Summary

Preliminary studies have shown that vanillin and other phenolics increase as the curing proceeds, moisture content decreases similarly water activity with marginal decrease. Microorganisms were isolated and identified by PCR amplification of 16S rDNA for bacteria, ITS for yeasts and light microscopy for fungi. A total of 18 Bacterial isolates, six fungal species and four yeasts were identified. PCR yielded 1.5 kb of amplicons for bacteria and various size amplified products were obtained for yeast. The amplified products were purified and sequenced. *Bacillus* was the major group of bacteria identified. Species identified includes *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus megaterium* and *Bacillus cereus* and yeasts isolates includes *Candida parapsilosis*, *Pichia guilliermondii* and *Pichia anomala*. DNA sequences were submitted to NCBI Gene bank and accession numbers are recorded. Fungal contamination was observed in two farms visited, some are mycotoxin producers and identified tentatively by TLC where fluorescent coloured bands were observed. Controlled fermentation was carried out using four different combinations of inoculums through traditional sun drying but increase in vanillin content was not observed, where as in the case of oven method even though substantial enhancement was not observed, there was a marginal increase in vanillin content (4.84, 1.95, 2.2 mg/gm in whole cured bean and 3.76, 8.7, 5.42 mg/gm in split cured bean) in the inoculated beans when comparing to control and was found to be statistically significant. Hence microbial association in vanillin formation cannot be ignored. Irradiation of vanilla did not enhance the vanillin yield either in green bean or in cured bean due to high radioresistance of glucovanillin as revealed in the Pulse radiolysis studies. A carbon cantered radical was noted in the pulse radiolysis studies. Phenolic site was unavailable due to glycoside linkage and bond breakage was not occurring. But there was a slight increase in the appearance of the radiation processed cured bean. Irradiation of cured bean at 5 kGy proved to be an optimum dose for the microbial decontamination in cured vanilla beans without the enhancement of vanillin. Of the five different pre treatments and controlled curing, none of the method yielded a positive result, only traditional cured beans found to be of good quality with the vanillin content of 19.49 mg/gm of bean. However the method adopted hydrolyzed the glucovanillin, but concurrent increase in the
yield was not observed. Vanilla beans can also be harvested when the whole bean turned to brown for a better yield of vanillin rather then the commonly followed blossom end yellow harvesting method. Brown bean on harvesting contains an average of 24.27 mg/gm of vanillin and upon curing yielded 31 mg/gm of vanillin when comparing to the traditionally cured beans (21.81 mg/gm) of the same batch. In addition brown bean cured had good aroma when comparing to the green bean cured. The location of seed within the bean was clearly established with light microscopic studies. Vanilla seeds undergo slight biochemical changes during curing. For the first time the amount of vanillin was quantified in the seed and found to be 9.61 mg/gm of cured bean and negligible amount in green bean. Survey revealed, almost all farmers follow a standardised method for curing the bean with minor variation in terms of methods and materials used, producing high quality cured beans. Highest vanillin content of 26.02 and a lowest of 10.99 mg/gm in cured bean were obtained in the analysed samples. Physical parameters such as altitude, weather condition, including method of cultivation and other geographical conditions doesn’t influence vanillin content and the quality of beans and couldn’t be correlated for the cured beans obtained from different farms across south India and the ratio of aroma constituents estimated, only R1 & R5 were exceeded and R2, R3 and R4 were falling within the revised ranges given by DGCCRF.