CHAPTER 4

SUMMARY AND CONCLUSIONS
4.0. Phytochemicals as antioxidants are gaining importance due to their dual role in the food industry as lipid stabilizers and as chemo preventive agents that could modulate cancer, CVD, cataract, ageing etc. Although synthetic antioxidants like BHA, BHT, TBHQ etc. are widely used as antioxidants in foods, their adverse effects on long term coupled with consumer preference for natural products have resulted in the resurgence in the research on natural antioxidants and consequently food industry is in search of potential natural antioxidants from edible sources. The major dietary sources of antioxidant phytochemicals are cereals, legumes, fruits, vegetables, oilseeds, beverages, spices and herbs. The present study focused on rice bran and its byproducts with special emphasis on defatted rice bran (DRB), an abundantly available byproduct of the rice bran oil industry (RBO). The main objective of the present study was to investigate whether DRB could be a source material for natural antioxidants/ nutraceuticals which is otherwise used as cattle feed. Thus the approach has been to characterise rice bran from prominent rice varieties grown in the region for their antioxidant phytochemicals; development of protocols for extraction and enrichment of the phytochemicals; and evaluation of their antioxidant efficacy using standard in-vitro models.

4.1. Rice bran, from major cultivars of the region were analysed for their chemical profile. The mean values for major constituents were dry matter (89.1%), fat (16.8%), protein (10.1%), crude fiber (11.3%), ash (11.4%), and available carbohydrates (50.5%). The mean energy content was 393.5 Kcal/100g. The mean values (ppm) of various minerals followed the order P (13608) > K (9520) > Mg (3844) > Ca (362) > Fe (216) ~ Na (190) > Mn (99) > Zn (39) > Cu (4), with P and Cu being the most and least abundant minerals respectively.

4.2. Rice bran oil obtained from the major cultivars were analysed for their chemical characteristics as well as fatty acid composition. The values obtained for FFA, saponification value, iodine value, and unsaponifiable matter were 12.3%, 182.9, 97.3 and 4.8% respectively. RBO had exceptionally high
unsaponifiables as compared to that of other edible oils. Major fatty acids of RBO were 16:0, 18:0, 18:1, and 18:2 with mean values of 21.6%, 2.0%, 41.8%, and 32.5% respectively, with saturated to unsaturated ratio of approximately 1:3. The fatty acid profile of RBO is thus close to the ideal ratio of saturated: mono unsaturated: poly unsaturated of 1:1.5:1.

4.3. To understand the distribution of the major antioxidant phytochemicals (oryzanols, tocols, and ferulic acid) among full fat rice bran (FFB), defatted rice bran (DRB), and rice bran oil (RBO); these process streams from the selected cultivars were characterized for the above phytochemicals using standard extraction protocols and HPLC. This included a systematic approach to separation, identification, and quantitation of various oryzanol and tocol components by HPLC. The various oryzanol components identified in RBO include stigmasteryl ferulate, cycloartanyl ferulate, 24-methylene cycloartanyl ferulate, cycloartenyl ferulate, campesteryl ferulate and β-sitosteryl ferulate. Seven tocols except β-T3 were present in all the rice varieties studied. The total oryzanols varied between 2169-3473 ppm in FFB, 12648-19348 ppm in RBO and 94-217 ppm in DRB. Corresponding values for tocols were 183-316 ppm, 1042-1648 ppm, and 3-9 ppm. The ferulic acid content of FFB and DRB from the varieties ranged from 66-166 ppm and from 81-201 ppm respectively. However, ferulic acid was not present in the hexane extracted RBO. Thus DRB, as the byproduct of RBO extraction could contain substantial amounts of antioxidant phytochemicals that could be harnessed as a source for natural antioxidants.

4.4. Kinetic studies were designed to select appropriate solvent and to optimise other process parameters like material-solvent ratio, time, temperature etc. for extraction of antioxidants from defatted rice bran. Methanol was found to be the most efficient solvent, with respect to the yield of TPC, oryzanols and ferulic acid from DRB. Other optimized conditions included a material-solvent ratio of 1:15 and a time of extraction of 10 hours using a Soxhlet extractor. The yield of methanol extracts of DRB from the major cultivars ranged from 3.2 to
5.0%. The sugar content of the extracts ranged from 18.8 to 33.8%, protein from 17.9 to 25.0%, TPC from 5.3 to 8.4% and ash from 3.9 to 5.1 %. The oryzanol content of the extracts ranged from 2358 to 6602 ppm, ferulic acid from 2541 to 4376 ppm and tocols from 110 to 284 ppm. 24-methylene cycloartanyl ferulate (~45%), and cycloartenyl ferulate (~25%) represented the major oryzanols of the DRB extracts. γ-tocotrienol (~70%), and α-tocopherol (~10%) were the major chromanols.

4.5. Enrichment of antioxidants in crude methanol extract was achieved by sequential extraction and fractionation. For this, the CME was re-extracted with less polar organic solvents like ethyl acetate, acetone, ether etc. From this, acetone was found to be the best solvent for ferulic acid and tocols. For further purification of the acetone extract (AE), sequential extraction technique was employed. For this, the dry AE was re-extracted with hexane to give a soluble fraction enriched in lipophilic compounds (AE-LP) and a residue enriched in polar compounds (AE-PP). Considering the bioactive phytochemicals of interest, AE-LP was enriched in oryzanols, and tocols and AE-PP in ferulic acid. Column chromatography was employed to isolate components present in the crude extract. The two pure compounds obtained were identified to be β-sitosterol and tricin based on UV, IR, NMR and MS data. Of these, the flavone tricin is of special phytochemical interest because of its rare occurrence.

4.6. The crude extract (CME), the enriched fractions (AE, AE-LP, and AE-PP) and the pure phytochemicals (oryzanols, tocols, ferulic acid, tricin and sterol) were then subjected to a number of antioxidant and antiradical activity assays using standard in-vitro models. To evaluate the antioxidant potential of the extracts and its phytochemical constituents in bulk oils, Schall Oven Test method and differential scanning calorimetry (DSC) were used. The results demonstrated that in bulk oils, some of the DRB extracts (AE-PP) were either equally efficient or better than BHT and that at identical concentrations AE-PP,
AE-LP, and AE performed better than the phytochemical constituents ozyzanols, ferulic acid and tocols with respect to PV, DV and DSC data. The increase in activity with fractionation might be due to the enhanced levels of antioxidants in the resultant fractions compared to CME. To evaluate the antioxidant potential in food relevant systems, linoleic acid emulsion method and the β-carotene bleaching test were used. The DRB extracts and its phytochemical constituents proved to have significant activity in these emulsion models as well. Contrary to the bulk oil system (SOT & DSC), where the DRB extracts (AE-PP) were either equally efficient or better than BHT, the latter was more effective in emulsions. None of the pure phytochemicals tested performed better than BHT or TBHQ, both in bulk oils and emulsions indicating that the synergistic effects of phytochemicals in the extracts including that of proteins, sugars and unidentified polyphenols could be contributing to the observed efficacy of DRB extracts. The results further suggest that the DRB extracts could be used both in bulk oils and in food emulsions as natural antioxidants.

4.7. The antiradical efficacies of DRB extracts and their phytochemical constituents were studied using the stable DPPH radical and the superoxide radicals generated in-situ by the xanthine-xanthine oxidase system. The DPPH radical scavenging activity of ferulic acid, and Tmix was greater than that of BHT and the activity of AE-PP was equal to that of BHT. It was also found that the DPPH scavenging activity of the fractions AE, AE-LP and AE-PP could be largely attributed to the levels of TPC and ferulic acid in the fractions. H-donating capacity (as evaluated by the DPPH method here) is an important biologically significant property of antioxidants to convert potentially damaging ROS (oxyl and peroxyl radicals) into non-toxic species, and in this context DRB could be a good source of such antioxidants. For evaluating the superoxide scavenging activity of the extracts, the cytochrome C and NBT methods were used. The superoxide scavenging activities of the
extracts also followed the order of their TPC and ferulic acid contents. Moreover, the various phytochemical constituents of DRB extracts viz. ferulic acid, tricin and T_mix also exhibited excellent superoxide radical scavenging activity thus directly supporting the superior antiradical efficacies of DRB extracts.

Quest for health friendly phytochemicals as food additives, chemo preventive and therapeutic agents is far more stronger than ever before. The resurgence in natural products in recent past and consequent scientific studies have brought out a variety of potential natural sources of such phytochemicals. However from the commercial point of view, not many sources have been identified to meet the requirements for food products, nutraceuticals and therapeutic agents. Because of the abundant availability and renewability, rice bran could be an economically viable source for antioxidant phytochemicals for the future as demonstrated through the results of this study. However more work is suggested for further studies on complete chemical characterisation and development of a process to obtain a fraction with higher purity active compounds.