# 3.0 Materials and methods

Millets are becoming one of the most important foods for both low-income and high- income countries. The nutrient composition of millets indicates that it is a good source of energy, proteins, carbohydrates, vitamins and minerals including the trace elements. Traditional treatments such as soaking, cooking, germinating and fermenting have been used to improve nutritional quality of the millets. The present study entitled **"A Comprehensive Study on changes in the selected biochemical composition and antioxidant levels of five millets on soaking and germination"** is focused to analyse the effect of soaking and germination on the alleviation of antioxidants and biochemical composition in the five commonly used minor millets in Tamil Nadu namely Cumbu (Pearl millet), Ragi (Finger millet), Samai (Little millet), Thinai (Foxtail millet) and Varagu (Kodo millet). The various materials and experimental procedure employed in the present study are described under the following headings:

**3.1. Materials**

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**3.2.1. Processing of millets: soaking and germination**

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**3.2.3.1. Estimation of superoxide dismutase**

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**3.2.3.4. Estimation of ascorbic acid**

**3.2.2.5. Estimation of total phenolics**

**3.2.2.6. Estimation of flavonoids**

**3.2.4. Statistical analysis**

## 3.1. Materials

### 3.1.1. Millets

The selected five minor millets such as Perl millet/Cumbu (*Pennisetum glaucum*), Finger millet/Ragi (*Eleusine coracana*), Little millet/samai (*Panicum sumatrens*), Foxtail millet/Thinai (*Setaria italica*) and Kodo millet/varagu (*Paspalum scrobiculatum*) were purchased from Department of millets, Tamil Nadu Agriculture University (TNAU) in Coimbatore. All cereal grains were of whole grain quality, dehusked and without any thermal or chemical treatment. They were purchased and stored in air tight containers at room temperature. The five millets selected for the present study is presented in **Plate 3.1.**

**Plate 3.1. Five different millets used in the study**


### 3.1.2. Chemicals

All chemicals and reagents were of analytical grade quality. Chemicals used for this study were purchased from HiMedia unless otherwise mentioned. The solvents used are of LR grade.

### 3.2. Methods

#### 3.2.1. Processing of millets: soaking and germination

Dry grains were used as control (C). 1 g of each whole grains were soaked in 10 mL distilled water in a beaker for different time period which includes 6 and 12 h at room temperature. The soaking treatments were termed as S1 and S2 respectively. Grains (1 g) from S1, S2 were germinated for 24 h and 48 h, using a moist white muslin cloth at room temperature. The germinated samples were named as S1G1 (6 h soaking + 24 h germination), S1G2 (6 h soaking + 48 h germination), S2G2 (12 h soaking + 24 h germination) and S2G2 (12 h soaking + 48 h germination). The grains and seeds (soaked or germinated) were dried at the end of each time point, freeze-dried and used for further studies.

#### 3.2.2. Determination of important biochemical composition of selected five minor millets during two different processing

Soaked and germinated grains were ground to fine powder. The resultant flours were packed in air tight containers and stored in refrigerator until use for analysis. The biochemical constituents such as protein, total carbohydrate and total free sugars were estimated in the treatments C, S1, S2 and G1 to G4 of five different minor millets by standard procedures. The amount of respective biochemical constituent is expressed in mg/g of sample. The experiments were repeated thrice.

##### 3.2.2.1. Estimation of protein

Protein was estimated by Lowry's *et al*. (1951) method **(Appendix-1).**

##### 3.2.2.2. Estimation of Total carbohydrate

The carbohydrate was analyzed by anthrone method (Hedge and Hofreiter, 1962) (**Appendix-2**).

##### 3.2.2.3. Estimation of total free sugar

Free sugar estimated by the method of Hedge and Hofreiter with modification (1962) **(Appendix-3).**

#### 3.2.3. Assessment of selected enzymic and non enzymic antioxidant levels in the five minor millets during two different processing

The powdered samples of C, S1, S2 and G1 to G4 were analyzed for the presence of enzymic antioxidants namely superoxide dismutase, polyphenol oxidase and

peroxidase and the nonenzymic antioxidants such as ascorbic acid, phenolics and flavonoids by following standard methods. The analysis was carried out in three replicates.

**3.2.3.1. Estimation of superoxide dismutase**

Assay of superoxide dismutase was carried out by the method of Misra and Fridovich, (1972) as explained in **Appendix-4.**

**3.2.3.2. Estimation of polyphenol oxidase**

The method of Esterbauer*et al.* (1977) was followed for the determination of polyphenol oxidase as stated in **Appendix-6.**

**3.2.3.3. Estimation of peroxidase**

The peroxidase activity of powdered millet samples with various treatments were estimated by the method of Reddy *et al.* (1995) as described in **Appendix-7.**

**3.2.3.4. Estimation of ascorbic acid**

The ascorbic acid content of each sample was assessed as described by Roe and Kuether (1953).

**3.2.2.5. Estimation of total phenolics**

Total phenolic content (TPC) was determined in sample extracts using the Folin-ciocalteau reagent (Malik and Singh, 1980).

**3.2.2.6. Estimation of flavonoids**

Flavonoid content of each sample was quantified colorimetrically using Zhishen *et al.* (1999).

**3.2.4. Statistical analysis**

The datas were analyzed using WASP 2.0 software. The datas were subjected to one-way analysis of variance (ANOVA) test and the differences between the means were compared for their significance at $P \leq 0.05$. The analysis was carried out in completely randomized design.