# 5 GENERAL CONCLUSION

## *5.1 Folates and Rice*

Like iron (Fe) and vitamin A, folate deficiency in nutritionally-at-risk groups (pregnant women and children) has been recognized as a problem of the highest priority. Folate is needed for DNA production, maintenance of cell division, metabolism and homeostasis by all living organisms. Deficiency in folate is linked to increased risk of birth defects called fetal neural tube defects (which affect the brain and spinal cord), cancer, anaemia, cardiovascular diseases, and also observed to retard growth in children (Gregory III *et al*., 2005; Quinlivan *et al*., 2002; Rader and Schneeman, 2006).

Rice is the staple food for more than half of the world's population. However, among the cereals, rice contains the lowest amount of folates which is ~19 µg per 100 g brown rice (Juliano, 2003). Increased folate content in this crop can, however, offer an alternative solution to current strategies to enhance intakes of natural food folates (Finglas *et al*., 2006; Tucker, 2003). Biofortification offers advantages over supplementation and fortification strategies in Asian countries as seeds from the previous harvest can be re-planted every season (Foyer *et al*., 2006; Bouis, 2009) without additional costs.

Due to the importance of both folates and rice in human health, the availability of a reliable technique for the determination of its naturally occurring forms is an important tool in obtaining the information needed to provide a better understanding of the accumulation and distribution of folate in important crops such as cereals (Rebeille *et al*., 2006; Bekaert *et al*., 2008; Gregory III, 1998).

## *5.2 Folate Analyses in Rice*

Basic information on the folate composition and range of intra-species diversity can be used as a basis for developing folate-enhanced cultivars. The LC-MS/MS technique used in this study proved to be a powerful tool not only in analyzing folates from rice but other cereals of similar starchy matrix as well. The development of the novel LC-MS/MS method revealed detailed new information about rice folate forms while the existing microbiological assay facilitated the rapid screening of a number of cultivars for total folate concentration. Method validation confirmed that the analytical procedure employed for rice folate measurement is appropriate and provided the intended results.

Microbiological and HPLC (UV/FLD and MS/MS) results obtained in this study differed. It may be that the HPLC based methods underestimate folate by approximately one third or MA overestimate the total folate as nonfolate compounds may have stimulated the growth of the microorganism. Part of the HPLC underestimation could be due to not detecting $H_4$PteGlu and 5,10-$CH^+$-$H_4$PteGlu. This and other reasons for the discrepancy are evidently complex and require further studies. Based on the results of this work, the use of reference material, CRM 121, and spinach, as in-house QC can be regarded as essential in folate determination.

New data were obtained on the total folate content and its variation in rice cultivars using MA. Results indicated that genotype may have a significant effect on the folate content. However, further studies are needed to evaluate the extent of this variation. Using LC-MS/MS, the novel information on the polyglutamated folate forms in rice was obtained. The folate vitamer distribution pattern of rice was characterized by a large proportion of 5-$CH_3$-$H_4$PteGlu and formylated vitamers. The folate species found in unpolished rice grains were: 5-$CH_3$-$H_4$PteGlu, 5/10-CHO-$H_4$PteGlu, 5-$CH_3$-$H_4$PteGlu$_4$, 5-$CH_3$-$H_4$PteGlu$_5$ and 5/10-CHO-PteGlu$_5$.

All the folate species were identified by retention time, MRM transition and by reference to the mass spectral library created in an earlier work by Santoyo Castelazo (2009).

## *5.3 Biofortification of Folates in Rice employing genetic and transgenic approaches*

The LC-MS/MS method developed in this work, has been employed to provide more comprehensive information about monoglutamated and polyglutamated folates in transgenic lines with overexpressed genes at the post biosynthetic pathway and folate binding proteins. The optimized method enabled the simultaneous detection and at the same time allows confirmation of the folate identity. We cannot, however, overemphasize the importance of validating the method using quality controls (QCs) or in-house QCs and certified reference materials (CRMs) to avoid uncertainties with the results. Folate forms in the *FPGS Os03g02030* knockout showed a significant decrease in mono-, polyglutamated and total folate levels. Conversely, profiling of transgenic rice lines (i.e. with overexpressed FPGS and expressed cow's and rat's FBPs) revealed a remarkable change in folate vitamers abundance including a dramatic increase in 5-$CH_3$-$H_4$PteGlu$_4$, 5/10-CHO-$H_4$Pteglu$_5$, 5-$CH_3$-$H_4$PteGlu$_6$, and 5/10-CHO-$H_4$Pteglu$_6$ levels, and 2.5 to 8.8-fold increase in total folate concentration in the unpolished grain.

## *5.4 Future Directions*

Although the results from a number of studies and from this study show that there is no doubt that folate can be enhanced in rice and in other staple crops, one matter that needs to be considered is the bioavailability of the enhanced vitamin. Assumptions whether the folate can be readily absorbed by humans have to be tested. Several studies showed that monoglutamated folates are more bioavailable than the polyglutamated forms (McKillop *et al*., 2006; Wei *et al*., 1996; Gregory III, 1991; Melse-Boonstra *et al*.,, 2004;) but in the absorption process from the intestinal tract to storage in the liver, the conversion rate from poly- to monoglutamyl is efficient enough to make the vitamin at least 50% bioavailable (Seyoum and Selhub, 1998; Castenmiller *et al*., 2000; Rogers *et al*., 1997). Some studies suggest that FBP-rich foods enhance the bioavailability of natural folates but would also depend on interactions with other components of the diet (Jones, *et al*., 2003; Verwei, 2004; Mason and Selhub, 1988; Verwei *et al*., 2005). The social and economic impacts of folate enhanced transgenic rice must also be addressed for the strategy to be effective in reaching the target population.