Evolution of Trypsin Inhibitor Activity during Germination of Lentils

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The effect of light and seed rinsing during the germination of lentil seeds (*Lens culinaris* var. Vulgaris cv. Magda-20) on trypsin inhibitor activity (TIA) has been studied. TIA decreased progressively during germination, while hydration grade and light exposure had less influence on TIA. In general, there was no significant change in TIA after 3 days of germination, except when seeds were rinsed only once and the experiment was conducted in the dark, showing a 10% TIA decrease. Six-day germination produced TIA decreases from 7% to 18%. In this case, the number of rinses had no significant effect on the final TIA, but the activity of protease inhibitors was significantly higher in seeds illuminated for 6 h per day. Maximal TIA decreases were found in seeds germinated in darkness and rinsed daily. Under these optimum conditions, TIA first decreased slowly (12% loss of this activity after 6 days) followed by a faster decrease (up to 45% of control seeds values) after 10 days of germination.

Keywords: *Lentils; germination; antinutrients; trypsin inhibitor activity*

INTRODUCTION

Lentil seeds (*Lens culinaris*) are a promising source of protein and starch, although their nutritional composition varies widely depending on cultivar, growth conditions, and agricultural practice (Adsule *et al.*, 1989). The lentil proteins, composing 22–31% of the seed dry weight, have an amino acid composition similar to those of other legumes, with methionine and cysteine being limiting (Majeed and Chang, 1977). Protein digestibility, however, also may be affected by the presence of trypsin inhibitor compounds, which inhibit the enzyme trypsin. They are mainly low molecular weight proteins, although some other compounds are included as trypsin inhibitors. They are found in most grain legumes and are capable of binding to the trypsin enzyme and inhibiting its activity (Liener and Kakade, 1980). In this sense, it is important not only to isolate the protease inhibitors but also to determine the total trypsin inhibitor activity (TIA) present in legumes. Several studies have shown the presence of trypsin inhibitors in lentils, and their nutritional significance has been extensively investigated (Chavan and Kadam, 1989). A high level of trypsin inhibitors in the diet stimulates pancreatic juice secretion and causes pancreatic hypertrophy (Liener and Kakade, 1980) and poor growth performance in animals (Jondreville *et al.*, 1992; Fernandez *et al.*, 1993).

Simple processes such as soaking, cooking, autoclaving, germination, and fermentation have been shown to reduce the levels of these antinutritional compounds in several legumes. Soaking slightly reduces the levels of TIA (Trugo *et al.*, 1990; Vidal-Valverde *et al.*, 1994), while heat treatments destroy it (Batra *et al.*, 1986; Vidal-Valverde *et al.*, 1994). Germination has been suggested as an inexpensive and effective technology for improving the quality of legumes, by enhancing their digestibility (Reddy *et al.*, 1989), increasing the level of amino acids (Chang and Harrold, 1988), and reducing the content of antinutritional factors (Ghorpade and Kadam, 1989; Vidal-Valverde and Frias, 1992). The sprouting of soybeans and mung beans was developed by the Chinese centuries ago, but it was not until the second quarter of this century that it was recognized that the process of germination increased the nutritive value of legumes (Rudra, 1938; Everson *et al.*, 1944). Later studies have shown that germination reduces the levels of hemaglutinins, TIA, and tannins and increases the *in vitro* protein digestibility and nitrogen solubility (El-Mahdy *et al.*, 1985).

Despite extensive studies on the effect of germination on TIA in legumes, there is still some controversy in the literature about the increase, decrease, or stability of TIA depending on the legume and the treatment conditions (Liener and Kakade, 1980). Most studies on the effect of germination on TIA have been conducted using a single set of germination conditions.

The main objective of this paper was to germinate lentil seeds in different light, hydration, and time conditions to optimize those that give processed lentils with the lowest TIA.

EXPERIMENTAL PROCEDURES

Germination. *L. culinaris* var. Vulgaris cv. Magda-20 from Albacete, Spain, harvested in 1991 and 1992, were used for the germination experiments, which were performed in duplicate. The germination procedure for lentil seeds harvested in 1991 (experiment A) was as follows: 100 g of seeds was washed with 0.7% sodium hypochlorite, soaked in 500 mL of water at room temperature for 6 h, and shaken every 30 min. The water was then drained off, and the seeds were transferred to a separating funnel, where different germination conditions were applied as follows: Some samples were germinated in darkness or were given 6 h daily of illumination. Some samples were hydrated by rinsing with distilled water every 24 h or on alternate days (Table 1). The germination was carried out at 20 °C. A total of 90–100% of the seeds germinated, and the sprouts reached 5–8 cm in length. The sprouts and the seeds were ground and freeze-dried for analysis.

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0021-8561/95/1443-2231$09.00/0

Table 1. Germination Conditions for Lentil Seeds Used in Experiment A

expt A	germination time (days)	light exposure (h of light/day)	no. of rinses (4 mL of water/g of lentil)
control A	0	0	0
A1	3	0	3 (daily)
A2	3	6	3 (daily)
A3	3	0	1 (alternate days)
A4	3	6	1 (alternate days)
A5	6	0	6 (daily)
A6	6	6	6 (daily)
A7	6	0	2 (alternate days)

Table 2. Germination Conditions for Lentil Seeds Used in Experiment B[a]

expt B	germination time (days)	no. of rinses (4 mL of water/g of lentil)
control B	0	0
B1	2	2 (daily)
B2	4	4 (daily)
B3	6	6 (daily)
B4	8	8 (daily)
B5	10	10 (daily)

[a] This germination experiment was carried out in the dark.

Table 3. Trypsin Inhibitor Activity during Germination of Lentils (Experiment A)[a]

expt A	germination conditions[b]	TI activity[c]	% reduction[d]
control A	0, 0, 0	5.05 ± 0.08^{a}	
A1	3, 0, 3	4.79 ± 0.27^{b}	5.1
A2	3, 6, 3	$4.91 \pm 0.14^{a,b}$	2.8
A3	3, 0, 1	4.54 ± 0.21	10.1
A4	3, 6, 1	$4.97 \pm 0.20^{a,b}$	1.6
A5	6, 0, 6	4.12 ± 0.30^{c}	18.0
A6	6, 6, 6	4.69 ± 0.29^{b}	7.1
A7	6, 0, 2	4.29 ± 0.22^{c}	15.0

[a] Values are the mean of six determinations ± standard deviation. The same superscript in the same column means no significant difference ($P \leq 0.05$). [b] Germination conditions (days, hours of light exposure per day, number of rinses). [c] Trypsin inhibitor units (TIU/mg of dry matter). [d] % reduction = 100 − [100 × (TI activity sample/TI activity control)].

The germination procedure for lentil seeds harvested in 1992 (experiment B) was as follows: 25 g of lentil seeds was soaked in 125 mL of distilled water for 6 h at room temperature and shaken every 30 min. The water was drained, and the seeds were transferred to a separating funnel and kept in the dark at 20 °C for a total germination time of 10 days. Every 24 h the seeds were moistened with 100 mL of distilled water and carefully shaken. Samples of the seeds were taken at 2, 4, 6, 8, and 10 days. A total of 90−100% of the seeds germinated, and the sprouts reached a length from 0.2 cm after 2 days to 10 cm after 10 days of germination. The sprouts and the seeds were ground and freeze-dried for analysis (Table 2).

Trypsin Inhibitor Analysis. TIA was determined using the method of Kakade *et al.* (1974) as modified by Valdebouze *et al.* (1980).

Statistical Analysis. Multifactor analysis of variance was applied to the data using Statgraphics Statistical Graphics Systems Software 5.0 with a PC.

To allow prediction of response over the full time of the germination experiment, polynomial regression curves for TIA were obtained using Origin 3.0 Technical Graphics and Data Analysis (MicroCal Software, Inc., Northampton, MA).

RESULTS

No differences were found in TIA between 1991 (Table 3) and 1992 (Figure 1) harvested material.

The effects of period of germination, light exposure, and rinsing frequency during germination of lentil seeds harvested in 1991 on TIA are shown in Table 3 (experiment A). The effect of germination time was shown by keeping constant light hours and hydration grade (experiments A1/A5, A2/A6, and A3/A7). Three-day germination did not bring about significant changes in TIA, except for germination carried out with no light exposure and only one rinse (experiment A3), for which a 10% decrease was obtained. When the period of germination was extended to 6 days, a decrease in TIA ranging between 7% and 18% was observed.

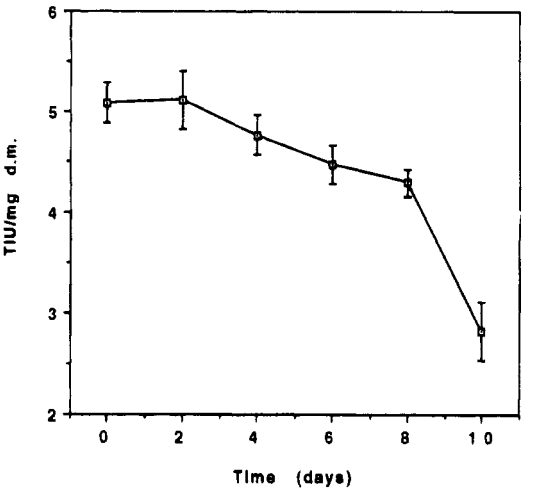

Figure 1. Effect of germination on trypsin inhibitor activity of lentils grown with no light exposure and rinsed daily (experiment B).

The effect of light on TIA during germination was observed by comparing experiments A1/A2, A3/A4, and A5/A6 (Table 3), in which time of germination and number of rinses were kept constant. No light exposure on the germinated seeds produced a slight but significant decrease in TIA compared to those with 6 h of light exposure, except for experiments A1 and A2 (3 days of germination and a daily rinse), for which light did not produce a significant difference.

The effect of rinse frequency was shown when germination time and light exposure were kept invariable (experiments A1/A3, A2/A4, and A5/A7). When germination of lentils was carried out for 3 days, the number of rinses affected TIA slightly but significantly, and a higher reduction of its activity was observed when only one rinse was performed and germination was carried out in darkness (experiment A3). However, TIA was not significantly modified by the number of rinses during 6 days of germination (Table 3).

The highest decrease of TIA was obtained when germination was performed for 6 days, in darkness and with daily rinses. A germination experiment using these conditions was designed, lasting 10 days with sampling every 2 days, to study the kinetics of these compounds during germination (Figure 1). Germination did not bring about appreciable changes in the TIA during the first 2 days. After 4 and 6 days of germination, decreases in TIA of 6% and 12%, respectively, were found. The reduction obtained after 8 days (16%) was not significantly greater than after 6 days. The major decrease in TIA was obtained after 8 days, and by the end of the experiment the activity had decreased by 45% (Figure 1).

Results obtained for TIA sampled every 2 days (experiment B) were fitted by order 2 polynomial regression curves using the equation $y = 4.77 + 6.43x - 0.019x^2$, showing a correlation coefficient of 0.873, a

square of correlation coefficient of 0.7612, and 0.405 for standard deviation of the fit.

DISCUSSION

The TIA in a large range of legume seeds has been described in the literature. Most studies have been conducted on soybean, and the amount of TIA in other legumes is based on soybean as reference, which has been shown to range from 16.5 to 29 units, depending on the variety (Collins and Sanders, 1976). For lentil seeds, Soni *et al.* (1978) indicated that TIA was 25% of that found in soybean, which corresponds to 5.7 units. Savage (1988) reported TIA values for lentils between 0.2 and 5.1 units, depending on the variety. Vidal-Valverde *et al.* (1994) reported values of 5.34 and 6.38 units for *L. culinaris* var. Vulgaris and *L. culinaris* var. Variabilis, respectively. The results presented in this paper give values of 5.0–5.1 units for *L. culinaris* var. Vulgaris cv. Magda-20, with no apparent differences between consecutive harvested years.

Germination has been investigated as a means of reducing the antinutritional effects of protease inhibitors, but the effect of germination remains controversial due to the fact that different results have been found depending on legume type and germination conditions (Collins and Sanders, 1976; El-Mahdy *et al.*, 1985; Lee and Karunanithy, 1990; Vidal-Valverde *et al.*, 1994). Most of the studies have been carried out on the effect of time on TIA, while not much work has been done on the effect of rinsing and light exposure during germination of lentils.

Frequent rinsing has been shown to increase the percent of germination and sprout development (Hsu *et al.*, 1980). There are few studies, however, to determine the effect of rinsing on TIA during germination. Collins and Sanders (1976) attributed the slight loss of TIA in soybeans to leaching during the daily washing of the sprouts. Our work, however, demonstrated that the number of rinses did not appear to affect the TIA.

To date, we have found no reports in the literature concerning the influence of light exposure on TIA during germination of lentils. Most of the work has been carried out in darkness. Our results showed that germination in darkness also brings about a greater reduction in TIA.

Some information has been found about the influence of germination time on TIA. Collins and Sanders (1976) reported that after 3 days of germination, the activity decreased 12.3% in soybean var. Kanrich and 8% in var. Dare, while var. Soylima did not show any apparent decrease. In lentils, El-Mahdy *et al.* (1985) observed 42% and 46% reductions in TIA after 24 h of germination for *L. culinaris* var. Syrian and Giza, respectively. Batra *et al.* (1986), however, found that germination of lentils for 3 days decreased TIA only slightly, while 6 days of germination lowered it substantially (21–54%). Similarly, Weder and Link (1993) observed that 72 h of sprouting did not alter the TIA of lentils, while Vidal-Valverde *et al.* (1994) observed 24% and 28% reductions in TIA after 6 days of germination of var. Vulgaris and Variabilis, respectively. The results of the present study showed that 3 days of germination was not sufficient to significantly reduce TIA, while 6 days of germination produced 18% reduction when it was carried out in the dark with daily rinsing. Our results showed that lentil germination lasting 10 days in the dark with daily rinsing showed a substantial decrease of TIA (45%), which could indicate that these compounds may be utilized during the seed germination as a source of energy.

ACKNOWLEDGMENT

We thank Mr. H. López-Corcoles for providing the lentil seeds, Dr. C. de la Cuadra for preparation of experiment A samples, and Dr. M. Dabrio for helping on the polynomial regression analysis.

LITERATURE CITED

Adsule, R. N.; Kadam S. S.; Leung, H. K. Lentil. In *CRC Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization*; Salunkhe, D. K., Kadam, S. S., Eds.; CRC Press: Boca Raton, FL, 1989; Vol. 2, pp 133–152.

Batra, V. I. P.; Vasishta, R.; Dhindsa, K. S. Effect of heat and germination on trypsin inhibitor activity in lentil and pigeon pea. *J. Food Sci. Technol.* **1986**, *23*, 260–263.

Chang, K. C.; Harrold, R. L. Changes in selected biochemical components in vitro protein digestibility and amino acids in two bean cultivars during germination. *J. Food Sci.* **1988**, *53*, 783–787.

Chavan, J. K.; Kadam, S. S. Protease inhibitors. In *CRC Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization*; Salunkhe, D. K., Kadam, S. S., Eds.; CRC Press: Boca Raton, FL, 1989; Vol. 1, pp 123–133.

Collins, J. L.; Sanders, G. G. Changes in TI activity in some soyabean varieties during maturation and germination. *J. Food Sci.* **1976**, *41*, 169–172.

El-Mahdy, A. R.; Moharram, Y. G.; Abou-Samaha, O. R. Influence of germination on the nutritional quality of lentil seeds. *Z. Lebensm. Unters. Forsch.* **1985**, *181*, 318–320.

Everson, G. J.; Steenbock, M.; Cederquist, M. The effect of germination, the state of maturity and the variety upon the nutritive values of soybean proteins. *J. Nutr.* **1944**, *27*, 255–259.

Fernandez, M. M.; Aranda, P.; Lopez-Jurado, M.; Urbano, G.; Estrella, E.; Sotomayor, C.; Diaz, C.; Frias, J.; Prodanov, M.; Vidal-Valverde, C. Effect of processing on some antinutritional factors of beans: influence on protein digestibility and food intake in rats. *Recent Advances of Research in Antinutritional Factors in Legume Seeds*, Wageningen Press: Wageningen, The Netherlands, 1993; pp 481–485.

Ghorpade, U. M.; Kadam, S. S. Germination. In *CRC Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology and Utilization*; Salunkhe, D. K., Kadam, S. S., Eds.; CRC Press: Boca Raton, FL, 1989; Vol. 3, pp 165–206.

Hsu, D.; Leung, H. K.; Finney, P. L.; Morad, M. M. Effect of germination on nutritive value and baking properties of dry peas. *J. Food Sci.* **1980**, *45*, 87–92.

Jondreville, C.; Grosjean, F.; Buron, G.; Peyronnet, C.; Beneytout, J. L. Comparison of four pea varieties in pig feeding through digestibility and growth performance results. *J. Anim. Physiol.* **1992**, *68*, 113–122.

Kakade, M. L.; Rackis, J. J.; McGhee, J. E.; Puski, G. Determination of trypsin inhibitor activity of soy bean products: a collaborative analysis of an improved procedure. *Cereal Chem.* **1974**, *51*, 376–382.

Lee, C. K.; Karunanithy, R. Effects of germination on chemical composition of *Glycine* and *Phaseolus* beans. *J. Sci. Food Agric.* **1990**, *51*, 437–445.

Liener, I. E.; Kakade, M. L. Protease inhibitors. In *Toxic Constituents of Plant Foodstuffs*; Liener, I. E., Ed.; Academic Press: New York, 1980; pp 7–71.

Majeed, A.; Chang, Y. O. Nutritional evaluation of lentils proteins. *Indian J. Nutr. Diet.* **1977**, *14*, 127–135.

Reddy, N. R.; Sathe, S. K.; Salunkhe, D. K. Carbohydrates. In *CRC Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology and Utilization*; Salunkhe, D.

K., Kadam, S. S., Eds.; CRC Press: Boca Raton, FL, 1989; Vol. 1, pp 65–131.

Rudra, M. N. Vitamin content of some germinated cereals and pulses. *Nature* **1938**, *141*, 203–205.

Savage, G. P. The composition and nutritional value of lentils (*Lens culinaris*). *Nutr. Abstr. Rev. (Ser. A)* **1988**, *58*, 319–343.

Soni, S. G.; Singh, T. P.; Singh, R. Comparative studies on the effect of certain treatments on antitrypsin activity of the common Indian pulses. *Indian J. Nutr. Diet.* **1978**, *15*, 341–347.

Trugo, L. C.; Ramos, L. A.; Trugo, N. M. F.; Souza, M. C. P. Oligosaccharide composition and trypsin inhibitor activity of *Phaseolus vulgaris* and the effect of germination on the alpha-galactoside composition and fermentation in the human colon. *Food Chem.* **1990**, *36*, 53–61.

Valdebouze, P.; Bergeron, E.; Gaborit, T.; Delort-Laval, J. Content and distribution of trypsin inhibitors and haemaglutinins in some legume seeds. *Can. J. Plant Sci.* **1980**, *60*, 695–701.

Vidal-Valverde, C.; Frias, J. Changes in carbohydrates during germination of lentils. *Z. Lebensm. Unters. Forsch.* **1992**, *194*, 461–464.

Vidal-Valverde, C.; Frias, J.; Estrella, I.; Gorospe, M. J.; Ruiz, R.; Bacon, J. Effect of processing on some antinutritional factors of lentils. *J. Agric. Food Chem.* **1994**, *42*, 2291–2295.

Weder, J. K. P.; Link, I. Effect of treatments on legume inhibitor activity against human proteinases. *Recent Advances of Research in Antinutritional Factors in Legume Seeds*; Wageningen Press: Wageningen, The Netherlands, 1993; pp 481–485.

Received for review December 27, 1994. Revised manuscript received May 2, 1995. Accepted May 22, 1995.[⊗] This work was supported by Spanish Comision Interministerial de Ciencia y Tecnologia ALI-91-1092 and forms part of the BSc of C.D.-P. J.F. is grateful to the European Union for financial support through an individual bursary (AIR3-BM93-1118).

JF9407331

[⊗] Abstract published in *Advance ACS Abstracts,* July 1, 1995.