EXPERIMENTAL PROCEDURE

3. EXPERIMENTAL PROCEDURE

The experimental procedure employed to compare the antioxidant level of detroit dark red and Ooty beetroot varieties are discussed under the following headings.

3.1 Collection of plant materials

The beetroot varieties namely detroit dark red and Ooty were collected in and around Coimbatore, Tamilnadu. The fresh beetroots are collected and air dried in the shade at room temperature for 20 days.

3.2 Antioxidant

Antioxidants are notable for boosting immune system because immune systems in the blood stream are so easily accessed by free radicals as well as antioxidants (Tretter, 2004).

3.2.1 Enzymic antioxidants.

Natural antioxidant enzymes manufactured in the body provide an important defence against free radicals. Catalase, superoxide dismutase, peroxidase, glutathione-S-transferase, glutathione peroxidase, glutathione reductase, polyphenol oxidase are the most important enzymic antioxidants (Schriner, 2005).

3.2.1.1 Estimation of Catalase

Catalase and glutathione peroxidase seek out hydrogen peroxide and convert it to water and diatomic oxygen. Catalase activity was estimated by the method of Luck (1974) and detailed procedure is given in Appendix-I

3.2.1.2 Estimation of Peroxidase

The most important role of peroxidases is the scavenging of hydrogen peroxide and organic peroxides (Smeet *et al.*, 2005). Peroxidase activity was determined by the method of Reddy *et al.* (1995) as described in Appendix-II.

3.2.1.3 Estimation of Superoxide Dismutase (SOD)

SOD is an endogenously produced intracellular enzyme present in the body. The Superoxide dismutase activity was estimated by the method described by Mirsa and Fridovich (1972) and is explained in Appendix-III.

3.2.1.4 Estimation of Polyphenol oxidase

Polyphenols have antioxidant characteristics with potential health benefits. This polyphenol antioxidant may reduce the risk of cardiovascular diseases and cancer (Arts and Hollman, 2005). The polyphenol oxidase activity of the plant sample was estimated by the method of Esterbauer *et al.* (1977). The detailed procedure is stated in Appendix-IV.

3.2.1.5 Estimation of Glutathione –S-transferase

Glutathione-S-transferase plays an essential role in eliminating toxic compounds by conjugation (Chandra *et al.*, 2000). The activity was determined by the method proposed by Habig *et al.* (1974) and the procedure is described in Appendix -V.

3.2.1.6 Estimation of Glutathione peroxidase (GSH-PX)

GSH-PX is an important antioxidant enzyme that plays a role in elimination of hydrogen peroxides and lipid hydroperoxides and reduces peroxides by using reduced glutathione as a hydrogen donar (Ilham *et al.*, 2004). The method of Rotruck *et al.* (1973) was followed to assess the activity of glutathione peroxidise and the same is given in Appendix -VI.

3.2.1.7 Estimation of Glutathione reductase

Reduction of oxidized form of glutathione (GSSG) is catalysed by glutathione reductase. The activity of glutathione reductase was determined by the method proposed by David and Richard (1983) and the procedure is elaborated in Appendix-VII.

3.2.2 Non enzymic antioxidants

The nutrients that most profoundly have important immune function are vitamin C, vitamin E, glutathione, polyphenol, total carotenoids and lycopene (Meydani, 1990).

3.2.2.1 Estimation of Vitamin C

Ascorbic acid (Vitamin C) is water soluble antioxidant present in citrus fruits, tomatoes and green leafy vegetables. Ascorbic acid was estimated by the method of Roe and Keuther (1953) and the procedure is given in Appendix-VIII.

3.2.2.2 Estimation of Vitamin E

Vitamin E is an essential nutrient that functions as an antioxidant in the human body. It is essential by definition, because the body cannot manufacture vitamin E by its own and thus it must be provided by foods and supplements. Vitamin E was estimated by Emmerie-Engel method as described by Rosenberg (1992).The detailed procedure is given in Appendix-IX.

3.2.2.3 Estimation of Glutathione

Glutathione is an important nonenzymatic antioxidants which promotes the detoxification of several toxic metabolites (Karthikeyan and Rani, 2003). The content of reduced glutathione of the sample was determined by the method of Moron *et al.* (1979) as given in Appendix-X.

3.2.2.4 Estimation of Polyphenol

Phenolics are group of non essential dietary components that have been associated with inhibition of atherosclerosis and cancer by chelating metals, inhibiting lipooxygenase and scavenging of free radicals (Lakshmi *et al.*, 2005). The procedure of Malik and Singh (1980) was followed to estimate the total phenolic content as explained in Appendix-XI.

3.2.2.5 Estimation of Total Carotenoids and Lycopene

Carotenoids and Lycopene were analysed by the method of Zarkaria *et al.* (1979) as described in Appendix-XII.

3.3 Statistical analysis

Statistical analysis like arithmetic mean and critical difference were employed to predict the results of the experiment.