Biochemical Characteristics and Microbial Profile during Sprouting Process in Grains of *Eleusine coracana* L. (Finger Millet)

Mandyam C. Varadaraj* and Akira Horigane

Resource Crop Quality Evaluation Laboratory, Department of Crop Physiology and Quality, National Agriculture Research Center, Tsukuba, Ibaraki 305, Japan

The associated biochemical and microbiological changes during sprouting in the seeds of *Eleusine coracana* L. (finger millet) common to India were studied. The initiation of physiological process as well as an increase in weight of seeds (0.6-fold) occurs during the stage of presoaking for 8 h at room temperature (23 °C). The levels of soluble protein, total soluble sugar, proteinase, and α-amylase activities, which were 1.22 mg, 0.6 mg, 0.44 unit of activity, and 14 units per gram of resting seed, respectively, increased significantly to 1.38 mg, 1.53 mg, 2.89 units, and 404 units per gram during sprouting for 48 h at 25 °C. The level of globulin increased from an initial level of 0.15 to 0.75 mg g^{-1} of seed. The electrophoretic mobility of isolated globulins from presoaked and sprouted (48 h at 25 °C) seeds shows more bands relative to the resting seeds as determined by sodium dodecyl sulfate−polyacrylamide gel electrophoresis. Sedimentation analysis of globulin and prolamin fractions indicates a probable self-associating system. During sprouting, there was the buildup of lactic acid bacteria, a desirable microflora. The associated changes in the seeds of finger millet can lead to the development of traditional foods with beneficial attributes.

Keywords: *Eleusine coracana; finger millet; sprouting; presoaking; proteinase activity; a-amylase activity; globulin; prolamin; electrophoretic mobility; protein associations*

INTRODUCTION

Changing trends worldwide have emphasized plant materials as human foods, with an increase in demand for convenience foods. In this situation, seeds constitute a major source of proteins for humans, of which cereals provide >50% of this protein supply. Foods, especially those of indigenous nature, have a traditional association with the country of origin and available natural food resources. A majority of these foods are cereal- and/or legume-based. In most of the cereals, prolamins constitute the major protein fraction; hence, these cereals are of relatively poor quality. In contrast to the low-lysine prolamins, globulins are considered to be nutritionally superior (Draper, 1973).

With an increasing population and greater demand for major cereals, it is essential that emphasis is placed on underutilized food grains which are potential source of nutrients to mankind (Haq, 1989). In this regard, millets, which form the staple food of a large segment of the population in India and tropical Africa, constitute a potential reserve of nutrients but are less explored due to the lack of suitable scientific and technological inputs in bringing about desirable quality products. Among the millets common to India, *Eleusine coracana* L. (finger millet) has a high nutritional status (Pore and Magar, 1979; Hemanalini et al., 1980). The grains are small, only 1−2 mm in diameter, and reddish brown in color. The grains are directly ground into a flour to make a thick porridge or allowed to germinate and processed into dumpling-like products, beer, and malt (Purseglove, 1976; Malleshi and Desikachar, 1979, 1986). Besides, germinated finger millet either by itself or in combination with greengram (*Phaseolus aureus*), is used to make baby foods with reduced viscosity and increased calorie density (Hemanalini et al., 1980; Malleshi and Desikachar, 1982).

Prolamins constitute the major protein fraction of finger millet; they are moderately deficient in lysine but have adequate amounts of other essential and related amino acids required by humans (Virupaksha et al., 1975). Sprouting of finger millet is a household practice among the Indian population. The process of controlled sprouting in cereals leads to an increase in their nutritional status (Virupaksha et al., 1975).

Although soaking and sprouting of finger millet have been the prerequisites of many traditional foods of Asia and Africa, the biochemical changes occurring during these processes are far less studied. In the present work, the prime objective was to evaluate a few of the important biochemical characteristics and associated microflora during the process of presoaking and sprouting, with special reference to quantitative and qualitative changes in protein moiety.

MATERIALS AND METHODS

The chemicals of general nature used in this study were from Wako Pure Chemicals, Osaka, Japan. Throughout the study, Milli Q water (Milli-Q SP. TOC, Millipore, Bedford, MA) was used. All of the experimental trials were carried out independently, three times. The mean values of each variable are presented with statistical analysis.

Samples. Mature seeds of *E. coracana* L. (finger millet) were harvested from plants grown under field conditions.

Presoaking and Sprouting. Seeds were washed well and soaked in Milli Q water for 8 h at room temperature (23 °C). Following soaking, water was drained off and seeds were placed at 4 °C for 15 h to initiate sprouting in a synchronous

* Address correspondence to this author at the Department of Food Microbiology, Central Food Technological Research Institute, Mysore 570 013, India (fax +91-821-517233).

S0021-8561(97)00495-0 CCC: $15.00
Published on Web 04/17/1998

pattern. Subsequently, seeds in 10-g aliquots were subjected to sprouting at 18 and 25 °C, respectively, for a period of 12, 24, 36, and 48 h. The sprouting process was carried out in Pyrex glass Petri plates (20 × 120 mm), with the seeds placed crease side down on a moistened filter paper saturated with 8 mL of water. For each incubation time−temperature combination, seeds were placed in individual Petri plates. In the case of incubation periods of 36 and 48 h, the filter paper was resaturated with 5 mL of water after 24 h of incubation. Radicle formed during sprouting was measured and recorded. The weight of seeds after presoaking and time intervals of sprouting was also recorded.

Samples for Analysis. These included resting seeds, seed that had been presoaked for 8 h, and seeds that had germinated for 12, 24, 36, and 48 h, respectively, at 18 and 25 °C. For the purpose of running electrophoresis, sedimentation analysis of isolated proteins, and microbial profile, germinated seeds of only 24- and 48-h incubations were included. Presoaked and sprouted seeds were blotted well with a filter paper and kept at 40 °C for 30 min to remove the surface moisture.

Moisture Content. This was determined in 2-g samples in accordance with the standard AOAC method (Baker, 1984).

Determination of Protein and Total Soluble Sugar in Water Soluble Extractives. The method of Redgwell (1980) was followed to prepare water soluble extractives from 1-g samples of seeds in an extraction mixture of methanol/chloroform/water/formic acid (12:5:2:1). The final extract was dried at 40 °C under vacuum in a rotary evaporator (Eyela, Tokyo, Japan). Dried material was dissolved in 5 mL of water and placed over KOH pellets in a desiccator under vacuum, so as to remove vapors of formic acid. The extract in 1-mL aliquots was dispensed into Eppendorf tubes and dried at 30 °C under vacuum in a centrifugal concentrator (VC-960, Taitec, Saitama, Japan), and the same was used in the following determinations:

For determination of protein, the concentrated extract in an Eppendorf tube was dissolved in 1 mL of 50 mM tris-(hydroxymethyl)aminomethane (Tris) in 1 M NaCl of pH 8.5. Protein content was determined using Folin−Ciocalteu reagent according to the method of Lowry et al. (1951).

For determination of total soluble sugar, the concentrated extract in an Eppendorf tube was mixed with 1 mL of ethanol/water (80:20). Total soluble sugar was determined following the modified method of phenol−sulfuric acid using glucose as the standard (Buysse and Merckx, 1993).

Assay for Proteinase Activity. The method described by Abe et al. (1977) was followed to extract proteinase from 5-g samples of seed in 10 mL of 25 mM sodium phosphate adjusted to pH 7.0. The dialysate was lyophilized and dissolved in 5 mL of 25 mM sodium phosphate adjusted to pH 5.5 containing 8 mM 2-mercaptoethanol. From this, a 1-mL aliquot was used to assay proteinase according to the procedure of Abe et al. (1977) using bovine hemoglobin (Sigma, St. Louis, MO) as substrate. Absorbance values of the supernatants were measured at 280 nm in a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). The proteinase activity in sample extracts was calculated by plotting the absorbance values on the standard curve prepared with tyrosine. One unit of activity was arbitrarily defined as 100 μg of tyrosine liberated from the reaction of enzyme−substrate mixture in 60 min at 37 °C.

Assay for α-Amylase Activity. The extraction of α-amylase and its assay were performed according to the procedure of Cornford et al. (1987). The extract was prepared from 3-g samples of seed in 6 mL of 100 mM Tris-maleate (Sigma) buffer of pH 6.2 containing 1 mM $CaCl_2 \cdot 2H_2O$. Before assay, the supernatant was heated at 70 °C for 15 min in a water bath. The assay was carried out using amylopectin azure (Sigma) as substrate. The final supernatant was read at 620 nm in a UV-1601 spectrophotometer (Shimadzu), and the absorbance values were converted to enzyme units by plotting the values on a standard curve prepared with barley malt α-amylase (Sigma).

An indication of starch degrading activity (qualitative) of the seeds was observed by cut-seed assay (Okamoto and Akazawa, 1979). The clearing zone, which formed around the seed halves on an agar medium containing starch after the plates were flooded (incubation at 25 °C for 60 min) with KI solution, provided an indication of the activity.

Isolation of Globulins and Prolamins. Globulins were extracted and precipitated, respectively, from 12-g samples of seed according to the methods described by Robert et al. (1983a) and Yamagata et al. (1982). Seed samples in quantities of 3 g each were extracted in 100 mL of 50 mM Tris in 1 M NaCl of pH 8.5 containing 2 mg mL^{-1} sodium bisulfite. From the final supernatant, globulins were precipitated overnight at −20 °C with 2 volumes of acetone, recovered by centrifugation (22000*g*, 30 min, 4 °C), and lyophilized.

Prolamins were also extracted from 12-g samples of seed following the procedure of Robert et al. (1983b). Seed samples in quantities of 3 g each were extracted at 60 °C for 2 h in 100 mL of 550 μL mL^{-1} 2-propanol containing 2 mg mL^{-1} sodium bisulfite, replacing the use of 20 μL mL^{-1} 2-mercaptoethanol. Prolamins were precipitated overnight at 4 °C from the supernatants obtained by centrifugation (15000*g*, 30 min, 20 °C) of the extracts by mixing with an equal volume of 40 mg mL^{-1} NaCl. Precipitated prolamins were recovered by centrifugation (22000*g*, 45 min, 4 °C) and lyophilized.

Dried globulins and prolamins were weighed and stored in screw-capped tubes for subsequent analysis. Protein content and associated total soluble sugar were determined in 5-mg samples of lyophilized fractions of globulins and prolamins as earlier described and expressed as milligrams per gram of seed and micrograms per milligram of protein, respectively.

Electrophoresis. This was restricted only to the isolated globulin fractions. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) was carried out under reducing and nonreducing conditions with only the isolated fractions of globulins according to the method of Laemmli (1970) in a Bio-Rad Mini-Protean II cell using a separating gel strength of 120 mg mL^{-1} in 375 mM Tris-HCl of pH 8.8. Samples (1 mg of protein mL^{-1}) for separation under nonreducing conditions were prepared by 1:4 dilution in 500 mM Tris-HCl (pH 6.8) containing 100 μL mL^{-1} glycerol, 20 mg mL^{-1} SDS, and 8 M urea. For separation under reducing conditions, the protein samples were prepared in the same buffer containing 50 μL mL^{-1} 2-mercaptoethanol and heated in a boiling water for 3 min. Aliquots of 12 μL (12 μg of protein) were loaded into the wells. Electrophoresis was conducted at a constant current of 30 mA per gel for 60 min. After electrophoresis, the gels were fixed in 125 mg mL^{-1} trichloroacetic acid (TCA) for 30 min and stained in 1 mg mL^{-1} Coomassie blue R-250 in methanol/acetic acid/water (4:1:4) for 6 h. Destaining of the gels was achieved through frequent changes in methanol/acetic acid/water (4:1:4). The molecular masses of protein subunits were calculated using the markers (Sigma) containing bovine albumin (66 kDa), egg albumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.2 kDa).

The final destained gels were dried in a gel dryer and used in subsequent documentation.

Sedimentation Analysis. This was carried out with isolated fractions of globulins and prolamins from resting, presoaked, and sprouted seeds for 24 h at 18 and 25 °C, respectively, in phosphate buffer of pH 7.6 (20 mM NaH_2PO_4 + 26.6 mM Na_2HPO_4 + 400 mM NaCl) in a Beckman Optima XL-A analytical ultracentrifuge according to their published procedures (Beckman Corp., 1991). The samples included aliquots of 110 μL of a protein concentration of 250 μg mL^{-1} (OD at 280 nm in the range of 0.5−1.0). The rotor used was an An-60 Ti, and XL-A running parameters were 28000 rpm for 26 h at 4 °C. After a running time of 24 h, sample aliquots in the housing cells of the rotor were subjected to a radial scan at 280 nm over a radius of 6.5−7.2 cm. The results were analyzed using Optima XL-A data analysis software program version 2.0 (Beckman Corp., 1993). The absorbance values saved in respective files were analyzed for important parameters such as (i) residual plot versus dependent variables, to determine the species nature of the proteins, and (ii) molecular

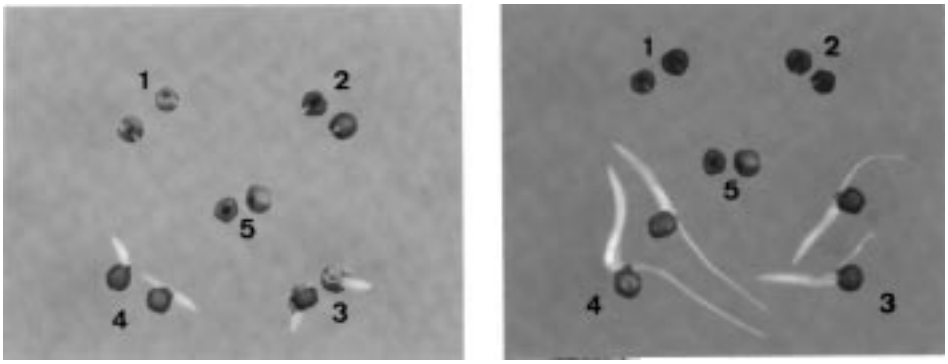

Figure 1. Sprouting pattern among the seeds of *E. coracana* at various time intervals during 18 °C (A, left) and 25 °C (B, right) incubation, respectively: (1) 12 h; (2) 24 h; (3) 36 h; (4) 48 h; (5) resting seeds.

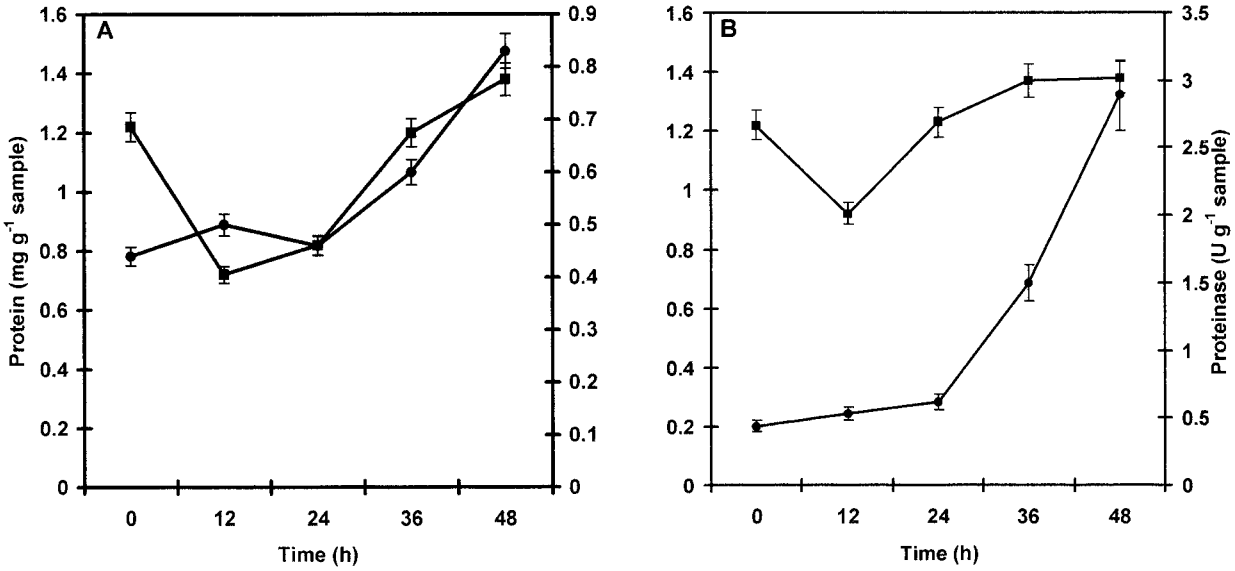

Figure 2. Soluble protein content (■) and proteinase activity (●) on (dry matter basis) in the seeds of *E. coracana* during sprouting at 18 °C (A) and 25 °C (B) respectively.

weight versus concentration, to determine the highest associative order (dimer or trimer) in a self-associating system.

Microbial Profile. Dilutions in sterile 0.85% saline were prepared from the initial sample homogenate of 1 g of seeds in 99 mL of saline. Appropriate dilutions (in duplicate) were pour-plated individually for the counts of mesophilic aerobes and lactic acid bacteria using Difco plate count and MRS *Lactobacillus* agars, respectively. In another set, the above plating media were amended with 1% soluble starch. Plates were incubated at 37 °C for 24−48 h. Colonies formed in the incubated plates were counted and expressed as colony forming units per gram (CFU g^{-1}).

Statistical Analysis. The data relating to soluble protein, total soluble sugar, proteinase, and α-amylase activities and levels of protein in fractions of prolamin and globulin were evaluated using analysis of variance, and the mean values were separated by Duncan's new multiple-range test (Bender et al., 1982).

RESULTS AND DISCUSSION

Sprouting. During presoaking and sprouting, moisture uptake by the seeds showed a gradual increase with time in the incubation period. The increase was more uniform in the case of 25 °C incubation (Table 1), and there was also the emergence of radicle as well as initiation of a shoot, which was more prominent after 48 h at 25 °C (Figure 1). At 18 °C incubation, the seeds required almost 36 h for the formation of radicle. However, at 25 °C, the seeds were able to form radicles in 12 h and all of the seeds in sample aliquots were able to sprout more uniformly. As the seeds of finger millet are used mainly in tropical countries, the uniform sprouting pattern at 25 °C enables better use for the preparation of many traditional foods.

Table 1. Moisture Uptake and Radicle Lengths Formed by *E. coracana* Seeds during Sprouting

incubation		moisture	length (mm)	
period (h)	temp (°C)	content (g kg^{-1})	radicle	shoot
resting seed		143		
8 (soaking)	23	300		
sprouting for				
12	18	307		
24	18	300		
36	18	400	2	initiation
48	18	476	5	formation
12	25	375	1	
24	25	412	2	
36	25	512	5	5
48	25	654	10	7

As a consequence of presoaking for 8 h, the gain in weight by the resting seeds was 600 mg g^{-1}. This gain in weight remained the same in seeds sprouted for 48 h at 18 °C, while in seeds sprouted for 48 h at 25 °C there was a decrease of 100 mg g^{-1} (Table 3).

Proteinase Activity. In comparison with the resting seeds, moisture uptake resulted in the initiation of physiological activities. This initiation occurred during the 8-h presoaking. Soluble protein concentrations and

Table 2. Protein Content in Lyophilized Prolamin and Globulin Fractions from 12 g of *E. coracana* Seeds during Sprouting[a]

incubation		prolamin fraction (mg g^{-1})		globulin fraction (mg g^{-1})	
period (h)	temp (°C)	dried material	seed protein	dried material	seed protein
resting seed		23.75	0.99^{ef}	7.58	0.15^{a}
8 (soaking)	23	26.25	0.67^{cd}	14.41	0.59^{c}
sprouting for					
12	18	22.33	0.83^{de}	13.16	0.37^{b}
24	18	22.50	0.86^{de}	14.41	0.30^{b}
36	18	22.33	0.55^{bc}	9.00	0.27^{ab}
48	18	25.16	0.67^{cd}	10.83	0.36^{b}
12	25	21.75	0.45^{ab}	14.50	0.65^{cd}
24	25	21.16	0.36^{a}	19.50	0.60^{c}
36	25	18.41	0.83^{de}	17.66	0.61^{cd}
48	25	14.50	1.13^{f}	14.41	0.75^{d}
SE_m (20 df)			±0.060		±0.046

[a] Values are the mean of three independent trials. Means in the same column with different superscript letters are different ($P < 0.05$)

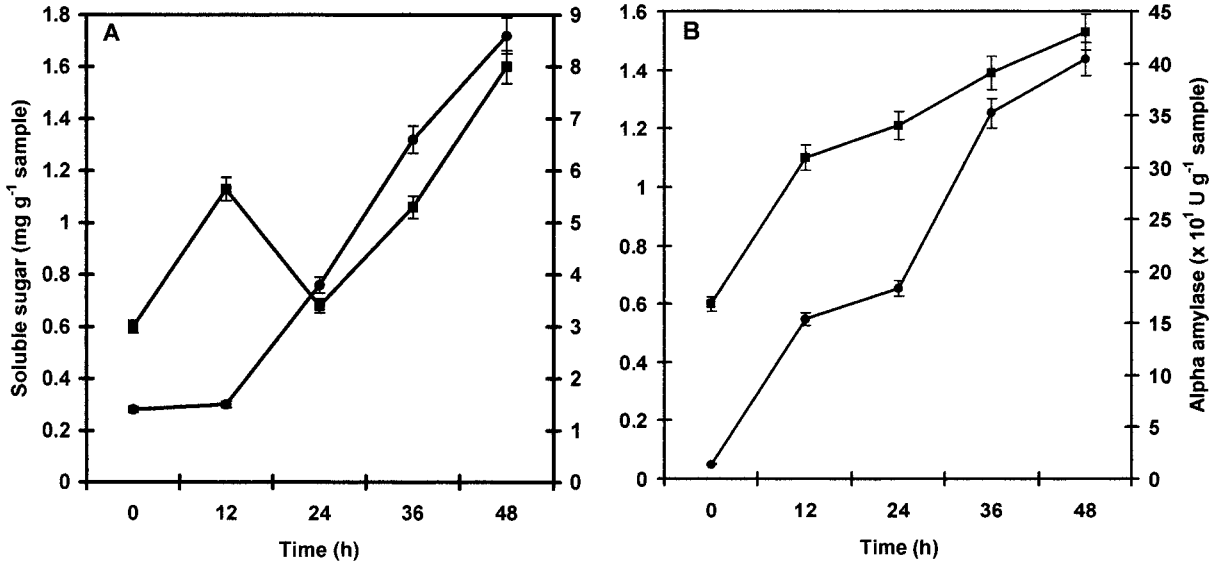

Figure 3. Relationship between α-amylase activity (●) and total soluble sugar (■) (on dry matter basis) in the seeds of *E. coracana* during sprouting at 18 °C (A) and 25 °C (B), respectively.

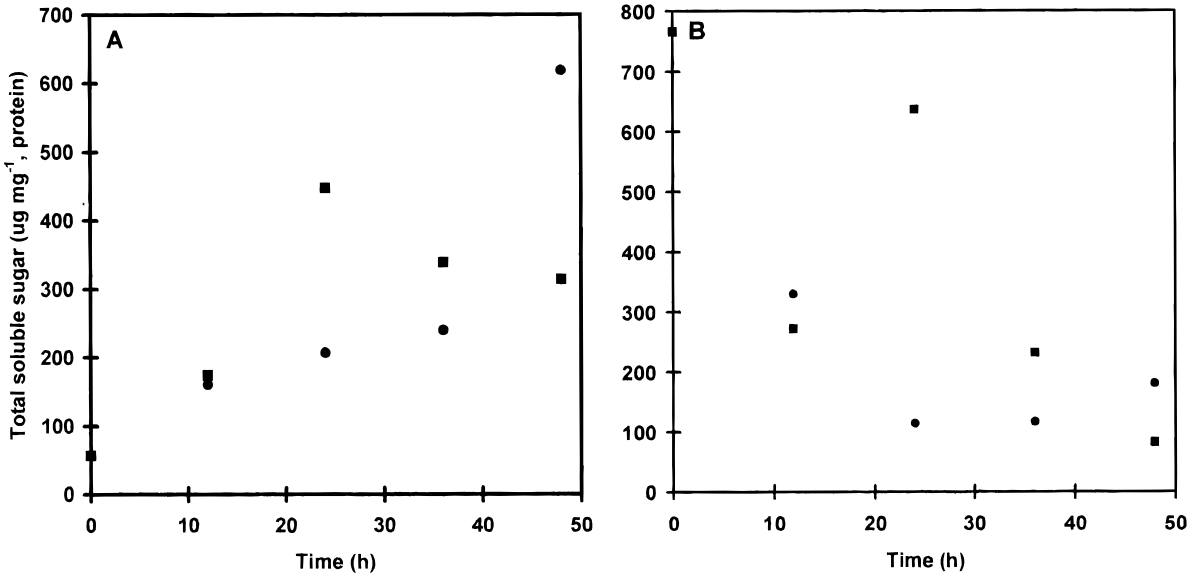

Figure 4. Total soluble sugar associated with protein fractions of prolamin (A) and globulin (B) during sprouting of *E. coracana* seeds at 18 °C (●) and 25 °C (■), respectively.

proteinase activity during presoaking and sprouting were different ($P < 0.05$). At 18 °C, the soluble protein content decreased during the initial stages of sprouting (12 and 24 h) and subsequently increased during later periods (36 and 48 h). At 25 °C, the increase in soluble protein levels was more uniform (Figure 2). At the same time, there was a progressive increase in proteinase activity during sprouting. At 18 °C, the increase was

Table 3. Quantitative Changes of Specific Biochemical Attributes in the Seeds of *E. coracana* during Sprouting

attribute	resting seed	presoaking for 8 h	sprouting for 48 h	
			18 °C	25 °C
seed weight (mg)	1000	1600	1600	1500
soluble protein (mg g^{-1})	1.22	1.21	1.38	1.38
proteinase (units g^{-1})	0.44	0.48	0.83	2.89
total soluble sugar (mg g^{-1})	0.60	1.25	1.60	1.53
α-amylase (units g^{-1})	14	85	86	404
prolamin[a] (mg g^{-1})	0.99	0.67	0.67	1.13
globulin[a] (mg g^{-1})	0.15	0.59	0.36	0.75

[a] The values are for the protein content in the lyophilized material of isolated fractions from 12 g of seeds

only 2-fold in 48 h, while at 25 °C, the increase was ~7-fold (Figure 2).

Earlier studies with cereals have indicated that resting grains contain little proteinase activity, which can increase to ~20-fold during sprouting for 3−5 days. This increase in activity has been attributed to proteinases synthesized in the scutellum and aleurone layer and secreted into starchy endosperm (Mikola, 1987). Similar studies are lacking for the seeds of finger millet except for the observation that proteinase inhibitory activities were markedly reduced during the process of germination (Veerabhadrappa et al., 1978). Changes observed in the levels of soluble protein and proteinase activity were reasonably expected (Ching, 1972).

α-Amylase Activity. Seeds of finger millet contain a greater fraction of starch. The process of presoaking and sprouting enabled an increase in α-amylase activity and total soluble sugar. The values obtained for these two parameters during sprouting were different ($P < 0.05$). Of the two incubation temperatures studied, 25 °C showed a greater increase in α-amylase activity. In 48 h at 25 °C, the activity was 40.4×10^1 units (g of sample)$^{-1}$ in contrast to an initial activity of 1.4×10^1 units g^{-1} (Figure 3). The presoaking of seeds enabled an increase of activity to 8.5×10^1 units (g of sample)$^{-1}$. The quantification of amylase activity related well with the qualitative cut-seed assay performed in starch agar plates.

In a similar manner, sprouting at 25 °C resulted in a gradual increase in total soluble sugar over a period of 48 h ($P < 0.05$). The soluble sugar content increase was from 0.60 mg g^{-1} for resting seeds to 1.53 mg g^{-1} in seeds sprouted for 48 h. Although there was a similar increase at 18 °C, it was not uniform (Figure 3). The mere process of presoaking for 8 h brought a 2-fold increase in soluble sugar [1.25 mg (g of sample)$^{-1}$] relative to the level in resting seed.

The observed changes were correlated with the moisture uptake by the seeds and formation of radicles. A greater α-amylase activity occurred in seeds sprouted for 36 and 48 h at 25 °C ($P < 0.05$). This could be related with greater moisture uptake (Table 1) and differentiation of root and shoot (Figure 1).

Considering the unavailable carbohydrate in finger millet, which was reported to be 18% (Kamath and Belavady, 1980), the process of presoaking and sprouting enables greater availability of carbohydrates. A gradual buildup of soluble sugar in the seeds during presoaking and sprouting relating to the initial level provides beneficial aspects in the preparation of several traditional fermented foods, similar to that reported for obtaining the best bean sprouts (Sato, 1966). The activity of amylases and proteinases during sprouting provides a potential application for these enzymes in food processing (Teichgraber et al., 1993).

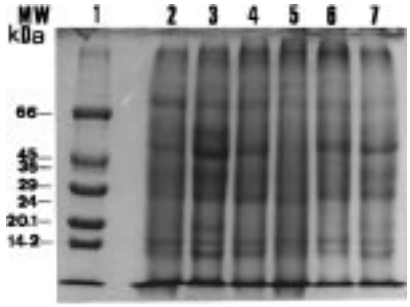

Figure 5. SDS−PAGE pattern of isolated globulin fractions (under reducing conditions) from the seeds of *E. coracana* during sprouting at 18 and 25 °C, respectively: (lane 1) protein molecular mass markers; (lane 2) resting; (lane 3) presoaked; (lanes 4 and 5) sprouted for 24 and 48 h at 18 °C; (lanes 6 and 7) sprouted for 24 and 48 h at 25 °C.

Levels of Prolamin and Globulin. The protein contents in the extracted prolamin and globulin fractions from 12-g samples are shown in Table 2. Presoaking of seeds for 8 h and sprouting for 48 h at 18 °C resulted in a decrease of prolamin content ($P < 0.05$). In 48 h at 25 °C, the prolamin content was unrelated to the level recorded in resting seeds, but there was a decrease in the initial stages of 12- and 24-h sprouting ($P < 0.05$). Relative to the levels in resting seeds of finger millet, presoaking and sprouting resulted in an increase of globulin content (Table 2). The increase was almost 2-fold in presoaked seeds and 2- and 5-fold in sprouted seeds for 48 h at 18 and 25 °C, respectively ($P < 0.05$). Replacement of 2-mercaptoethanol with $NaHSO_3$ enabled sufficient extraction of prolamin and globulin fractions from the samples of seed.

The total soluble sugar associated with protein fractions of prolamin and globulin resulted in a very few data points at the respective sprouting temperatures. These points are insufficient to fit into any regression function. The same are plotted as a scatter graph (Figure 4). An increase of almost 11-fold occurred in the level of total soluble sugar associated with prolamin fraction in seeds sprouted for 48 h at 18 °C relative to the level in resting seeds. At 25 °C, an increase of almost 8-fold occurred in 24 h, and subsequently, there was a decrease (Figure 4A). In the globulin fraction, total soluble sugar content decreased during sprouting and, after 48 h at 25 °C, it was only 11% of the initial sugar content in resting seeds (Figure 4B).

In general, cereals contain greater amounts of prolamins as the major storage protein fraction; these are devoid of essential amino acids that constitute the globulins. Similarly, the finger millet protein is moderately deficient in lysine, due to a lesser level of globulin, but has adequate amounts of other essential and related amino acids required by adult humans (Virupaksha et al., 1975). Besides, it has been shown that prolamin fractions are quite rich in tryptophan (Indira and Naik, 1971). Our findings show an increase in levels of globulin fraction during the process of sprouting in seeds of *E. coracana* and the degree of change is affected by the incubation.

Changes in biochemical attributes occurring as a result of presoaking and sprouting relative to their initial level in resting seeds are presented in Table 3. The table shows that, as against the use of resting seeds of *E. coracana* in the preparation of traditional foods

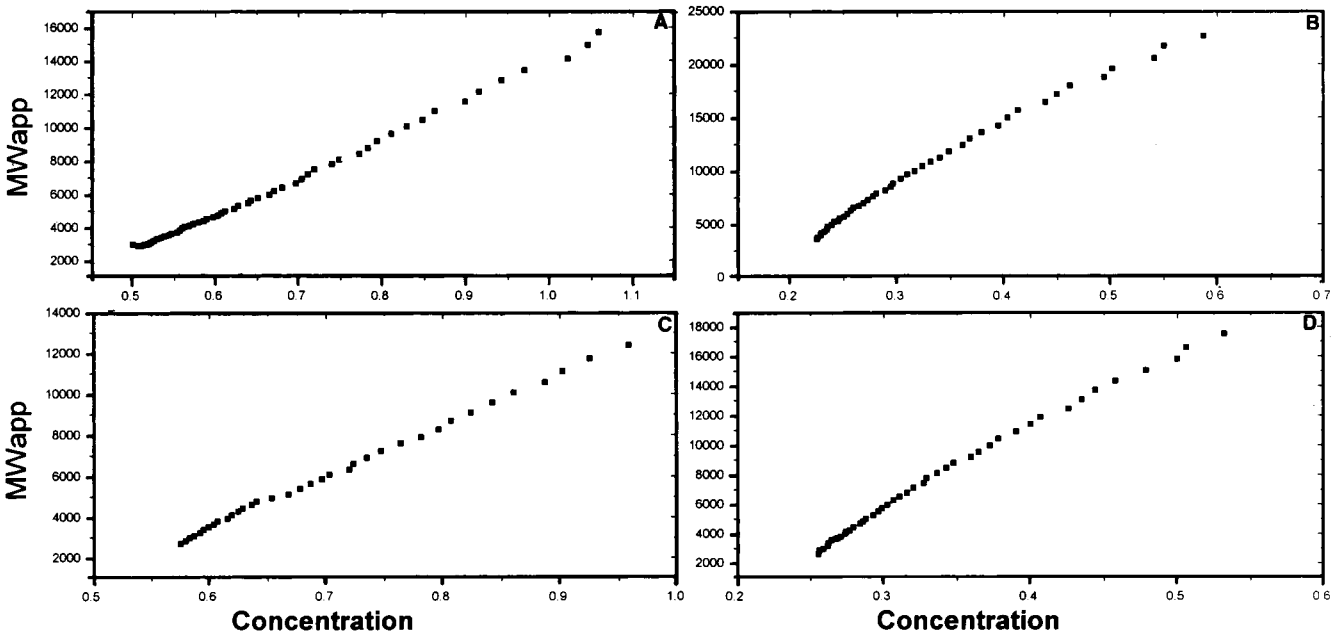

Figure 6. Relationship between apparent molecular weight and concentration (absorbance) in isolated globulin fractions from resting (A), presoaked (B), and sprouted seeds of *E. coracana* for 24 h at 18 °C (C) and 25 °C (D), respectively, determined by sedimentation analysis.

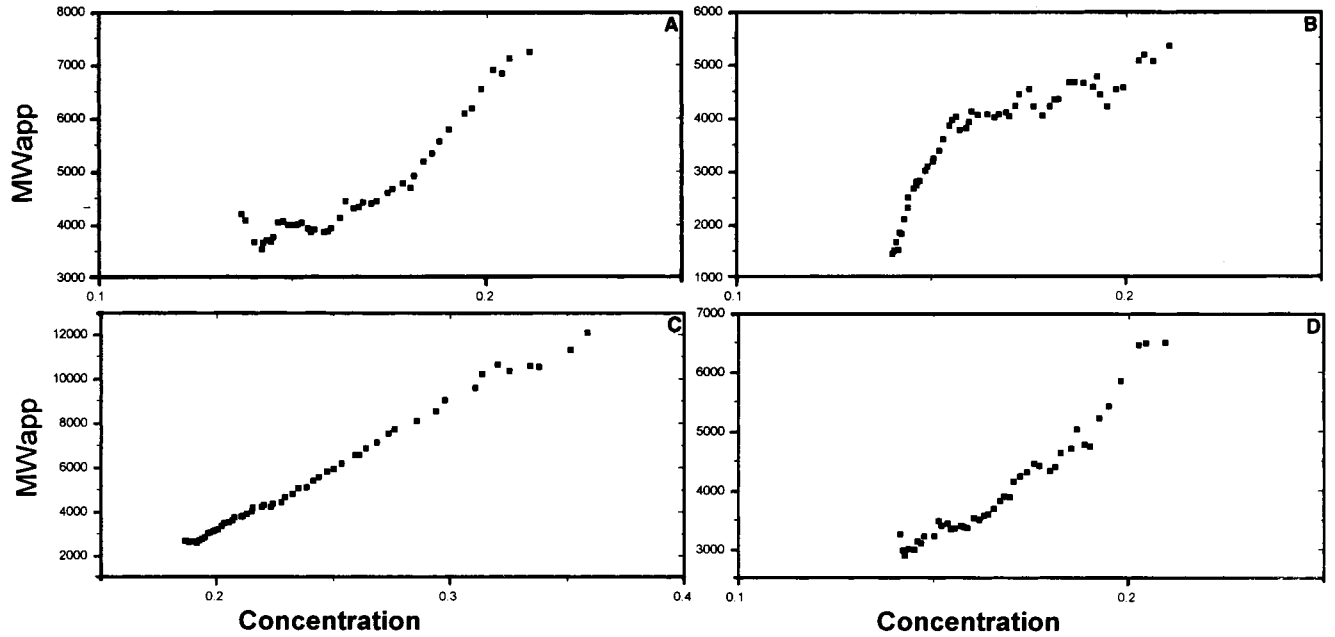

Figure 7. Relationship between apparent molecular weight and concentration (absorbance) in isolated prolamin fractions from resting (A), presoaked (B), and sprouted seeds of *E. coracana* for 24 h at 18 °C (C) and 25 °C (D), respectively, determined by sedimentation analysis.

(more of fermentation-based), the use of presoaked and sprouted seeds is beneficial in terms of increased levels of desirable biochemical attributes, available either directly or indirectly for human consumption. Although resting and sprouted seeds contain the same levels of prolamin, the process of sprouting at 25 °C enabled an increase of globulin. This change is highly desirable for people whose major source of protein comes from finger millet.

Electrophoretic Characterization. The electrophoretic profile of the isolated fractions of globulins from resting, presoaked, and sprouted seeds under reducing conditions of finger millet is shown in Figure 5. Under both reducing and nonreducing conditions, there appeared to be no differences in the electrophoretic mobilities of the proteins as performed in SDS−PAGE (results not shown). The molecular masses of the protein molecules were in the range of 15−67 kDa. The resting seed had protein bands of various mobilities (lane 2) corresponding to molecular masses of 16.5, 18, 28, 35, 45, and 67 kDa, respectively. However, these bands were not prominent, particularly those of 35 and 28 kDa, which were faint. An almost identical pattern existed in the case of globulins isolated from sprouted seeds of finger millet for 24 h at 18 and 25 °C (lanes 4 and 6), respectively.

In comparison with the resting seeds, the profile of seeds presoaked (lane 3) and sprouted for 48 h at 25 °C (lane 7) had extra bands in the globulins and the mobility pattern was almost the same. The bands

corresponding to molecular masses of 33−35 and 27−29 kDa in these two fractions were clear and prominent, and a band of molecular mass 20 kDa was also apparent.

In the literature, electrophoretic characterization of protein fractions from major and minor cereals including a few millets has been extensively documented (Altschul et al., 1966; Robert et al., 1983a; Burgess and Shewry, 1986). Although finger millet seeds are an essential raw material of many of the traditional foods of the tropics, information is lacking about the electrophoretic pattern of either globulins or prolamins. Considering *E. coracana* as a minor millet, a comparison with a few other millets of common use in some kinds of traditional foods has relevance. Studies with proteins of proso millet had shown that the alcohol soluble fractions move slowly relative to the salt soluble fractions (Jones et al., 1970). Sreeramulu (1997) has shown that a homology exists among the proteins of cereals, with minor variations in the subunits. However, there is little information relating to the type of protein subunits in either resting or sprouting seeds of millets commonly used in many traditional foods.

Protein Associations. The results of sedimentation analysis of isolated fractions of globulin and prolamin are presented in Figures 6 and 7. It appears that the globulin and prolamin fractions of finger millet have a probable self-aggregating system.

The sedimentation equilibrium data for prolamin and globulin fractions were analyzed as a single ideal species. The line obtained through plotting of apparent molecular weight against absorbance (concentration) indicates the nature of aggregation of molecular weights of all species present. Besides, it also enables determination of closest associative order in a self-associating system. The nature of plotted lines obtained in the case of globulin fractions comprising seeds rested, presoaked, and sprouted for 48 h at 18 and 25 °C, respectively, is shown in Figure 6. The apparent molecular weight increases with the concentration. Such a pattern indicates binding of monomers into possible dimers, trimers, tetramers, and further. The pattern observed in all of the above samples was rather uniform, with slight differences in molecular weights of initial monomers between presoaked and the other types. It appears that globulins obtained from presoaked and sprouted seeds for 48 h at 25 °C contain an association of higher order, with monomers binding into polymers (panels B and D of Figure 6). This could well be related with the protein content of globulin fractions (Table 2).

The isolated prolamins also showed an aggregation of molecular weights. However, in seeds rested, presoaked, and sprouted for 48 h at 25 °C, a slight heterogeneity in the binding nature of monomers appears relative to that observed in seeds sprouted for 48 h at 18 °C (Figure 7); here, the aggregation pattern was more uniform with the increasing concentration. Prolamins of sorghum exhibit the unique ability to form gels at low protein concentrations in a variety of solvents (Beckwith and Jones, 1972). The observed self-associating system in prolamins and globulins of finger millet appears to be similar to those reported for protein fractions of major cereals such as wheat (Altschul et al., 1966).

Microbial Profile. The associated changes in the counts of mesophilic aerobes and lactic acid bacteria during the stages of presoaking for 8 h at 23 °C and sprouting for 48 h at 18 and 25 °C, respectively, in comparison with the resting seeds are presented in Table 4. The count of mesophilic aerobes was almost the same at all stages. The count of lactic acid bacteria showed no change at stages of presoaking and sprouting relative to that in resting seeds ($P > 0.05$). The predominance of lactic acid bacteria during the process of sprouting provides desirable attributes in traditional fermentations. This phenomenon has been observed in many fermented foods; natural fermentation is still the backbone of product characteristics (Steinkraus, 1983).

Table 4. Changes in the Microbial Profile in the Seeds of *E. coracana* during Sprouting[a]

incubation		bacterial counts [$\log_{10}$(CFU g^{-1})]	
period (h)	temp (°C)	mesophilic aerobes	LAB
resting seed		4.3[a]	3.7[b]
8 (soaking)	23	4.2[a]	5.2[b]
sprouting for			
24	18	3.9[a]	5.3[b]
48	18	4.4[a]	5.4[b]
24	25	3.4[a]	5.3[b]
48	25	4.4[a]	5.5[b]
SE_m (12 df)		±0.266	±0.551

[a] Values are the mean of three independent trials. Means in the same column with same superscript letters are not different ($P > 0.05$)

In conclusion, beneficial attributes (both biochemical and microbiological) are derived through the process of presoaking and sprouting of finger millet seeds. Use of such enriched seeds is important in improving the quality characteristics of traditional fermented foods of the tropics.

ACKNOWLEDGMENT

We are thankful to the Director-General, NARC, Tsukuba, Japan, for providing the facilities. We gratefully acknowledge Mr. B. S. Ramesh, CFTRI, Mysore, India, for assistance in statistical analysis.

LITERATURE CITED

Abe, M.; Arai, S.; Fujimaki, M. Purification and characterization of a protease occurring in the endosperm of germinating corn. *Agric. Biol. Chem.* **1977**, *41*, 893−899.

Altschul, A. M.; Yatsu, L. Y.; Ory, R. L.; Engleman, E. M. Seed proteins. *Annu. Rev. Plant Physiol.* **1966**, *17*, 113−136.

Baker, D. Cereal foods. In *Official Methods of Analysis of the Association of Official Analytical Chemists*, 14th ed.; Williams, S., Ed.; Arlington, VA, 1984; p 249.

Beckman Corp. An-60 Ti analytical rotor, cells and counterbalance. Spinco Business unit, Beckman Instruments, Fullerton, CA, 1991; p 30.

Beckman Corp. Optima XL-A data analysis software. Spinco Business unit, Beckman Instruments, Fullerton, CA, 1993; version 2.0 User's Manual.

Beckwith, A. C.; Jones, R. W. Physical chemical characterization of grain sorghum prolamin fractions and components. *J. Agric. Food Chem.* **1972**, *20*, 259−261.

Bender, F. E.; Douglass, L. W.; Kramer, A. *Statistical Methods for Food and Agriculture*; AVI Publishing: Westport, CT, 1982; p 345.

Burgess, S. R.; Shewry, P. R. Identification of homologous globulins from embryos of wheat, barley, rye and oats. *J. Exp. Bot.* **1986**, *37*, 1863−1871.

Buysse, J.; Merckx, R. An improved colorimetric method to quantify sugar content of plant tissue. *J. Exp. Bot.* **1993**, *44*, 1627−1629.

Ching, T. M. Metabolism of germinating seeds. In *Seed Biology*; Kozlowski, T. T., Ed.; Academic Press: New York, 1972; Vol. I, pp 103−206.

Cornford, C. A.; Black, M.; Daussant, J.; Murdoch, K. M. Alpha amylase production by pre-mature wheat (*Triticum aestivum* L.) embryos. *J. Exp. Bot.* **1987**, *38*, 277−285.

Draper, S. R. Amino acid profiles of chemical and anatomical fractions of oat grains. *J. Sci. Food Agric.* **1973**, *24*, 1241−1250.

Haq, N. Crop plants: Potential for food and industry. In *New Crops for Food and Industry*; Wickens, G. E., Haq, N., Day, P., Eds.; Chapman and Hall: London, U.K., 1989; pp 246−256.

Hemanalini, G.; Umapathy, K. P.; Rao, J. R.; Saraswathi, G. Nutritive evaluation of sprouted ragi. *Nutr. Rep. Int.* **1980**, *22*, 271−277.

Indira, R.; Naik, M. S. Nutrient composition and protein quality of some minor millets. *Indian J. Agric. Sci.* **1971**, *41*, 795−797.

Jones, R. W.; Beckwith, A. C.; Khoo, U.; Inglett, G. E. Protein composition of proso millet. *J. Agric. Food Chem.* **1970**, *18*, 37−39.

Kamath, M. V.; Belavady, B. Unavailable carbohydrates of commonly consumed Indian foods. *J. Sci. Food Agric.* **1980**, *31*, 194−202.

Laemmli, U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680−685.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.

Malleshi, N. G.; Desikachar, H. S. R. Malting quality of new varieties of ragi (*Eleusine coracana*). *J. Food Sci. Technol.* **1979**, *16*, 149−150.

Malleshi, N. G.; Desikachar, H. S. R. Formulation of a weaning food with low hot paste viscosity based on malted ragi and greengram. *J. Food Sci. Technol.* **1982**, *19*, 193−197.

Malleshi, N. G.; Desikachar, H. S. R. Nutritive value of malted millet flours. *Plant Food Hum. Nutr.* **1986**, *36*, 191−196.

Mikola, J. Proteinases and peptidases in germinating cereal grains. In *IV International Symposium on Pre-harvest Sprouting in Cereals—Proceedings*; Mares, D. J., Ed.; Westview Press: Boulder, CO, 1987.

Okamoto, K.; Akazawa, T. Enzymic mechanisms of starch breakdown in germinating rice seeds. *Plant Physiol.* **1979**, *63*, 336−340.

Pore, M. S.; Magar, N. G. Nutrient composition of hybrid varieties of finger millet. *Indian J. Agric. Sci.* **1979**, *49*, 526−531.

Purseglove, J. W. Millets—*Eleusine coracana*. In *Evolution of Crop Plants*; Simmonds, N. W., Ed.; Longman: London, U.K., 1976; pp 91−93.

Redgwell, R. J. Fractionation of plant extracts using ion-exchange Sephadex. *Anal. Biochem.* **1980**, *107*, 44−50.

Robert, L. S.; Matlashewski, G. J.; Adeli, K.; Nozzolillo, C.; Altosaar, I. Electrophoretic and developmental characterization of oat (*Avena sativa* L.) globulins in cultivars of different protein content. *Cereal Chem.* **1983a**, *60*, 231−234.

Robert, L. S.; Nozzolillo, C.; Altosaar, I. Molecular weight and charge heterogeneity of prolamins (Avenins) from nine oat (*Avena sativa* L.) cultivars of different protein content and from developing seeds. *Cereal Chem.* **1983b**, *60*, 438−442.

Sato, T. Field crops in Thailand. In *Reports on Research in Southeast Asia*; Natural Science Series 1; Center for Southeast Asian Studies, Kyoto University: Kyoto, Japan, 1966; p 152.

Sreeramulu, G. Biochemical and molecular genetic investigations on wheat seed storage proteins. Ph.D. Thesis, University of Mysore, India, 1997; p 204.

Steinkraus, K. H. *Handbook of Indigenous Fermented Foods*; Dekker: New York, 1983; p 671.

Teichgraber, P.; Zache, U.; Knorr, D. Enzymes from germinating seeds—potential application in food processing. *Trends Food Sci. Technol.* **1993**, *4*, 145−149.

Veerabhadrappa, P. S.; Manjunath, N. K.; Virupaksha, T. K. Proteinase inhibitors of finger millet (*Eleusine coracana* Gaerta.). *J. Sci. Food Agric.* **1978**, *29*, 353−358.

Virupaksha, T. K.; Geeta, R.; Nagaraju, D. Seed proteins of finger millet and their amino acid composition. *J. Sci. Food Agric.* **1975**, *26*, 1237−1246.

Yamagata, H.; Sugimoto, T.; Tanka, K.; Kasai, Z. Biosynthesis of storage proteins in developing rice seeds. *Plant Physiol.* **1982**, *70*, 1094−1100.

Received for review June 9, 1997. Revised manuscript received January 20, 1998. Accepted January 23, 1998. M.V. is grateful to the United Nations University, Headquarters, Tokyo, Japan, for awarding him with a UNU-KIRIN Fellowship (1995−1996) and to CSIR, New Delhi, India, for the opportunity to undertake the fellowship at Japan.

JF970495K