It is the ancient Totonaco Indians of Mexico who were the first keepers of the secrets of vanilla. When they were defeated by the Aztecs, they were demanded to relinquish their exotic fruit of the Tlilxochitl vine, vanilla pods. When, in turn, the Aztecs were defeated by the conquering Spaniard, Hernando Cortez, he returned to Spain with the precious plunder - vanilla beans.

Hernan Cortez was the first European to taste vanilla in Mexico in 1520. As he entered the Aztec capital, Montezuma the intrepid Aztec emperor, handed him the royal beverage, chocolatl, a beverage of cocoa beans, corn, vanilla pods and honey served in golden goblets. "Tlilxochitl", the Aztec word for vanilla, was derived from "tilli" for black and "zachitl" meaning pod. Vanilla beans were considered to be among the rarer tributes paid to the Aztec emperor by his subject tribes.
INTRODUCTION

Vanilla (*Vanilla planifolia* Andrews) is a tropical, climbing orchid belongs to the family orchidaceae (Childers, 1959). It is cultivated for producing the valuable beans, the natural source of vanillin and the most important flavor molecule used in the food and beverages industries, cosmetic and pharmaceutical industry etc. Native of Mexico, nowadays cultivated in Madagascar, Indonesia and Comoros (Priefert *et al.*, 2001) including Tonga, West Indies, South America, Rodriguez, Mauritius and Seychelles, Uganda, Fiji, India in smaller proportions. Manual pollination of flowers to initiate bean development and laborious curing procedure lasting anywhere between 3-6 months makes this commercially important spice crop very expensive in the international market. In addition to the above, vanillin is found to be a good antioxidant and antimicrobial agent and has the potential to be used as a food preservative (Buri *et al.*, 1989; Davidson and Naidu, 2000). In addition there is some evidence of having anti mutagenic property as well (Keshava *et al.*, 1998).

It is cultivated in some selected countries around the world with over 37,525 ha and a production of 4403 tonnes. World demand for vanilla is around 32,000 tonnes and the demand for natural vanillin is increasing at 7–10 per annum as the world is shifting towards herbal products of which less than 0.5% of the vanillin is isolated from vanilla beans (Anilkumar, 2004; Priefert *et al.*, 2001) the rest is produced synthetically, mostly from petrochemicals such as guaiacol and lignin (Clark, 1990). The price of vanillin extracted from beans is between US $ 1200-4000/kg, where as the price of vanillin synthesized chemically from guaiacol is less than US$15/ kg (Serra *et al.*, 2005).

India is the largest producer and consumer of spices in the world and for a very long time, has been among the leading spice exporting country. While the value of Indian spice exports has been in the range of US$ 300–400 million in recent years, the estimated domestic retail market value is around US$ 4 billion. India exports spices to around 120 countries (Kumar and Muraleedharan, 2007). In the international market, average domestic price (export) for cured vanilla beans as on 07.02.2009 (dec'08) was 830.00 (Rs/kg). During January 2008 USA imported 11.20 MT cured vanilla beans worth 16.79 (US$/kg) from India. During 2007-08 India exported 200
MT of cured vanilla beans worth 1775 lakhs (4.41 MLN US$) (DGCIS & S, Calcutta).

In India this crop was introduced as early as 1835 (Rajeev et al., 2005). Total area under vanilla is around 2545 ha and is proposed to expand its cultivation to 5000 ha. Karnataka occupies the largest area of vanilla cultivation in India with 1465 ha followed by Kerala (812 ha) and Tamilnadu (268 ha) though introduced in India in the 18th century. In recent years, vanilla has gained importance and doubled the cultivation in the last 2 years, from 1600 ha in 2001–2002 to 3427 ha in 2003–2004 and the production from 60 to 131 tonnes during this period (Anil kumar, 2004). Indian share of vanilla in the international market is very small.

Freshly harvested beans are green in colour, flavorless and are subjected to a rigorous curing process for 3 to 6 months or longer. The curing process is comprised of four major stages i.e., killing, sweating, drying and conditioning (Balls and Arana, 1941; Ranadive, 1994). In green beans, flavour precursors are present in the form of vanillin glycoside (Glucovanillin). The action of β-glucosidase on glucovanillin releases the aglycone (free form of aroma compound)(Arana, 1943; Sorensen and Mehlium, 1956). Glucovanillin is the major and the most abundant glycoside present in green bean. In addition to this there are several other glycosides (Odoux, 2006). It is established that hydrolytic enzymes, such as β-glucosidase and other glycosyl hydrolases are separated from glucovanillin or other flavor precursors. The purpose of the curing process is to make contact between the flavour precursors and the enzymes that catalyze the hydrolysis of precursor to produce vanillin and other flavour compounds. Approximately 250 aroma compounds have been identified in the cured bean (Adedeji et al., 1993).

Considerable research and development have been carried out in order to speed up the curing process and to produce the quality beans with good aroma and higher vanillin yield. Ranadive, (1994) made some early improvements and some of the process were patented (Towt, 1952; Graves et al., 1958; Karas et al., 1972; Kaul, 1967). Broderick (1956) suggested the right stage for harvesting of beans and this was supported by Ranadive et al., (1983) and Tokoro et al., (1990). Kaul (1967) proposed the use of plastic sheets for curing the beans. Broderick (1956) and Jones and Vicente (1949) suggested use of hot water treatment and freezing of beans was investigated by Ansaldi et al., (1990). Mane and Zucca (1993) obtained vanillin after enzymatic treatment and Brunerie, (1993) obtained patent for enzymatic processing of green
vanilla pods. Conventional curing supplemented with hot-air drying has been attempted by Theodose (1973) where as others used solar energy based drying equipment (Kamaruddin and Mursalim, 1997; Ratobison, 1998). Influence of natural factors in the quality of cured vanilla bean has been investigated by Jones and Vicente, (1949). Cervantes et al, (2004) showed that oven curing to be an alternative process in order to reduce the curing time and also to get a uniform product. Obolensky, (1958) attempted ultrasonic treatments and able to enhance 25-35 \% vanillin and 10 -15 \% oleoresin content in treated vanilla pods. Recently Sreedhar et al, (2007) used auxins and ethrel in combination with different pretreatments at 38°C for 40 days and also certain elicitors to enhance the flavour compounds to accelerate curing (Sreedar et al., 2009).

Microbiological studies of traditional postharvest processing of vanilla beans (curing) was examined by Rolling et al., (2003). He reports the association of microbial communities and identified thermophilic and thermotolerant Bacillus sps, fungi and yeast on the green beans during curing and suspected for their role in the vanillin formation and proposes the future work to find out the role of microorganism in the vanillin enhancement. Bacillus species are known to positively contribute to cacao and several legume food fermentations in Asia and Africa (Cook, 1994; Schwan et al., 1995). Hence the role of microorganism in the vanillin formation cannot be ruled out and has to be established. Hence one of the objectives of the present study was to find out the role of microorganisms in vanilla aroma development.

The exact mechanism of vanillin formation in vanilla beans during curing is not understood fully. Research has shown that activity of $\beta$-glucosidase, the enzyme responsible for the hydrolysis of glucovanillin is lost in the first few days of curing (Dignum et al., 2002; Ranadive et al., 1983) and some amount of glucovanillin is still present at the end of curing. Where as the large part of vanillin and other aroma is developed during the later stages of curing.

Studies have shown that radiation processing improves the quality of food products by radiolytic breakdown of certain glycosides in saffron, nutmeg (Zareena et al., 2001; Ananthkumar et al., 2006) and enhancement of antioxidant content of soybeans (Variyar et al., 2004) etc. Influence of gamma radiation on cured vanilla beans in the dose range of 5-50 kGy have been investigated by Bachman et al., (1995). In which survival of contaminating microflora and the effect on chemical constituent's like vanillin and sugars were studied. Thus there is a need for improving
yield of natural vanillin obtained from vanilla beans for economic reasons. Hence the unhydrolysed glucovanillin present in the vanilla beans (Dignum et al., 2002; Ranadive et al., 1983) may be hydrolysed upon irradiation.

In addition to the above, irradiation is generally used to decontaminate microflora of food products for safe consumption. Contamination of food with microorganism's particularly pathogenic organisms is one of the most important public health problems. Joint Food and Agriculture Organization (FAO), International Atomic Energy Agency (IAEA), World Health Organization (WHO) expert Committee recommended that food may be irradiated with absorbed doses up to 10 kGy (WHO, 1981). The U.S. Food and Drug Administration (FDA) set a limit for irradiation of culinary herbs, seeds, spices, vegetable seasonings and blends of these aromatic vegetable substances up to 30 kGy (Bendini et al., 1998; Olson, 1998). Radiation processing of spices has been successfully commercialized in several European countries and in U.S.A recent years (IAEA, 1988; Loaharanu, 1987).

Curing of vanilla pods using the traditional method is inefficient in terms of vanillin yield. Aroma of the properly cured vanilla bean is much more complex than just vanillin alone. Natural vanilla flavor is still very much in demand by the food industry. There is no nature-identical vanilla aroma available that equals the vanilla extract. Although some of the above described processes seem successful in shortening the curing process with higher yield, they are not widely used in industry and they depend on the traditionally cured beans. By keeping the above facts in mind developing and/or improving the existing methods of curing is the need of the hour and a detailed research work is required in this angel.

Hence, in the present study an attempt was made to increase the vanillin content and also to enhance the overall quality of the beans with the following objectives,  
1) Isolation and identification of the microorganisms involved in the vanilla curing/fermentation.
2) Controlled fermentation of green bean with known microorganism (co-culture/cocktail inoculums) previously isolated from the beans undergoing curing to enhance the vanillin yield and also to find out the role of microorganisms in vanillin formation.
3) Radiation processing of fresh green and cured vanilla beans to hydrolyze vanillin glycoside (Glucovanillin) to enhance the yield of vanillin and also to decontaminate microflora of the cured bean.
4) Controlled curing using fermenter for preserving the loss of vanillin during sunning and also to shorten the curing period with better quality.

5) Studies on the effect of maturity and standardisation of vanilla harvesting period for the better yield and aroma of the cured bean.

6) Preliminary biochemical studies on hitherto unexplored vanilla seeds.

7) Survey and evaluation of cured vanilla beans of south Indian to find out the influence of curing methods, agroclimatic factors and other geographical difference if any in the quality of cured bean.