Summary

Humans may accrue much health benefits from plant pigments such as carotenoids, anthocyanins and curcuminoids; many of which are antioxidants. Considerable amounts of these secondary metabolites occur in the leaves of sweet potato varieties. However, their instability in the isolated state and poor bioavailability hinder their wider use and therefore improved stability and bioavailability are desirable. These may be achieved by their incorporation into other biopolymer carriers such as starch. With this consideration in view, the present study focuses on the isolation of natural pigments from natural sources, their characterisation, stability evaluation, bioactivity assays and incorporation in native cassava starch and starch nanoparticles. The thesis is organized in seven sections. Section I presents review, background and objectives of the present project and the results are presented and discussed in the subsequent six sections, followed by a summary and a list of references.

The results of the extraction and isolation studies reported in this thesis show that anthocyanins of sweet potato leaves can be easily extracted with methanol and HCl preferably at pH levels less than 2 and at an extraction temperature of 40°C. The foliar anthocyanins of the sweet potato genotypes S-406 and S-purple are now found to be more acylated compared to the anthocyanins of grape pomace as determined by their UV-visible spectroscopy. Among the above two sweet potato anthocyanins studied, the S-purple anthocyanins are more acylated and hence are more stable than that of S-406. The foliar anthocyanin content of S-406 and S-purple is found to be in the range 150-200mg/100g fresh weight respectively. This anthocyanin content is higher than the reported value of about 40–60 mg anthocyanins/g fresh weight for the purple-fleshed sweet potatoes developed in Japan (Furuta, et al., 1998).
Our studies on the pigments of sweet potato genotypes Orissa-3 and S-purple using HPLC indicate that the predominant pigments present in the sweet potato leaves studied are cyanidin and peonidin based anthocyanins, which are acylated with caffeic, ferulic and coumaric acids. This acylation with aromatic acids imparts a greater stability to these pigments. The results of our study establish that the sweet potato leaves can be used as a low-cost source of anthocyanin pigments. The present findings would pave the way for further optimization studies to design a commercial production of anthocyanins from sweet potato leaves.

Results presently gathered based on our stability studies showed that the stability of anthocyanins from the leaves of sweet potato during storage depends on the structural characteristics of the respective anthocyanins, temperature of storage, light intensity during storage and the presence of sugars and salts in the storage medium. The addition of sugars and salts had a deleterious effect on the anthocyanin stability from the sweet potato and grape pomace sources, under all the experimental conditions presently studied. The S-purple anthocyanin is more stable than S-406 anthocyanin in the model systems used in our experiments. Both the S-purple and S-406 anthocyanins exhibit remarkable stability in comparison with that of the grape pomace anthocyanins. Such a better stability may be attributed to the acylation of the anthocyanins from S-purple and S-406, with caffeic, ferulic and coumaric acids. The half-lives ($t_{1/2}$) of the sweet potato anthocyanins were found to be higher than the corresponding values for grape pomace anthocyanins in the buffer solutions presently used. These results show that the viability of the addition of anthocyanin natural extract to soft drinks would strongly be influenced by the chemical structure of the anthocyanin source. In short, we now report that the sweet potato leaves are economical, renewable source for anthocyanin pigments that are comparatively more stable than those reported from other natural sources.
The present study on the antioxidant capacity of Anthocyanin Rich Extracts (AREs) has now shown that the AREs from the young emerging leaves of the sweet potato and *Dioscorea* crops, which have high anthocyanin contents, exhibit good antioxidant activity. The antioxidant capacity of AREs presently studied is higher than that of quercetin. This encouraging result indicates that the above two tuber crops could be considered as commercially viable, novel natural antioxidant sources for the functional food and dietary supplement industry. A comparison of the antioxidant activities exhibited by various sweet potato genotypes can be readily tailored into sweet potato breeding efforts and food processing research programmes.

Our present results on anti-cancer activity studies show that the foliar anthocyanin extract from sweet potato variety Orissa-3 displayed significant cytotoxic activity against human leukemia cell line K562 and human cervical cancer cell line HeLa. The high anticarcinogenic activity and antioxidant capacity indicate that the above two crops are worthwhile candidates for natural antioxidant and anticancer resources. Nevertheless, further research is required to replicate and substantiate these results. The present findings are also important because the leaves are available throughout the year and are usually considered to be a waste material after harvesting the tubers. Moreover, our study appears to be the first report on the effect of the ARE from Orissa-3 leaves inhibiting human cervical cancer and the human leukaemia cell growth in an *in vitro* preclinical model. Overall, these results support the further clinical development of anthocyanins as potential cancer chemo preventive agents.

We have also extracted carotenoids from selected tuber crops and curcuminoids from dried rhizomes. The extraction and estimation of carotenoids present in tuber crop samples studied indicate that, these crops can be utilized as a source of vitamin A. Curcuminoids were fractionated and the antioxidant
activities of curcumin, dimethoxycurcumin and bisdemethoxycurcumin have
been studied with *in vitro* model system. The antioxidant capacities of
curcuminoids were found to decrease in the order: curcumin >
demethoxycurcumin > bisdemethoxycurcumin.

We have next demonstrated that the two natural pigments, curcumin and
anthocyanin can be effectively incorporated into cassava starch. The pigment
content and colour retention is satisfactory during the storage period studied for
six months. The stability of this incorporation could be attributed to the
formation of inclusion complexes with amylase in starch.

Subsequently starch nanoparticles were prepared from cassava starch by
sulphuric acid hydrolysis. TEM and AFM images indicate that the particle size
of these starch nanoparticles is in the nano scale; with majority of particles
having particle size less than 100 nanometers and are spherical in shape. These
starch nanoparticles are stable for several months, under refrigeration. The
main advantage of this method over the mechanical grinding procedure
(Szymonska *et al.*, 2008) is that the nanoparticles attained inherent stability due
to the presence of sulphate groups at their surface resulting from the H$_2$SO$_4$
treatment (Angellier *et al.*, 2004). Anthocyanidins are superior to anthocyanins
in terms of chemopreventive properties (Wang and Stoner, 2008). We have
now successfully incorporated anthocyanidins into starch nanoparticles. We
have also developed fluorescent biodegradable nanoparticles, (by loading
curcumin) which are of great importance in nanotechnology.

The present work also showed that the anthocyanin, curcumin and
carotene, when incorporated into native cassava starch, have enhanced stability
in comparison with these pigments in their native form. These plant pigments,
with their significant chemopreventive properties, when incorporated into an
excellent drug carrier such as the starch nanoparticles, are expected to have
wide pharmaceutical applications.