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Effect of Processing on Some Antinutritional Factors of Lentils

Concepcion Vidal-Valverde,*,† Juana Frias,† Isabel Estrella,† Maria J. Gorospe,‡ Raquel Ruiz,§ and Jim Bacon§

Instituto de Fermentaciones Industriales, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain, Departamento de Produccion y Tecnologia de Alimentos, Instituto Nacional de Investigaciones Agrarias, CIT, Apdo. 8111, 28080 Madrid, Spain, and Food Molecular Biochemistry Department, Institute of Food Research, Colney Lane, Norwich NR4 7UA, U.K.

Changes in the trypsin inhibitor activity and in the phytic acid, tannin, and catechin content of lentils (*Lens culinaris* var. Vulgaris) were investigated after soaking in distilled water, citric acid, and sodium bicarbonate solutions. The effect of cooking, after the seeds were presoaked in the above-mentioned solutions and both the soaking and cooking solutions were discarded, was also studied. Finally, two varieties of lentils (*L. culinaris* var. Vulgaris and Variabilis) were germinated for 6 days, and the effect on the trypsin inhibitor activity and the phytic acid, tannin, and catechin contents was also measured. Soaking did not modify the trypsin inhibitor activity, decreased the phytic acid content, and increased the tannin and catechin contents. Cooking the presoaked seed brought about the total removal of trypsin inhibitor activity, a reduction of the phytic acid level, and an increase of the content of tannins and catechins. The trypsin inhibitor activity and the phytic acid content showed a large decrease after 6 days of germination, while amounts of tannins and catechins in the two lentil varieties studied increased. Cooking and germination seem to be good procedures to improve the quality of lentil flour from the nutritional point of view, despite the fact that a large variation on the effects of processing, related to the different legume varieties, has been observed.

Keywords: *Lentils; germination; soaking; antinutritional factors; tannins; phytic acid; trypsin inhibitor activity*

INTRODUCTION

Lentils, as a pulse crop, are a very important component of tropical agriculture and provide a highly nutritious and protein-rich food. Containing about 25% protein, 56% carbohydrate, and 1.0% fat in seeds, lentil is one of the best and cheapest sources of vegetable protein. Although lentils are considered to be one of the most nutritious pulses, they contain several antinutritional factors which could limit their consumption (Salunkhe and Kadam, 1989). Included among these are trypsin inhibitors (TI), which inhibit the proteolytic activity of the digestive enzyme trypsin and can lead to reduced availability of amino acids and reduced growth (Liener and Kakade, 1980). Condensed tannins have been reported to occur in appreciable amount in lentils. They can cross-link with protein by reacting with lysine or methionine, making them unavailable during digestion (Davis, 1981). However, the degree of polymerization of these polyphenolic compounds plays an important role in both the effect on protein digestibility and the availability of vitamins and minerals (Suschetet, 1975). Phytate content in legumes has been involved in reducing the bioavailability of minerals (Deshpande and Cheryan, 1984) and inhibiting the activity of several enzymes (Knuckles et al., 1989).

Removal of undesirable components is essential to improve the nutritional quality of legumes and effectively utilize their full potential as human food. It is widely accepted that simple and inexpensive processing techniques are an effective method of achieving desirable changes in the composition of seeds. Different authors have reported that soaking, cooking, and germination improve the quality of legumes because of the removal of some antinutritional factors. In many instances, usage of only one method may not effect the desired removal of antinutritional compounds and a combination of two or more methods is required.

Soaking could be one of the processes to remove soluble antinutritional factors, which can be eliminated with the discarded soaking solution, but some metabolic reactions can take place during soaking, affecting the content of some compounds (Vidal-Valverde et al., 1992).

Cooking generally inactivates heat-sensitive factors such as trypsin and chymotrypsin inhibitors and volatile compounds. The cooking water may be discarded, but some other soluble compounds could be removed. Bressani and Elias (1980) observed that about 30–40% of polyphenols can be removed from *Phaseolus vulgaris* by cooking and discarding the cooking water solution. Trypsin inhibitory activity (TIA) in dry beans was appreciably destroyed by high temperatures (Antunes and Sgarbieri, 1980), although resistance of trypsin inhibitors to heat has also been reported (Elias et al., 1976).

Germination has been documented to be an effective treatment to remove antinutritional factors in legumes, mobilizing secondary metabolic compounds which are thought to function as reserve nutrients (e.g., phytates and raffinose oligosaccharides). Phytic acid has been suggested to be a source of phosphorus, cations, phosphates, and inositol (Reddy et al., 1978). Germination can lower the phytate content in legume seeds depending upon the type of bean and germinating conditions. In lentils, Belavady and Banerjee (1953) observed 53% phytic acid hydrolyzed after 5 days of germination. Vidal-Valverde and Frias (1992) observed the total

† CSIC.
‡ CIT.
§ Institute of Food Research.

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elimination of raffinose oligosaccharides in lentils after 6 days of germination. The effect of germination on enzyme inhibitor activity remains controversial because different results have been reported, depending on the legume type and the germination conditions.

The aim of the present work was to study the effect of simple processes—soaking, removing the soaking medium; soaking plus cooking discarding the soaking and cooking solutions; and germination—on the antinutritional factors content—trypsin inhibitor, phytic acid, and tannins—in two varieties of lentils widely consumed in Spain to improve the nutritional quality of the final product for human consumption.

EXPERIMENTAL PROCEDURES

Samples. Two varieties of lentils, *Lens culinaris* var. Vulgaris and *L. culinaris* var. Variabilis, were purchased at a local market. The seeds were submitted to the following processes.

Soaking. This process was performed on *L. culinaris* var. Vulgaris seeds in three types of solutions: distilled water, 0.1% citric acid (pH 4.95 ± 0.02), and 0.07% sodium bicarbonate (pH 7.85 ± 0.02). The proportion of seed to soaking medium was 1:3 w/v. The soaking period, 9 h at room temperature, was chosen to obtain maximum seed weight and hydration. The soaking solution was drained, and the soaked seeds were weighed and some ground and freeze-dried.

Cooking. Some of the seeds from the soaking process were boiled for 35 min in distilled water (seed:water ratio 1:6.7 w/v). The cooking liquid and seeds were separated using a strainer and were weighed; the seeds were ground and freeze-dried.

Germination. *L. culinaris* var. Vulgaris and *L. culinaris* var. Variabilis (25 g of seeds) were soaked in distilled water at room temperature and shaken every 30 min. After 6 h, the water was drained and the lentils were transferred to a separating funnel and kept in the dark at 20 °C to germinate for 6 days. Every 24 h the seeds were moistened with distilled water and carefully shaken and drained. The germinated seeds were ground and freeze-dried.

Trypsin Inhibitor Analysis. Trypsin inhibitor activity was determined using the method of Kakade et al. (1974) as modified by An et al. (1993).

Phytic Acid Analysis. The method of Latta and Esking (1980) as modified by Vaintraub and Lapteva (1988) was used to quantify phytic acid in raw and processed lentils.

Tannin Analysis. The condensed tannin content was determined according to the method of Reed et al. (1982) and the catechin content according to the method of Swain and Hillis (1959).

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

RESULTS AND DISCUSSION

The effects of soaking, cooking, and germination on the levels of trypsin inhibitor activity in lentils are shown in Table 1. Soaking lentils in distilled water, 0.1% citric acid solution, or 0.07% sodium bicarbonate solution for 9 h at room temperature did not bring about appreciable changes in the trypsin inhibitor content, although it seems that water and basic soaking solutions caused a slightly larger loss of the trypsin inhibitor activity (11% and 9% reductions, respectively; Table 1) than acid soaking solution (4% loss). This finding agrees with the observation of some other authors. Deshpande and Cheryan (1983) reported a retention of 98–99% of TIA in many cultivars of *P. vulgaris* after the seeds were soaked in water for 18 h. Liu and Markakis (1987) also observed that soaking of soybeans in water at 22 °C for 24 h had no effect on the TIA value.

Table 1. Effect of Different Processes on Trypsin Inhibitor Activity of Lentils (Dry Matter)[a]

variety and treatment	trypsin inhibitor activity	difference	% reduction
L. culinaris var. Vulgaris			
unprocessed	5.3 ± 0.1		
water soaking	4.7 ± 0.2	0.6	11
water soaking + cooking			100
citric acid soaking	5.1 ± 0.1	0.2	4
citric acid soaking + cooking			100
sodium bicarbonate soaking	4.8 ± 0.1	0.5	9
sodium bicarbonate soaking + cooking			
germination	4.1 ± 0.1	1.3	23
L. culinaris var. Variabilis			
unprocessed	6.4 ± 0.1		
germination	4.6 ± 0.1	1.8	28

[a] Values are the mean of four determinations ± standard deviation. Significant differences ($P \leq 0.05$) were found between all values.

In the same way, Dhurandhar and Chang (1990) soaked navy and red kidney beans for 16 h in water at ambient temperature, and both cultivars showed insignificant decreases in TIA. Trugo et al. (1990) also did not find any loss of activity when black beans were soaked in water for 16 h. However, soaking of lentil seeds for 24 h in distilled water resulted in a 58–66% decrease in TIA (Batra et al., 1986). A substantial amount of trypsin inhibitors has also been reported to leach out of Great Northern beans by soaking in acidic or alkaline solutions (Eicher and Satterlee, 1988). In addition, Fernandez et al. (1993) also observed that after soaking faba beans (in distilled water, in 0.1% citric acid solution, and in 0.07% sodium bicarbonate solutions), a decrease in TIA took place, except in the case of citric acid soaking, due probably to the stability of the inhibitor in acidic pH. This is highly in accordance with our results; therefore, it seems that there is a tendency of TI to be more stable in acidic pH.

Cooking lentil seeds (having been presoaked in distilled water, 0.1% citric acid, or 0.07% sodium bicarbonate solutions) for 35 min in distilled water resulted in a 100% loss in trypsin inhibitor activity (Table 1).

The effect of cooking on TIA followed the pattern usually found for other legumes and varieties of lentils: Soaking during 24 h plus cooking for 20 min was necessary for the complete disappearance of TI in mature soybean seeds (Liu and Markakis, 1987). Kadam and Smithard (1987) observed that cooking of presoaked winged beans in boiling water for 30 min was sufficient to inactivate TI. Cooking for 60 min at 100 °C was sufficient to inactivate over 90% of TIA in *P. vulgaris* (Trugo et al., 1990). Mulimani and Paramjyothi (1993) found that TIA was eliminated completely on heating soaked redgram seeds in boiling water for 5 min. TIA of presoaked faba beans decreased significantly after they were cooked 35 min (Fernandez et al., 1993). The same behavior was observed after chickpea seeds were cooked for 40 min (Savage and Thompson, 1993), while Nestares et al. (1993) found a complete destruction of TI after cooking presoaked chickpeas for 35 min. Weder and Link (1993) observed that soaking whole seeds overnight followed by boiling for 2 h almost completely abolished TIA of beans, chickpeas, lentils, and peas. These results show that heat treatment can inactivate completely the action of trypsin inhibitor, potentially improving legume protein digestibility.

Table 2. Effect of Different Processes on Phytic Acid Content of Lentils (Dry Matter)[a]

variety and treatment	phytic acid (mg/g)	difference	% reduction
L. culinaris var. Vulgaris			
unprocessed	6.2 ± 0.1		
water soaking	4.5 ± 0.3	1.7	27
water soaking + cooking	3.8 ± 0.1[a]	2.4	39
citric acid soaking	3.9 ± 0.1[a]	2.3	37
citric acid soaking + cooking	4.2 ± 0.1	2.0	32
sodium bicarbonate soaking	4.8 ± 0.1	1.4	23
sodium bicarbonate soaking + cooking	4.4 ± 0.1	1.8	29
germination	2.1 ± 0.1	4.1	66
L. culinaris var. Variabilis			
unprocessed	8.1 ± 0.1		
germination	4.5 ± 0.1	3.6	44

[a] Values are the mean of four determinations ± standard deviation. The same superscripts in the same column indicate no significant differences ($P \leq 0.05$).

Germination did not bring about the total removal of trypsin inhibitor activity but was found to be more effective at lowering it than soaking treatment (23% and 28% reductions were observed for *L. culinaris* var. Vulgaris and Variabilis, respectively; Table 1).

Many different results regarding the effect of germination on the trypsin inhibitor activity of legumes have been reported. During germination, the TIA decreased in horsegrams, mothbeans (Subbulakshmi et al., 1976), soybeans (Collin and Sanders, 1976; Bates et al., 1977), red kidney beans (El-Hag et al., 1978), vicia faba beans (El-Mahdy et al., 1981), faba beans (Rahma et al., 1987), jack beans (Babar et al., 1988), French beans (Nielsen and Liener, 1988), and chickpeas (Savage and Thompson, 1993). However, TIA was not lost during germination of chickpeas (Khaleque et al., 1985), winged beans (King et al., 1987), navy beans (Chang and Harrold, 1988), vicia faba beans (Ndzondzi-Bokuaogo et al., 1989), and cowpeas (Abudu and Akinyele, 1990). Chang and Harrold (1988) also observed an increase in TIA in pinto beans. Therefore, the effect of germination on TIA remains controversial due to differing results found depending on the legume type and the germination conditions.

There is little information about the effect of germination on TIA in lentils. El-Mahdy et al. (1985) observed a marked reduction in TIA after 24 h of germination and then the rate decreased, which may indicate that these compounds are utilized in the first stage of germination as a source of energy. However, Batra et al. (1986) found that germination for 3 days decreased TIA only slightly, while 6-day germination lowered it substantially (21–54%). More recently, Weder and Link (1993) observed that 72 h of sprouting did not alter the total inhibitor content of lentils.

Our results showed a substantial decrease of TIA (23–28%), which is in good agreement with those of Batra et al. (1986), but they cannot be compared with those of Weder and Lind (1993) because of the different germination times.

The effect of the different processes on the phytic acid content of lentils is shown in Table 2. Soaking the seeds in sodium bicarbonate did not seem to be as efficient as water in reducing the phytic acid content (27% vs 23%), whereas the citric acid solution was more efficient than water alone (37% vs 23%). These results are in agreement with those of Ford et al. (1978), who investigated the removal of phytic acid from soybean concentrates using changes in the pH level, obtaining the highest reduction with a pH level of 5.5. Notwithstanding, our results differ from those of Deshpande and Cheryan (1983), who observed that a greater reduction in phytic acid content was attained by soaking beans in 2% sodium bicarbonate than in distilled water for 12 h, but the difference could be attributed to the different sodium bicarbonate concentrations.

Table 3. Effect of Different Processes on Tannin and Catechin Content of Lentils (Dry Matter)[a]

variety and treatment	tannin (mg/g)	catechin (mg/g)	tannin/ catechin
L. culinaris var. Vulgaris			
unprocessed	3.9 ± 0.4[a]	0.5 ± 0.1	7.8
water soaking	5.9 ± 0.4[b]	1.8 ± 0.1	3.3
water soaking + cooking	6.1 ± 0.4[b]	1.2 ± 0.1[a]	5.1
citric acid soaking	8.0 ± 0.3[c]	1.4 ± 0.1[b]	5.7
citric acid soaking + cooking	7.7 ± 0.6[c]	1.4 ± 0.1[b]	5.5
sodium bicarbonate soaking	5.9 ± 0.3[b]	1.5 ± 0.1[b]	3.9
sodium bicarbonate soaking + cooking	6.9 ± 0.7[d]	1.4 ± 0.1[b]	4.9
germination	5.9 ± 0.4[b]	1.2 ± 0.1[a]	4.9
L. culinaris var. Variabilis			
unprocessed	3.9 ± 0.6[a]	0.4 ± 0.1	9.7
germination	6.2 ± 0.6[b,d]	1.0 ± 0.1	6.2

[a] Values are the mean of four determinations ± standard deviation. The same superscripts in the same column indicate no significant differences ($P \leq 0.05$).

The cooking of soaked seed with distilled water reduced further the phytic acid content in lentils. This same effect was observed by Khan et al. (1988) using chickpeas and by Sievwright and Shipe (1986), who found that black beans lost 17–27% of their phytate content after 24 h of soaking and 30 min of cooking. When the bicarbonate soaking solution was used, boiling in water also decreased the phytic acid content (29% vs 23%). However, when the soaking was performed with citric acid, the subsequent cooking slightly increased this content.

Germination is the most effective process for the reduction of phytic acid content in legumes. These losses may be attributed to the activity of the enzyme phytase. Phytic acid serves as an important reserve of phosphate generated by the action of phytase during seed germination for the developing seedling. Reddy et al. (1978) noted that phytic acid was hydrolyzed during germination, resulting in an increase in available inorganic phosphorus. In our case, it is evident that germination reduces phytic acid content (66% and 44%) at a higher rate than soaking or cooking.

The contents of tannins and catechins in raw and processed lentils are collected in Table 3. Both varieties of lentils (*L. culinaris* var. Vulgaris and Variabilis) investigated here had similar initial tannin and catechin contents. However, both samples had a brown seed coat, and the seed coat color of lentil has been associated with tannin content (Nozolillo and de Bezeda, 1984).

Soaking *L. culinaris* var. Vulgaris in either distilled water, 0.1% citric acid, or 0.07% sodium bicarbonate solutions brought about a large increase in the amount of the two types of phenolic compounds studied (Table 3), the citric acid soaked seeds showing the highest increase. After the soaking process, tannins, which are originally placed in the interior of the cell, could move, being more accessible to subsequent analysis.

On the other hand, tannins can be linked to proteins, carbohydrates, and vitamins, forming more or less

stable complexes depending on their degree of polymerization. Soaking and cooking processes liberate these complexes, therefore, the tannin levels increase. This coincides with the observed decrease of α-galactosides (Vidal-Valverde et al., 1992) because these compounds are more soluble than tannins.

The largest increase in the tannin content was found after acid soaking. pH represents a very important factor in the stability of these complexes, being more stable at basic pH (Ya et al., 1989; Haslam, 1993). These results are in accordance with those of Fernandez et al. (1993), who reported an increase in the tannin and catechin contents after soaking faba beans in distilled water, 0.1% citric acid, and 0.07% bicarbonate solutions.

Cooking lentil seeds (having been presoaked in distilled water, 0.1% citric acid, and 0.07% sodium bicarbonate solutions) for 35 min in distilled water resulted in a 100% retention of the phenolic compound studied compared to those for soaked seeds (Table 3).

Tannins in lentil seed show a high degree of polymerization (tannin/catechin ratio) similar to that observed by Bartolome et al. (1994) and higher than observed in faba beans and chickpeas (Fernandez et al., 1993; Nestares et al., 1993). The degree of polymerization decreased significantly as a consequence of processing. The protein affinity of tannins has been shown to decrease together with the degree of polymerization (Hagerman, 1992).

Because of that reason, the catechin content increases after all types of processing and, contrary to tannins, decreases when presoaked seeds are cooked, although no large differences were found in the case of the basic soaking solution.

Tannin and catechin contents in lentils (*L. culinaris* var. Vulgaris and Variabilis) were also analyzed after 6 days of germination. Germination brought about an increase of tannin content for both varieties (152% and 162% retention percentages were observed, respectively). Catechin content showed an even larger increase, reaching a level twice that of the raw seed. The tannin/catechin ratio decreased after germination for both varieties, which suggests a smaller condensation of the phenolic compounds as a consequence of germination. Germination could make tannins move in the same way that soaking and cooking do, and the fact that these compounds are thermo- and photochemically labile makes them more easily accessible to the analysis.

In conclusion, although a large variation in the effect of processing has been observed in lentils, cooking and germination are recommended treatments at both domestic and industrial scales to prepare good quality lentil flour for human nutrition uses.

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