment whereas there exits considerable increase in vanillin content in the extract between 4 days and 5 days treatment. From this it may be concluded that percolation for 5 days may be considered optimum for maximum extraction of vanillin.

Table 4.4: Standardization of method of extraction

Method	Weight of chopped beans (gm)	Ethanol (%)	Water (%)	Ethanol (ml)	Water (ml)	Amount of Extract obtained (ml)	Vanillin (%)
Dipping	15	(60,30,15)	(40,70,85)	(30,15,7.5)	(20,35,42.5)	125	1.1
Percolation	15	(60,30,15)	(40,70,85)	(30,15,7.5)	(20,35,42.5)	130	1.4

Table 4.5: Standardization of set of concentration of ethanol

Weight of chopped beans (gm)	Ethanol (%)	Water (%)	Ethanol (ml)	Water (ml)	Total solvent used (ml)	Amount of extract obtained (ml)	Vanillin (%)
15	(60, 30,15)	(40,70,85)	(30,15,7.5)	(20,35,42.5)	150	130	1.36
15	(50,40,15)	(50,60,85)	(25,20,7.5)	(25,30,42.5)	150	128	1.27
15	(70,25,10)	(30,75,90)	(35,12.5,5)	(15,37.5,45)	150	90	1.02

Table 4.6: Standardization of days for extraction

Weight of chopped beans (gm)	Ethanol (%)	Water (%)	Ethanol (ml)	Water (ml)	No of days kept	Amount of extract obtained (ml)	Vanillin (%)
10	(60,30,15)	(40,70,85)	(20,10,5)	(13.3,23.3,28.4)	4	78	1.19
10	(60,30,15)	(40,70,85)	(20,10,5)	(13.3,23.3,28.4)	5	80	1.48
10	(60,30,15)	(40,70,85)	(20,10,5)	(13.3,23.3,28.4)	6	94	1.45

Based on the results of the experiments conducted and discussed, a percolation type extractor made of stainless steel with arrangements for heating and controlling the temperature was fabricated. It was found that a combination set of 60%,30% and 15% ethanol percolated for a period of fifteen days at the rate of 2 hours per day for 5 days for each concentration so as to produce and extract with 35% ethanol resulted in maximum extraction of vanillin.

4.3 TESTING OF VANILLA EXTRACTOR

The fabricated vanilla extractor was tested for its extraction efficiency and evaporative loss. Table 4.7 shows the average vanillin content of 25 samples of cured vanilla beans processed in the drying lab of the department of PHT & AP. These samples were analyzed for percentage vanillin at the Spices Board analytic lab by standard methods. It may be revealed from the results that average vanillin content of the raw material used for the production of vanilla extract using the developed prototype was 1.2%.

The results of the tests conducted for evaluating the performance of the extractor is presented in Table 4.8.It may be seen from that result that the developed vanilla extractor has a capacity of 500gm per batch and the extraction efficiency is 84.2%.The evaporative loss was only 12.9%. The developed extractor is simple in design, easy to fabricate, maintain and operate. Cost of fabrication and operation is less and is therefore economical for farmers and small scale industries. The capacity of the extractor may be increased by increasing the number of cups and overall dimensions.

Table 4.7 Average vanillin content of the cured vanilla bean

Sample number	**Weight of each sample(gm)**	**% vanillin**
1	**10**	**1.35**
10	**10**	**.24**
11	**10**	**1.62**
12	**10**	**1.21**
13	**10**	**1.51**
14	**10**	**1.79**
15	**10**	**1.25**
16	**10**	**1.79**
17	**10**	**1.18**
18	**10**	**0.93**
19	**10**	**1.73**
2	**10**	**1.05**
20	**10**	**1.84**
21	**10**	**1.52**
22	**10**	**0.49**
3	**10**	**1.77**
30	**10**	**0.17**
31	**10**	**1.99**

4	**10**	**0.94**
5	**10**	**0.67**
6	**10**	**1.6**
7	**10**	**1.14**
8	**10**	**0.52**
9	**10**	**0.47**
Average vanillin percentage=1.2		

Analytical Report from Spices Board, Cochin

Table 4.8 Performance evaluation of the vanilla extractor

Capacity (gm)	**500**
Amount of vanilla used for testing (gm)	**450**
Solvent used	**Ethanol (60%, 30%, 15%)**
Total quantity of solvent used (L)	**4.5**
Quantity of extract obtained (L)	**3.92**
Vanillin in the extract by spectrophotometer (%)	**1.01**
Extraction efficiency (%)	**84.2**
Evaporative loss (%)	**12.9**

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SUGGESTIONS for future work

CHAPTER V
SUGGESTIONS FOR FUTURE WORK

1. **A condenser may be incorporated with the fabricated extractor to minimize evaporation loss.**
2. **Capacity of the extraction unit can be increased by increasing the number of cups and overall dimensions.**
3. **Pump may be replaced by a low hp stainless steel pump which will facilitate more edible extract. A low discharge pump is best suited for complete extraction of vanillin from the cell sap.**
4. **The outer cylinder may be insulated to minimize the heat loss from the cylinder.**

SUMMARY AND CONCLUSION

CHAPTER VI
SUMMARY AND CONCLUSION

Vanilla being an important food flavoring agent, the demand for natural vanillin extracted from vanilla beans is bound to grow. Presently, the vanilla flavor in India is derived mainly from synthetic substitutes. As there is a visible trend of shifting towards natural produce, the consumption of synthetic vanilla may not grow at the present level. Even a one percent shift towards natural vanilla will ensure a substantial market for natural vanilla. Therefore the demand for natural vanillin would grow at an alarming rate. At present, there is dearth of indigenous techniques for cost effective efficient extraction of vanilla in any forms. Therefore a prototype percolation type vanilla extractor was developed and its performance evaluated.

Fabrication of the extractor was carried out after standardization of the parameters for extraction such as the solvent for extraction, concentration of solvent, set of concentrations, method of extraction and days of extraction. It was found from the experiments that ethanol is the best suited solvent for extraction and the set of concentration of extraction 60%, 30% and 15% ethanol, each percolated at the rate of 2 hours per day for 5 days so that the total number of days of percolation 15 gave maximum vanillin content in the extract. The extracts so collected after percolation of each concentration were then mixed so that the final mix has 35% ethanol which is the recommended limit for safe consumption.

The fabricated extractor consists of extraction cylinder, hot water jacket, cup assembly, frame assembly besides a heater, thermostat, pump, and power supply unit. The extractor was fabricated using 22 gauge stainless steel. There is a provision to heat the extract to a temperature of 45ºC and maintain the temperature of the extract at this temperature by an immersion heater and thermostat.

The extractor was then tested for its performance by estimating the extraction efficiency and percentage evaporative loss. The vanillin content of the raw material was

found at Spices Board Analytical Lab as per standard methods and vanillin content of the extract obtained from the extractor was found using UV Spectrophotometer. It was found that the extraction efficiency of the developed vanilla extractor was 84.2% and evaporative loss was 12.9%. The capacity of this batch prototype extractor was 500gm which could be increased by increasing the number of cups and overall dimensions. The developed equipment is simple in design, easy to operate and maintain. It can be fabricated locally with suitable modifications and refinements. This vanilla extractor could produce extract efficiently and economically.

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APPENDICES

Appendix 1: UV Spectrophotometer readings of standard vanillin solution

Volume of standard (ml)	Absorbance for sample with NaOH	Absorbancefor sample without NaOH	Optical density
2	0.165	0.084	0.081
4	0.327	0.143	0.184
6	0.501	0.249	0.252
8	0.659	0.274	0.385
10	0.823	0.339	0.484

Department of Biochemistry, College of Horticulture, Vellanikkara

Sample calculation:

Percentage vanillin content in the extract obtained from vanilla extractor is calculated using the formula given in section 3.7.7.2.

$$\text{Vanillin content (\%)} = \frac{(0.727\times80\times100\times3.921)\times100}{(0.385\times5\times1000\times450)} = 1.01$$

$$\text{Extraction efficiency(\%)} = \frac{1.01 \times 100}{1.2} = 84.2$$

$$\text{\% Evaporative loss} = \frac{4.5-3.921}{4.5} = 12.9$$

FABRICATION AND TESTING OF VANILLA EXTRACTOR

BY

DIVYA.E

NITHYA, N.S

PARVATHI.S

ABSTRACT OF THE PROJECT REPORT

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in

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Faculty of Agricultural Engineering and Technology

Kerala Agricultural University

Department of Post-Harvest Technology and Agricultural Processing

KELAPPAJI COLLEGE OF AGRICULTURAL ENGINEERING AND TECHNOLOGY

TAVANUR - 679 573, MALAPPURAM

KERALA, INDIA

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ABSTRACT

In view of developing an indigenous, cost effective method of extraction of natural vanilla from *vanilla planifolia*, a prototype percolation type vanilla extractor was developed and its performance evaluated. It was found from studies that ethanol is the best suited solvent for extraction and the combination of concentration 60%, 30% and 15% ethanol; each percolated at the rate of 2 hours per day for 5 days gave maximum vanillin content in the extract. The main components of the developed extractor were extraction cylinder, hot water jacket, cup assembly, frame assembly besides, a heater, thermostat, pump and power supply unit. It is made of 22 gauge stainless steel with arrangements for heating and controlling the temperature. The extractor was then evaluated for its performance. It was found that the extraction efficiency of the extractor was 84.2% and evaporative loss was 12.9%. The capacity was 500gm which could be increased by increasing the number of cups and overall dimensions. The developed equipment is simple in design, easy to operate and maintain and can be fabricated locally with suitable modifications and refinements.