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RELATIONSHIP BETWEEN PEROXIDASE ACTIVITY PROTEIN AND PHENOLICS AT DIFFERENT LEVELS OF GERMINATED LEGUMES

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Peroxidase enzyme was found to be more active in 24 h germinated legumes. Therefore it is presumed that enzyme plays a very important metabolic role in nitrogen metabolism, a-oxidation of fatty acid and in the oxidation of phenolics, with same time of germination, proteins and phenolics were decreased maximum.

Legumes, popularly known as pulses, are the most common nutritious seeds in the diets. Before legumes are consumed they are subjected to simple processes. Germination is a simple procedure of improving the nutritional quality of food and texture (Jood *et al.*, 1985).

The changes in the nutritive value are due to different seed metabolism. Certain antinutritional factors have been reported in ungeerminated legumes (Savage, 1988) which are reduced or metabolized during germination. Phenolics are one of the antinutritional factors which should be metabolized in presence of peroxidase enzyme during germination.

Little or no work has been done for peroxidase enzyme. It is presumed that it might play very important metabolic role in nitrogen metabolism, a-oxidation of fatty acid and in the oxidation of phenolics. Present study deals with the peroxidase enzyme activity and its relation with protein and phenolics at different stages of germination.

MATERIALS AND METHOD

Economically important legumes namely Bengal gram (*Cicer arietinum* L.), Cowpea (*Vigna unguiculata* L.), Lentil (*Lens esculenta* L.), Mung bean (*Phaseolus aureus, Roxb*), Moth beans (*Phaseolus aconitifolius, Jacg.*), and pea (*Pissum satiyum* L.) were used for this study.

Germination - Dry seeds were made free of foreign material, washed and soaked in water for 6 h at room temperature. Seeds were germinated by rapping them in the moistened cloth. The germinated seeds were removed after 24, 48 and 72 h of germination for the study. Experiments were also performed in dry and soaked (6 h) seeds.

Enzyme assay - The assay system consisted of 50 ml of 0.285N H_2O_2 in 0.2 M phosphate buffer (pH 7.0). The reaction was started by the addition of 1.0 ml of enzyme preparation and enzyme activity was determined titrimetrically after 15, 30, 45 and 60 min of incubation at 30°C.

The Velocity constant (K) of peroxidase enzyme at different time intervals was calculated by formula.

$$K = \frac{1}{t} \log \left(\frac{a}{a - x}\right)$$

Where K = Velocity constant
t = time
log a = reading at 0 time
log (a-x) = reading at different time interval.

With the help of velocity constant the katf values of peroxidase in terms of fresh weight of tissue and protein were calculated by using the formula -

$$\text{Katf in terms of fresh wt} = \frac{\text{Velocity constant (k) at zero time}}{\text{ml of enzyme extract in 50 ml } H_2O_2}$$

$$\text{Katf in terms of protein} = \frac{\text{Velocity constant k at zero time}}{\text{mg of protein/ml of 20\% homogenate}}$$

Estimation of moisture content (Soaking capacity) - The water uptake by the seeds was calculated which determined the soaking capacity.

Estimation of peroxidase enzyme - Peroxidase enzyme activity was calculated by the modified method of Raghuramulu *et al.* (1983). Enzyme activity was expressed as ml of O_2 liberated/ml of Enzyme extract/h.

Different optimal conditions like substrate concentration, optimal pH, enzyme concentration, time

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Table 1: Specific activity in terms of velocity mg^{-1} protein of peroxidase enzyme of different legumes at different hours of germination.

Hours after germination	Bengal Gram	Cow pea	Lentil	Mung bean	Moth bean	Pea
6	0.086	0.08	0.051	0.051	0.061	0.07
24	0.15	0.10	0.10	0.11	0.072	1.7

and temperature were also studied for peroxidase enzyme. Protein and phenolics were estimated by the method of Khanna *et al.* (1969) and Goldstein & swain (1963) respectively.

RESULTS AND DISCUSSION

Soaking in water and then germination is a very common household practice for processing of legumes which result in profound metabolic changes of protein, carbohydrate and fat. In different legumes soaking capacity was found to be in the range of 84% to 96% after 6 h. Chavan *et al.* (1983) observed 50% to 200% water uptake by dhal during cooking after one hour of soaking in water.

Peroxidase at different stages of germination- The velocity constant (K) of different legumes was calculated at different time intervals from which the kaft values on the basis of fresh weight as well as on the basis of protein were calculated at different stages of germination. It was found that the enzyme was more active when the legumes were germinated up to 24 h, after that it become almost constant, fig. 1 and 2 show activities of enzyme at 6 h and 24 h of germination respectively. Same results were found when katf values of enzyme were calculated in terms of fresh weight as well as in terms of protein. Table 1 shows the katf values of different legumes in terms of specific activity of enzymec at 6 h and 24 h of germination. Various enzymes have been studies by Jaya & Venkataraman (1981), Bendnakshi *et al.* (1987) in different germinated seeds.

Peroxidase enzyme was found to be active at pH 7 at 60 minutes of incubation and 30°C. The Km was found to be 3.74 g/litre. Several enzyme systems become active which bring about profound changes in the nutritive value of legumes (Deosthale, 1982). When the activity of enzyme of different legumes were compared, it was found that the enzyme was more advanced in Mung and Moth beans as compared to other legumes. Jaya & Venkataraman (1981) studied the influence of germination on the carbohydrate digestibility of chick pea and green gram and found the maximum b-amylase activity after 48 h of germination. From the results it is clear that the peroxidase enzyme with its different optimum conditions is active after 24 h of germination and is required either in nitrogen metabolism, Oxidation of phenolics or in oxidation of fatty acids. (West *et al.*, 1974; Lehniger, 1978; Mahadevan, 1978).

Protein - Table 2 represents the protein content of different legumes. It was observed that maximum decrease in protein content was found after 24 h to 48 h of germination. Similar results were found by King & Parvastien, (1987) who observed a decrease in protein nitrogen in winged bean with germination. Jood *et al.* (1985) have recommended 24 h germination as a reasonably good treatment for legumes for reduction of flatus production. Srinivas Rao (1988) concluded that digestibility is increased with increase in time of germination due to increase in the activity of hydrolytic enzymes.

Phenolics - Table 2 also indicates phenolic content of legumes. In dry legumes the phenolic content ranged between 18 mg to 49 mg/100 g of dry tissue. After 24 h germination the values were between 15 mg to 39 mg/100 g of dry weight.

A direct relationship between the metabolism of

Table 2: Protein and phenolic content (mg/100 g of dry tissue) of different legumes at different stages of germination

Legumes	Ungerminated seeds		TIME (h)							
			6		24		48		72	
	Phenolic	Protein	Phenolic	Protein	Phenolic	Protein	Phenolic	Protein	Phenolic	Protein
Bengal gram	39	610	32	550	21	300	18	250	14	240
Cow pea	40	585	36	450	29	305	28	290	26	260
Lentil	49	540	45	490	39	460	27	390	28	280
Mung bean	43	495	34	490	33	440	30	360	25	260
Moth bean	29.3	505	28.5	500	25	400	21	320	18.3	310
Pea	47	595	43	510	34	260	32	250	25	245

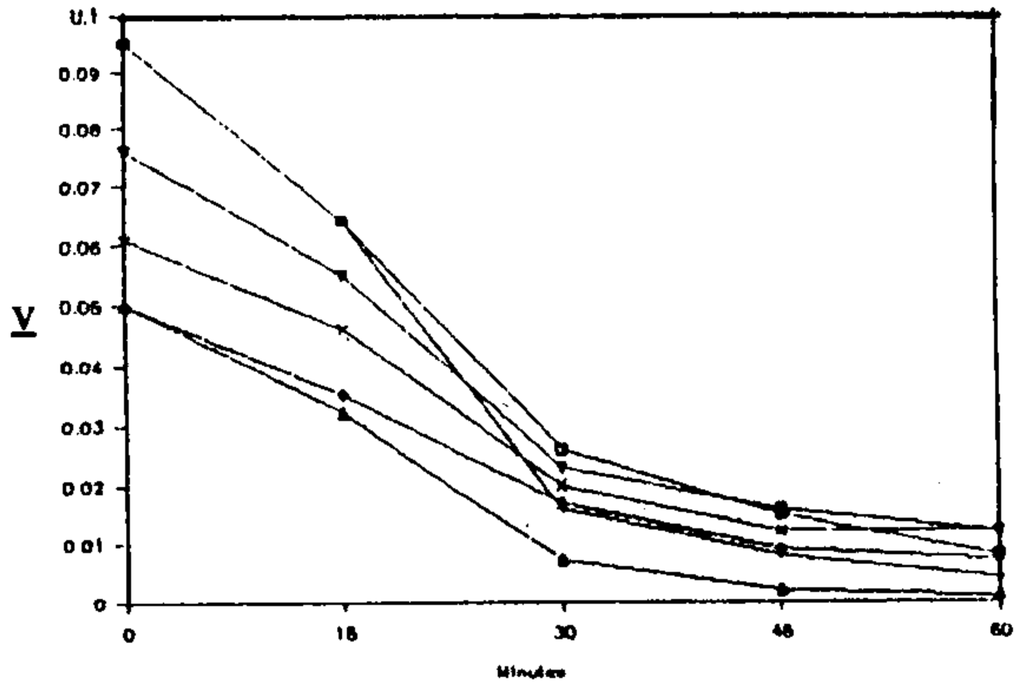

Fig. 1.Velocity constant (K) of different legumes germinated for 6 hours (: Bengal Gram, + : Cowpea, : Lentil, : Mung bean, x : Moth bean, : Pea)

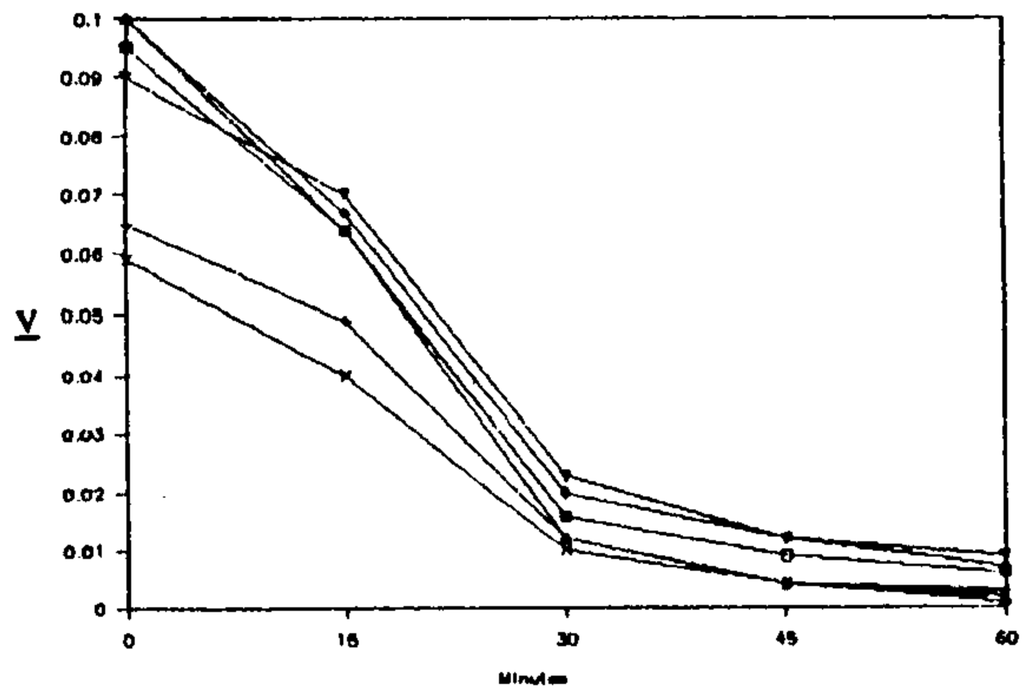

Fig. 2.Velocity constant (K) of different legumes germinated for 24 hours (: Bengal Gram, + : Cowpea, : Lentil, : Mung bean, x : Moth bean, : Pea)

peroxidase enzyme with protein and phemolics can be seen. During germination energy is more required by the system, the Respiratory Quotient is increased, therefore the activity of peroxidase enzyme may be useful to facilitate more oxygen for te juvenile seedlings.

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