Chapter - VII

CONCLUDING REMARKS

Production of Phulwara (*Madhuca*) fat from *M.butyracea* mature seeds is a home industry in the sub-Himalayan region of India and Nepal , the natural habitat of the plant. The fat is considered to be a high - value commodity by the local people as, in addition to its desirable cooking quality, it is believed to have some medicinal property particularly useful for aging people with weak heart. In spite of Phulwara being a palmitate rich highly saturated fat, contrary to our current concept, the local people are convinced that it is a healthy food based on their experience of cooking meals with it for hundreds of years. In the beginning, I began to wonder whether a high content of palmitate compared to a high content of stearate in a predominantly saturated fat makes some qualitative difference with respect to nutritive value in man! Unfortunately, no carefully controlled human or animal experiments seemed to have been carried out with Phulwara fat to support the belief of the local people that it is really good for health and a cure for weak heart.

Nevertheless, palm oil is a more well-known palmitate-rich fat (about 40% palmitate; ca 50% saturated fatty acid content) which has been evaluated as a dietary component for its effect on rat arteries. Palm oil diet in rat under carefully conditioned experiments did not show any tendency towards arterial degeneration, and actually it tended to decrease platelet aggregation (also lowered thromboxane/prostacycline ratio) compared with highly unsaturated sunflower seed oil; moreover, unlike high stearate, high palmitate in diet did not affect platelet membrane fluidity (Rand *et al.*, 1988). In human experiments also, dietary use of palm oil, contrary to our expectation, did not induce unfavourable plasma-lipoprotein profile, and platelet function remained normal during prolonged dietary intervention in human volunteers (de Bosch *et al.*, 1996). Also, dipalmitoyl phospholipids as surfactant play an important role in the maintenance of healthy lungs. It may be remembered here that fat in our mothers' milk

also contains 50% saturated fatty acids in which a major contribution is made by palmitic acid (25%). Therefore, as a model for high palmitate saturated fat, Phulwara really deserves more scientific attention from nutritionists' point of view.

There is another facet of *Madhuca butyracea* that makes it an extremely interesting system to study as it is a rare oil seed with a very high content of palmitic acid. At the beginning of this study I was really fascinated to know whether there exists a special mechanism unknown to biochemists that makes this unique fatty acid composition possible in its seeds. Of course, any practical application of this knowledge, such as using its genetic system for modification of normal fatty acid biosynthetic pathway in an oil seed cultivar, could be very exciting. When one considers the fact that 50% of the edible vegetable oils are hydrogenated for production of margarines and shortenings, the case for high palmitate Phulwara fat which is a good quality natural margarine becomes very strong for some basic studies. As most oils which are partially hydrogenated are oleate/linoleate rich but poor in palmitate (about 5-10%), margarines and shortenings are rich in stearate with a significantly high content of trans - fatty acid generated during catalytic hydrogenation step. Both trans fatty acids and high stearate content in partially hydrogenated fat are known to create health hazards in man. On the other hand, it is known that increased palmitate content in margarines improves their texture and quality (Safford *et al.*, 1993). There is no doubt that in future people are going to prefer a natural rather than artificial margarine.The possibility of production of Phulwara fat in large scale is remote as it is still a wild plant and it takes a decade to become mature to bear fruits. But to learn from its biochemical system how they manage to produce such uniquely high palmitate fat is certainly possible, and then apply the knowledge to genetically engineer any common oil seed plant like *Brassica* to modify its

fatty acid metabolic pathway to obtain palmitate rich margarine directly from seed is within our reach.

While our experiments on substrate specificity of acyl-ACP thioesterases of developing *Madhuca* seeds and fatty acid deposition in seed fat were going on merrily, Jones *et al.*, (1995) published their extremely important paper on plant fatty acyl chain terminating thioesterases (FATs) that distinguished long-chain specific Fat A and saturated medium-chain specific Fat B type. The paper also threw some new light on the evolutionary relationship between type A and typeB thioesterases. What affected our ongoing research directly was their observation that *Fat B*1 gene of *Cuphea hookeriana* in a transgenic situation could induce a high level expression of palmitate although the gene is normally poorly expressed in most plant tissues. There was at first a puzzlement because I could already observe during studies with *Madhuca* seed development that the fatty acyl-ACP substrate specificity of the predominantly expressed PO-FAT (palmitate: oleate: stearate = 100: 90: 30) could be correlated, more or less, to the fatty acid profile of the deposited fat (palmitate: oleate: stearate = 67: 28: 3). However, the relationship between fatty acid profile in deposited fat and the specificity of the expressed thioesterase(s) is not very simple. For example, Voelker *et al.*, (1997) found that in the cases of Elm and Nutmeg, *in vitro* fatty acyl specificity of their medium-chain specific thioesterases very poorly match with the corresponding genes ability to deposit fatty acids in seed fat. Nevertheless, the *Fat B*1 gene's potentiality to produce high palmitate fat in transgenic oil seed plants is already well-established (Jones *et al.*, 1995; Voelker *et al.*, 1997). We remain uncertain at this stage about the potentiality of the *PO-Fat* gene with respect to its capability to maintain high palmitate: oleate ratio in seed fat in a transgenic situation. I am excited by the fact that evolution has made use of PO-FAT for

producing high palmitate fat but not Fat B1 (as far as we know at this time) for that purpose. 5-10% palmitate is normally found in seed fat (e.g. in *Brassica* seeds) due to non-specific substrate hydrolytic activity of Fat A type (LC-FAT) that primarily acts on C18:1- ACP. In many developing oil seeds, active Fat B type enzymes are not encountered.

Fat A - Fat B model proposed by Jones *et al.*, (1995) for understanding the evolution of acyl-ACP thioesterase in plants and their role in the design of fatty acid composition of tissue lipids has been very illuminating at the initial stage. But I think the model is not quite adequate to explain all the additional facts about fatty acid metabolism that are being revealed since then. Our discovery of PO-FAT in *Madhuca* necessitates some revision of the Model. *PO-Fat* is evolved and sustained during evolution which can not be typified under *Fat A* or *Fat B* class. One inherent problem of the classification has been the degeneracy of substrate specificity of plastidial thioesterases, and the other one being the uncertainty about how the degenerate catalytic activities of these enzymes are regulated *in vivo* to design a particular fatty acid composition of the lipid. In *Madhuca*, only *PO-Fat* and *LC-Fat* are expressed in almost all tissues and no induction of any other thioesterases was noticed in the developing seeds, but the seeds fulfill the programme to contain about 67% palmitate in the fat. Fat B1 type enzymes do not appear to have any role in determining the fatty acid composition of this fat. I believe that not only *Fat A* type genes evolved from the primordial *Fat B* type but the *PO-Fat* gene type of *Madhuca* also evolved from the same source. *PO-Fat* can not be classified as either *Fat A* or *Fat B* type. Therefore, I suggest that the most appropriate name for these class of genes (*PO-Fat*) would be *Fat AB*. Further investigation in future with *Fat* with acyl-ACP thioesterases in different species might reveal the presence of more *Fat* AB type enzymes.

Because of the time constraint the study of *Madhuca* could not be continued at the genetic level. The logical extension of the work would be to clone the *PO-Fat* gene and study how it would modify the fatty acid composition of seed lipid in a heterologous system. However, it is naive to expect that the introduction of only one gene (*PO-Fat*) would alter fatty acid composition in a new host to make it palmitate rich like that in *Madhuca*. In *Madhuca* it is conceivable that besides PO-FAT other factors come into play to control fatty acid composition. A possible candidate could be KAS I (whose end product is palmitoyl-ACP), that might cooperate with PO-FAT in a special way to help high palmitate deposition in *Madhuca* seeds. Another obvious factor that should play an important role for generating C16:0 rich fat could be the enzyme that catalyzes the transfer of palmitoyl group from palmitoyl-CoA to the 2- position of lysophosphatidic acid (palmitoyl-CoA: lysophosphatidic acid 2-palmitoyl-transferase). *Madhuca* seed fat contain about 8% 1,2,3-tripalmitoyl glycerol (Vander Wal,1960; Coleman,1961). In a transgenic situation this type of host support may or may not be available to PO-FAT.

Finally, because of the obvious importance of preparing our favourite food with natural high palmitate margarine, instead of artificial one currently in use, I suggest that Phulwara fat be immediately evaluated with respect to its effect on human health as it has been done previously with palm oil. It would be my great pleasure if in near future genetically engineered seed fat, similar to Phulwara fat, could eliminate partially hydrogenated fat totally from our diet.