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Evaluation of Changes in Phytase, α-Amylase and Protease Activities of Some Legume Seeds during Germination

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Abstract—**The present study addresses the enzymatic changes taking place during soaking and germination of some legume seeds as a function of germination time with possible improvements in nutritional quality. Mung bean (Phascolus aureus), cowpea (Vigna catjang), lentil (Lens culinaris) and chickpea (Cicer arietinum) were used as raw materials. Untreated, soaked and germinated (for 24, 48 and 72 h) legume seeds were analyzed for phytase, α-amylase and protease activities. Enzymes activities increased significantly ($P<0.05$) on pre-germination soaking, except for phytase activity of chickpea, which did not differ significantly ($P<0.05$). Enzymatic activities of all legumes improved significantly ($P<0.05$) and reached maximum during the course of germination up to 72 h. However, maximum protease activity in mung bean was at 48 h germination and declined thereafter. Germination as a biotechnological technique improved enzymatic activities in all legume seeds.**

Keywords-Enzyme activity, soaking, cow pea, mung bean, lentil, chick pea

I. INTRODUCTION

Dry legumes are good sources of protein, energy and other nutrients in developing countries. However their use is limited because of high dietary bulk; presence of antinutritional factors, mainly phytic acid in most of the legumes; and low protein and carbohydrate digestibility [1,2,3,4]. Phytic acid is widespread in nature and is the principal form of phosphorous in many seeds. Phytic acid forms complexes with minerals and trace elements rendering them nutritionally unavailable [5,6]. There is also a significant ($P< 0.05$) negative correlation between phytic acid content and extent of starch and protein digestibility of legumes [7].

Phytic acid can be degraded by the enzyme phytase, which liberates myo-inositol and phosphoric acid [8]. Phytase is present in plants, microorganisms and certain animal tissues. Germination of seeds appears to be a relatively simple non-chemical technique for increasing phytase levels. It has been demonstrated in rye and barely [9] and horse gram and moth bean [10].

During germination of seeds, α-amylase and protease, that are developed, degrade starch granules and reserve proteins, respectively; thereby reducing the dietary bulk and improving the digestibility of starch and protein [4,11,12,13]. Germination was shown to increase monosaccharide and decreased disaccharide contents of legumes due to α-amylase [14]. Monosaccharides can be used as a good energy source and will also increase the palatability of seeds.

Hence, enhanced phytase, amylase and protease activities in germinated legumes may diminish the antinutritional effects of phytic acid and improve the nutritional quality, which make them important in biotechnological applications. The malting capacity and enzymatic changes of different cereals have been studied in great deal with regard to their use, especially the amylases in germinating cereals in several cereal species [11,15,16]. But a comprehensive data on changes of key enzymes (phytase, α-amylase and protease) in legume seeds is scarce, so the present was planned with an objective to obtain more information on enzymatic activities in germinating legume seeds.

II. MATERIALS AND METHODS

A. Materials

Samples of mung bean (*Phascolus aureus*), cowpea (*Vigna catjang*), lentil (*Lens culinaris*) and chickpea (*Cicer arietinum*) were obtained form local market. Legume seeds were cleaned, washed and soaked in 4-5 volumes of distilled water for 12h under ambient laboratory conditions (22-25^{o}C). At the end of the period, the water was drained and the seed samples were allowed to germinate under a wet muslin cloth in the dark at 25^{o}C for 24, 48 and 72 h. Seed samples were removed periodically after germination. All seeds (untreated, soaked, germinated for 24, 48 and 72 h) were dried in a cabinet air forced drier at 50^{o}C for 16-18 h and frozen. All samples were milled to flour in plate mill before analysis.

B. Chemical analysis

The dry matter content was determined gravimetrically by drying about 1 g of milled seeds for 24 h at 60^{o}C [17].

Phytase activity was assayed [8]. Inorganic phosphorous was determined colorimetrically [18]. Phytase activity was expressed in phytase units (PU) per g dry matter of seeds. 1 PU is equivalent to the enzymatic activity that liberates 1 μmol inorganic phosphorous per min.

α- Amylase activity was estimated [19]. Amylase activity was expressed in terms of mg of maltose (maltose units) produced by 1 ml of enzyme solution at 37°C for 30 min.

Protease activity was estimated [12]. The protease activity was expressed as micromoles of tyrosine released at 37°C in 60 min.

C. *Statistical analysis*

The analysis was carried out in four replicates for all determinations. The mean and standard deviation were calculated. The data were analyzed by one-way analysis of variance (ANOVA). A multiple comparison procedure of the treatment means was performed by Duncan's New Multiple Range Test [20]. Significance of the differences was defined as $P<0.05$.

III. Results And Discussion

Table I shows the phytase activity of soaked and germinated (24, 48 and 72 h) legume seeds. Phytase activity increased significantly ($P<0.05$) in all the legumes tested on soaking but in the case of chickpea the difference was not significant. There was about 14-46 % increase in phytase activity on soaking. Germination enhanced phytase activity significantly ($P<0.05$) in all the legumes studied. During the first 24h germination, enzymatic activity was comparatively less, followed by an increase after 48h and 72h in all the samples. The most pronounced increase in phytase activity was found in mung bean exceeding 300 % of the initial value. Phytase activity increased between 90% and 240 % in the other legumes. Cowpea had the highest initial enzyme activity of all the legumes in this study (0.35 PU/g dry matter). The highest final value (germinated 72 h) was found in mung bean (1.04 PU/g dry matter). Reference [8] have reported increases in phytase activity of soybean and white bean on 72 h germination, to maximum values of 0.77 and 0.47 PU/g dry matter. Similar results have been reported for faba bean showing phytase activity exceeding 450% of the initial value [21].

α-Amylase activity, as a function of germination time, is shown in Table II. The untreated seeds of the legumes exhibited very low amylase activity that ranged from 7.0 to 18.1 maltose units/g dry matter. The amylase activity increased insignificantly in all the legumes in this study on soaking, but only in the case of mung bean the difference was significant ($P<0.05$). The α-amylase activity improved by 10 to 150 % over the initial value with the lowest in cowpea and highest in mung bean samples. The α-amylase activity increased significantly ($P<0.05$) in all the legumes tested on progressive germination. Appreciable increase in enzyme activity was observed in mung bean from 8.1 to 280.2 maltose unit/ g dry matter at 0 and 72 h germination time, followed by cowpea, lentil and chickpea that had increases of exceeding 600, 500 and 200%, respectively over the untreated initial values. Reference [22] has also reported same results in the case of cowpea. He found that germination had a highly significant effect ($P<0.05$) on cowpea α-amylase activity. α-Amylase levels increased from 85.6 to 720.9 μmoles maltose/ ml of extract at 0 and 72 h germination time, respectively. Similar behavior was reported for cowpea, whose investigations indicated that α-amylase activity had attained a maximum at 3 days germination and had begun to decline at 4 days [15]. Reference [1] also showed improvement in α-amylase levels of horse gram, moth bean and field bean during germination. These findings agree with other reports regarding α-amylase production during germination of plant seeds other than legumes such as, maize [16], oats [23], millet [24] and sorghum [11,25].

The results of soaking and germination studies on protease activity of legumes are shown in Table 3. The untreated samples had 0.71 to 1.53 protease unit/ g dry matter and the lowest and highest values was belonging to mung bean and chickpea respectively. Soaking increased significantly ($P<0.05$) the protease activity of the legumes studied, by 17-36 % over the untreated samples. There was continuous and significant ($P<0.05$) increase in protease activity of the legumes tested with germination. However, in mung bean the maximum activity declined during the later stage of germination. Chickpea had the highest protease activity at 72 h germination (6.21 protease unit/ g dry matter) followed by lentil, cowpea and mung bean. Although, the maximum increase in enzyme activity at 72 h germination over the initial value was in cowpea (310 %), following by chickpea (306 %), lentil (232 %) and mung bean (216 %). However, mung bean had 311 % increase in enzyme activity in 48 h germination. Reference [27] studied about the effect of germination on protease activity of chickpea and mung bean which support these results.

Although, enzymes are synthesized during germination, reduction of some inhibitory factors such as phytic acid and protease inhibitor may increase the enzymes activity [27]. In conclusion soaking to some extent was useful in enzymes production but germination as a simple biotechnological technique can be used to bring about sufficient increase in enzymes activities, which in turn causes partial elimination of antinutrients (phytic acid) and predigestion of carbohydrate and protein in legume seeds, which make them useful in formulation of pediatric to geriatric foods.

TABLE I. PHYTASE ACTIVITY (PU*/G DRY MATTER)** OF UNTREATED, SOAKED AND GERMINATED LEGUME SEEDS

Samples	Control	Soaked	Germinated		
			24 h	*48 h*	*72 h*
Mung bean	0.24 ±0.02^{e}	0.35 ±0.03 ^{d} (46)	0.44 ±0.02 ^{c} (83)	0.90 ±0.04 ^{b} (275)	0.04 ±0.02 ^{a} (333)
Cowpea	0.35 ±0.03^{e}	0.40 ±0.02 ^{d} (14)	0. 45 ±0.03 ^{c} (28)	0.53 ±0.02 ^{b} (51)	0.67 ±0.02 ^{a} (91)
Chick pea	0.25 ±0.03^{d}	0.29 ±0.02 ^{d} (16)	0.36 ±0.03 ^{c} (44)	0.69 ±0.02 ^{b} (176)	0.75 ±0.03 ^{a} (200)
Lentil	0.26 ±0.03^{e}	0.31 ±0.03 ^{d} (19)	0.41 ±0.02 ^{c} (58)	0.55 ±0.03 ^{b} (111)	0.89 ±0.03 ^{a} (242)

* 1 Phytase unit (PU) is equivalent to the enzymatic activity which liberates 1 μmol inorganic

phosphate per min.

** Values represent mean ± standard deviation of triplicate analysis

Values in parentheses represent phytase activity in % of the initial (untreated) values.

All mean scores bearing different superscripts in rows are significantly different (P< 0.05).

TABLE II. AMYLASE ACTIVITY (MALTOSE UNIT*/G DRY MATTER)** OF UNTREATED, SOAKED AND GERMINATED LEGUME SEEDS

Samples	Control	Soaked	Germinated		
			24 h	*48 h*	*72 h*
Mung bean	8.1 ±0.2 [e]	21.3 ±3.5 [d] (159)	52.2 ±2.4 [c] (541)	139.4 ±3.5 [b] (1616)	280.2 ±5.8 [a] (3356)
Cowpea	18.1 ±1.8 [d]	20.1 ±2.9 [d] (11)	28.4 ±2.9 [c] (55)	93.5 ±2.9 [b] (416)	13.8 ±2.9 [a] (666)
Chick pea	7.0 ±0.9 [d]	8.5 ±0.9 [d] (16)	12.6 ±0.8 [c] (71)	19.4 ±0.8 [b] (171)	25.4 ±1.8 [a] (257)
Lentil	10.2 ±0.9 [d]	12.0 ±1.3 [d] (20)	18.7 ±1.7 [c] (80)	30.3 ±1.8 [b] (200)	67.7 ±2.9 [a] (570)

* 1 maltose unit is equivalent to the enzymatic activity, which liberates 1 mg of maltose in 30 min

** Values represent mean ± standard deviation of triplicate analysis

Values in parentheses represent α-amylase activity in % of the initial (untreated) values

All mean scores bearing different superscripts in rows are significantly different (P< 0.05).

TABLE III. PROTEASE ACTIVITY (PU*/G DRY MATTER)** OF UNTREATED, SOAKED AND GERMINATED LEGUME SEEDS

Samples	Control	Soaked	Germinated		
			24 h	*48 h*	*72 h*
Mung bean	0.71 ±0.04 [e]	0.93 ±0.03 [d] (31)	1.34 ±0.03 [c] (89)	2.92 ±0.04 [a] (311)	2.25 ±0.04 [b] (216)
Cowpea	0.93 ±0.04 [e]	1.27 ±0.04 [d] (36)	2.05 ±0.05 [c] (120)	2.73 ±0.03 [b] (273)	3.81 ±0.05 [a] (310)
Chick pea	1.53 ±0.04 [e]	1.84 ±0.03 [d] (20)	2.15 ±0.03 [c] (41)	2.37 ±0.04 [b] (55)	6.21 ±0.04 [a] (306)
Lentil	0.29 ±0.06 [e]	1.51 ±0.04 [d] (17)	2.34 ±0.03 [c] (81)	3.27 ±0.04 [b] (153)	4.29 ±0.05 [a] (232)

* 1 Protease unit (PU) is equivalent to the enzymatic activity, which liberates 1 µmol tyrosine in 60 min.

** Values represent mean ± standard deviation of triplicate analysis

Values in parentheses represent protease activity in % of the initial (untreated) values.

All mean scores bearing different superscripts in rows are significantly different (P< 0.05).

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