Influence of Processing on Trypsin Inhibitor Activity of Faba Beans and Its Physiological Effect

Concepción Vidal-Valverde,*,† Juana Frias,† Concepción Diaz-Pollan,† Mar Fernandez,‡ Maria Lopez-Jurado,‡ and Gloria Urbano‡

Instituto de Fermentaciones Industriales, Juan de la Cierva 3, 28006 Madrid, Spain, and Departamento de Fisiologia, Instituto de Nutrición, Facultad de Farmacia, Universidad de Granada, Campus Universitario de Cartuja, s/n 18071, Granada, Spain

Changes in the trypsin inhibitor activity (TIA) of faba beans (*Vicia faba* L. Major) were investigated after beans were soaked in distilled water, citric acid, and sodium bicarbonate solutions. The soaking solution was discarded. The effect of cooking—discarding both soaking and cooking solutions—was also studied. Finally, a dry-heating process was examined. Soaking treatment produced a slight decrease of trypsin inhibitor activity. Cooking the presoaked seeds brought about the total or partial removal of TIA, depending on the soaking solution used. TIA was partially removed after dry-heating. Trypsin inhibitor activity analyses were completed with 28-day biological trials for raw and dry-heated faba beans. For 21 and 28 days of experimental time food intake, protein intake, and weight gain were similar for rats fed raw and dry-heated faba beans. However, after 28 days protein efficiency ratio (PER) and food transformation index (FTI) improved in rats fed dry-heated faba beans compared with those fed raw faba beans. This was related to lower TIA values found in dry-heated faba beans.

Keywords: *Faba beans; trypsin inhibitor activity; soaking; cooking; dry-heating; protein efficiency ratio; food transformation index*

INTRODUCTION

Faba beans are a good source of protein of adequate nutritional quality. They provide sufficient quantities of essential amino acids, except for cysteine and methionine, to ensure normal development in growing rats, according to values recommended by the National Research Council (1978). However, with rations containing high levels of untreated faba bean meal, slow growth rates have been reported in rats (Marquardt and Campbell, 1974; Moseley and Griffiths, 1979; Martinez and Larralde, 1984). This has been attributed to antinutritional constituents such as α-galactosides and tannins (Fernandez et al., 1993) and the low content in some essential amino acid of faba bean protein (Marquardt et al., 1976; Huyghebaert et al., 1979; Bond, 1980; Boulter, 1982; Fernandez et al., 1996).

The low digestibility of legume proteins has been related to the presence of protease inhibitors, which are widely distributed in legumes (Savage, 1988). There is some speculation about the role of these compounds in plants. Some authors have suggested that protease inhibitor compounds act as defense tools against microorganisms and insects (Ryan, 1979; Richardson, 1981; Brown et al., 1985; Graham et al., 1985a,b). Other authors have demonstrated that some of these compounds are seed protective factors against endogenous proteolytic enzymes, and it has been suggested that protease inhibitors could exist as inactive enzyme-inhibitor complexes in plants (Preston and Kruger, 1976).

Protease inhibitors have the ability to inhibit the proteolytic action of certain enzymes. It has been proven that their presence in legume seeds led to a depletion of growth and alteration of the pancreatic function in rats (Krogdahl and Holm, 1979; Struthers et al., 1983; Bhatty and Christison, 1984; Sitren et al., 1985). In general, trypsin inhibitors are low molecular weight proteins formed by association of identical peptide chains of smaller size. These chains have between 1 and 8 disulfide bonds and, in general, they are devoid of carbohydrates (Mueller and Weder, 1990). Protease inhibitors from different sources have diverse structures, molecular weights, and chemical compositions; however, the amino acid sequence of the active sites is quite similar (Richardson, 1977; Liener, 1994). There are some other substances which inhibit digestive enzymes, such as tannins (Price and Buttler, 1980; Quesada et al., 1996) and several indigestible polysaccharides (Mercier, 1979; Price et al., 1988), although the contribution of these compounds to the total trypsin inhibitor activity (TIA) in most foods may be minimal (Ikeda and Kusano, 1983).

Levels of trypsin inhibitors in faba bean seeds seem to be lower than in other legumes such as soybeans (Valdebouze et al., 1980), chickpeas (Soni et al., 1978; Diaz-Pollan et al., 1997), and lentils (Vidal-Valverde et al., 1994). Simple and inexpensive methods have been suggested to remove trypsin inhibitors totally or partially from legume seeds (Antunes and Sgarbieri, 1980; Weder and Link, 1993; Vidal-Valverde et al., 1994). However, there is little information on changes in TIA of processed faba beans and its implication on their nutritive value. The present paper reports how simple processes, such as soaking in different solutions, cooking after the soaking process (discarding both soaking and cooking solutions), and dry heating affect TIA values. Long-term biological trials (28 days) were carried out with raw and dry-heated faba beans in order to correlate the modifications of TIA with nutritive value of faba

* Author to whom correspondence should be addressed.

† Instituto de Fermentaciones Industriales.

‡ Departamento de Fisiologia.

Table 1. Trypsin Inhibitor Activity of Raw and Processed Faba Beans

treatment	TIA[a] (TI units/mg dm)	% reduction
raw faba bean	2.62 ± 0.17[a]	
soaked in		
water	2.11 ± 0.28	19
citric acid 0.1%	2.77 ± 0.28[a]	
sodium bicarbonate 0.07%	1.98 ± 0.28	24
cooked in water after being soaked in		
water	ND	100
citric acid 0.1%	ND	100
sodium bicarbonate 0.07%	0.44 ± 0.03	83
dry-heated	0.81 ± 0.16	69

[a] The same superscript in the same column indicates no significant differences ($p \leq 0.05$). Values are the mean of four determinations ± standard deviation. ND: not detected.

bean, expressed as protein effeciency ratio (PER) and food transformation index (FTI).

MATERIALS AND METHODS

Samples. Faba bean seeds (*Vicia faba* L. Major) were purchased at a local market. The seeds were submitted to the following treatments.

Soaked Beans. Faba bean seeds were soaked in distilled water, 0.1% citric acid (pH 4.94 ± 0.02) and 0.07% sodium bicarbonate (pH 7.85 ± 0.02) for 9 h in the dark. The proportion of seed to soaking medium was 1:3 w/v. After this period, the soaking liquid was drained, and the soaked seeds were weighed, ground, and freeze-dried.

Cooked Beans. 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 the seeds were weighed, ground, and freeze-dried.

Dry-Heated Beans. Raw ground faba bean seeds were dry heated in an autoclave at 120 °C and 1 atm for 15 min. After this time, the legume flour was freeze-dried.

Chemical Analysis. Trypsin inhibitor activity was determined according to the method of Kakade et al. (1974), as modified by Valdebouze et al. (1980).

Biological Methods. *Diets.* Raw and dry-heated faba beans containing 4.5 and 4.1 mg of nitrogen per 100 g of diet (dm), respectively, as analyzed by Kjeldahl method (Fernandez et al., 1996), were used for the biological studies in rats. The findings of these experiments were compared with those obtained for the control diet (casein + 0.3% D,L-methionine; 3.2 mg of nitrogen/100 g of diet) (AIN, 1977).

Experimental Design. A total of 30 rats were divided into 3 groups of 10 animals. Two groups were fed either raw or dry-heated faba bean diets, and the third group was fed the control diet.

Animals. The animals were 4-week-old (recently weaned) Wistar albino rats with an initial body weight of 55 ± 5 g, reared in the University of Granada Animal Services Laboratory. The animals were divided into groups of 10 rats each (5 male, 5 female), which were housed in individual metabolic cages designed for the separate collection of feces and urine. The cages were located in a well-ventilated, thermostatically controlled room (21 ± 2 °C) with 12 h light/dark period (light on at 9:00 a.m.). A period of 3 days was allowed for adaptation to the diet, followed by either 14, 21, or 28 days experimental period. Food intake (the total amount consumed daily by each rat determined by weighing the amounts of diet given, refused, and spilled) and body weight were recorded at the beginning and weekly to the end of the experimental periods. Throughout the experimental period all rats had free access to double-distilled water and the diet was consumed *ad libitum*.

Biological Indexes. The following indexes and parameters were determined for each group: food intake (expressed as dry matter), protein intake (N × 6.25), (Fernandez et al., 1996), weight gain, PER, corrected protein efficiency ratio for each diet (CPER), FTI, and corrected food transformation index for each diet (CFTI). Food intake, protein intake, and body weight

$$\text{food intake} = \frac{\text{final diet wt} - \text{initial diet wt}}{\text{no. of days}}$$

$$\text{wt gain} = \frac{\text{final rat wt} - \text{initial rat wt}}{\text{no. of days}}$$

$$\text{PER} = \frac{\text{wt gain}}{\text{protein intake}}$$

$$\text{CPER} = 2.5 \times \frac{\text{PER (for each diet)}}{\text{reference casein PER}}$$

$$\text{FTI} = \frac{\text{food intake}}{\text{wt gain}}$$

$$\text{CFTI} = 100 \times \frac{\text{FTI (for each diet)}}{\text{reference casein diet FTI}}$$

were expressed as (g/rat)/day.

Statistical Methods. Multifactor analysis of variance was applied to the data using Statgraphics Statistical Graphics 5.0 System Software (Statistical Graphics Corporation, Rockville, MD) with an IBM Personal System/2 Model 20 Computer (International Business Machines Corp., U.K.).

RESULTS

Chemical Analysis. Table 1 summarizes TIA values in raw and processed faba beans. The TIA level of raw faba beans was 2.6 TI units/mg (dm). Soaking faba beans in distilled water and 0.07% sodium bicarbonate solution resulted in a 19 and 24% TIA reduction, respectively. Soaking seeds in 0.1% citric acid, however, did not cause significant changes ($p \leq 0.05$) in the values of faba bean TIA compared with those for raw seeds.

Cooking faba been seeds (having been presoaked either in distilled water or acidic solution) for 35 min in distilled water resulted in a 100% TIA loss. When cooking was carried out after the seeds were soaked in sodium bicarbonate, however, only a 83% TIA loss was obtained. In addition, dry-heating resulted in less reduction of TIA; a 69% loss was observed.

Biological Analysis. Table 2 shows food intake, protein intake, and weight gained results obtained from the biological assays carried out with animals fed raw and dry-heated faba beans for 14, 21, and 28 days. Casein diet was used as a control.

During the whole experimental time, food intakes (expressed as grams of diet per animal per day) of rats fed raw and dry-heated faba beans were lower than those fed the casein control diet. Comparing both faba bean diets, during the first 14 days a lower food intake was found for dry-heated faba beans, while no significant differences ($p \leq 0.05$) were found when the time was extended to 21 and 28 days (Table 2).

When food intake was expressed as g/100 g of animal weight per day for 21 days, no significant differences were found ($p \leq 0.05$) among results obtained for control and various faba bean diets. After 28 days, lower food intakes for both raw and dry-heated faba beans were obtained compared with those for the casein control diet.

Protein intake obtained for faba bean diets was as expected, considering their food intake and protein content (Table 2).

The weight gain was lower for animals fed raw and processed faba beans than for those animals fed a control diet throughout the 28-day experimental time.

Table 2. Food Intake, Protein Intake, Weight Gain, Protein Efficiency Ratio (PER), Corrected Sample PER (CPER), Food Transformation Index (FTI), and Corrected Sample FTI (CFTI) in Rats Fed Raw and Dry-Heated Faba Beans for 14, 21, and 28 Days[a]

diets	food intake (g/rat/day)	protein intake (g/rat/day)	food intake (g/100 g rat weight/day)	wt gain (g/rat/day)	PER	CPER	FTI	CFTI
control								
1−14 days	12.20 ± 0.17[a]	2.53 ± 0.03[a]	11.90 ± 0.14[b]	4.40 ± 0.15[a]	1.74 ± 0.04		2.78 ± 0.07[ab]	
1−21 days	12.30 ± 0.17[a]	2.54 ± 0.04[ab]	10.90 ± 0.16[c]	3.90 ± 0.14	1.55 ± 0.04[a]		3.13 ± 0.08[ab]	
1−28 days	13.80 ± 0.19	2.86 ± 0.04[a]	10.80 ± 0.18[c]	4.40 ± 0.24[a]	1.52 ± 0.07[a]		3.24 ± 0.17[b]	
raw faba beans								
1−14 days	9.30 ± 0.22[b]	2.40 ± 0.06[a]	12.10 ± 0.23[b]	1.90 ± 0.17[b]	0.79 ± 0.06[c]	1.13 ± 0.08[b]	5.20 ± 0.38[c]	187.00 ± 13.60[b]
1−21 days	8.60 ± 0.24[c]	2.22 ± 0.06[bc]	10.80 ± 0.19[c]	1.60 ± 0.23[b]	0.73 ± 0.09[c]	1.18 ± 0.14[b]	5.40 ± 0.37[c]	172.50 ± 11.95[b]
1−28 days	8.10 ± 0.26[c]	2.11 ± 0.06[c]	10.00 ± 0.16[d]	1.10 ± 0.12[c]	0.51 ± 0.04	0.84 ± 0.06	7.82 ± 1.01	241.38 ± 31.18
dry-heated faba beans								
1−14 days	8.50 ± 0.38[c]	2.39 ± 0.12[ab]	11.30 ± 0.49[bc]	1.60 ± 0.04[bc]	0.71 ± 0.04[c]	1.01 ± 0.06[bc]	5.20 ± 0.22[c]	186.30 ± 8.05[bc]
1−21 days	8.30 ± 0.27[c]	2.09 ± 0.19[c]	10.40 ± 0.33[cd]	1.40 ± 0.13[c]	0.66 ± 0.04[cd]	1.06 ± 0.07[bc]	5.70 ± 0.35[c]	182.20 ± 11.33[bc]
1−28 days	7.90 ± 0.34[c]	2.14 ± 0.09[c]	9.60 ± 0.39[d]	1.30 ± 0.08[c]	0.60 ± 0.04[d]	0.98 ± 0.05[c]	6.32 ± 0.29	195.09 ± 8.99[c]

[a] Values are means ± standard error of 10 Wistar rats. The same superscript in the same column indicates no significant differences ($p \leq 0.05$).

Table 3. Weekly Protein Intake, Weight Gain, and PER in Animals Fed with Raw and Dry Heated Faba Beans

diets	wt gain (g/rat/day)	protein intake (g/rat/day)	PER
control			
second week (7−14 days)	3.58 ± 0.15	2.56 ± 0.05[a]	1.39 ± 0.05[a]
third week (14−21 days)	3.00 ± 0.17[b]	2.54 ± 0.05[a]	1.16 ± 0.05[b]
fourth week (21−28 days)	5.69 ± 0.66[a]	3.84 ± 0.13	1.48 ± 0.16[a]
raw faba beans			
second week (7−14 days)	1.04 ± 0.26[c]	2.36 ± 0.07[a]	0.41 ± 0.10[c]
third week (14−21 days)	0.36 ± 0.19	1.86 ± 0.08[b]	0.16 ± 0.11[d]
fourth week (21−28 days)	0.21 ± 0.15	1.78 ± 0.07[b]	0.10 ± 0.09[d]
dry-heated faba beans			
second week (7−14 days)	1.07 ± 0.13[c]	2.48 ± 0.08[a]	0.41 ± 0.05[c]
third week (14−21 days)	0.66 ± 0.21[d]	2.04 ± 0.08[c]	0.32 ± 0.08[c]
fourth week (21−28 days)	0.64 ± 0.24[d]	2.11 ± 0.07[c]	0.41 ± 0.11[c]

[a] Values are means ± standard error of 10 Wistar rats. The same superscript in the same column indicates no significant differences ($p \leq 0.05$).

During the first 14 days, weight gain for animals fed raw and dry heated faba beans was 60% lower than for those animals fed a control diet (Table 2). This situation was even more acute when the experimental time was extended, and differences of 75 and 70% after 28 days for raw and dry-heated faba beans, respectively, were found.

Table 2 also shows PER, CPER, FTI, and CFTI for animals fed raw and dry-heated faba beans, as well as the control diet for 28 days. Animals fed both faba bean diets showed lower PER values than those fed a control diet. Throughout the experimental time of faba bean diets, PER decreased gradually, reaching a plateau after 21 days, with the exception of the continual decrease for those animals fed raw faba beans. When CPER was calculated for animals fed raw and dry-heated faba beans, no significant differences ($p \leq 0.05$) were found after 14 and 21 days. After 28 days, a different situation was found, since a higher CPER for animals fed dry-heated faba beans was obtained (Table 2).

Food transformation indices (FTI) were higher (worse) for animals fed raw and dry-heated faba beans than those calculated for the control diet (Table 2). For the same experimental time, there were no significant differences ($p \leq 0.05$) between FTI values calculated for those rats fed raw and processed faba beans, except in experiments performed for 28 days, where FTI levels for dry-heated faba beans improved compared with those for raw faba beans. Similar results were obtained when CFTI were calculated (Table 2).

Table 3 shows weight gain, protein intake, and PER values obtained weekly for rats fed control, raw, and dry-heated faba bean diets. Weight gain was lower for animals fed either raw or processed faba beans compared with those found for the casein diet. Comparing both faba bean diets, no significant differences in weight gain ($p \leq 0.05$) were found during the second week (7−14 days), while for the third (14−21 days) and fourth (21−28 days) weeks larger weight gains for animals fed dry-heated faba beans were found.

Protein intake for faba bean diets was similar to that found for the control during the second week. After the third and the fourth week, protein intake for rats fed faba beans was lower than for control diet (Table 3). Comparing both faba bean diets after the third and fourth weeks, larger protein intakes in animals fed dry-heated faba beans were found.

PER calculated weekly showed that during the second week no significant differences ($p \leq 0.05$) in PER for animals fed raw or processed faba beans were obtained; however PER was lower than in those fed a control diet. For the third and fourth week, PER was much higher for animals fed dry heated faba beans than in those fed raw seeds (Table 3).

DISCUSSION

TIA values have been reported for a large range of legume seeds. Soybean has been extensively studied and used as reference, showing a 16.5−29 unit range (Collins and Sanders, 1976). TIA values for raw faba bean seeds found here agree with those reported in the literature by Soni et al. (1978) and Hove and King (1979), who indicated TIA values of raw faba bean between 1.60 and 2.30 TI units/mg (dm). For faba bean seeds, Valdebouze et al. (1980) found TIA values ranging from 4.1 to 4.5 TI units/mg (dm) and Arntfield et al. (1985) reported values of 5.9 TI units/mg (dm), results which depend on the type and variety of the legume studied.

It is well known that the presence of trypsin inhibitors in the diet leads to the formation of the irreversible trypsin enzyme–trypsin inhibitor complex, causing a trypsin drop in the intestine and a decrease in the diet protein digestibility, leading to slower animal growth. Under this situation, the organism increases the secretory activity of the pancreas which could cause pancreatic hypertrophy and hyperplasia. Since pancreatic enzymes such as trypsin and chymotrypsin are particularly rich in the sulfur-containing amino acids, pancreatic hypertrophy and/or hyperplasia diverts these amino acids from the synthesis of body tissue protein to the synthesis of these enzymes. This loss in sulfur-containing amino acids exacerbates an established critical situation with respect to legume protein, which is inherently deficient in these amino acids, causing a significant reduction in growth (Liener and Kakade, 1980).

Heat treatments partially or completely inactivate TIA in legumes. A soaking procedure usually is a prior step to legume cooking in which aroma and palatability are enhanced. Some authors have reported that soaking hardly decreases TIA in kidney beans (Deshpande and Cheryan, 1983; Dhurandhar and Chang, 1990), soybeans (Liu and Markakis, 1987), black beans (Trugo et al., 1990), and faba beans (Sharma and Segal, 1992). Conversely, a substantial amount of trypsin inhibitors has been reported to leach out of lentils soaked in distilled water (Batra et al., 1986; Vidal-Valverde et al., 1994), kidney beans in water and salt solutions (Sathe et al., 1984), and Great Northern beans soaked in acidic and alkaline solutions (Eicher and Saterlee, 1988). Consequently it seems very important to discard the soaking solution. In addition, some metabolic reactions take place during the soaking procedure (Vidal-Valverde et al., 1992). Our results show that only when faba bean seeds were soaked in citric acid solution no modification of TIA levels was found; that is in agreement with Iyer et al. (1980). These authors pointed out that acid pH causes hardness of the seed coat during soaking–hydrating conducting to a less diffusion of some compounds such as those with trypsin inhibitor activity to the soaking liquid. Similar results were obtained in soaked lentil seeds; the stability of lentil protease inhibitors in acidic solution was shown (Vidal-Valverde et al., 1994).

Although most compounds with protease inhibitor activity are heat-labile, it has been found that the thermostability of TI in legumes varies not only with legume source but also with the different conditions used during processing, such as pH, humidity, time, temperature, and pressure (Marquardt et al., 1976; Ellenrieder et al., 1980; Padhye and Salunkhe, 1981; Trugo et al., 1990; Ziena et al., 1991; Mulimani and Paramiyothi, 1993; Savage and Thompson, 1993; Weder and Link, 1993; Vidal-Valverde et al., 1994; Urbano et al., 1995; Diaz-Pollan et al., 1997). In faba beans, several authors have reported that cooking and autoclaving produce larger TIA reductions than dry heating (Gallardo et al., 1974; Griffiths, 1983; Khalil and Mansour, 1995). However, Kozlowska et al. (1990a,b) found total inactivation of trypsin inhibitors isolated from faba bean seeds after autoclaving.

Heat treatments seem to improve legume protein digestibility which is attributed to the removal of the heat-labile antinutritional constituents (Sathe and Deshpande, 1989). Nestares et al. (1993) reported that the absense of TIA after cooking of soaked chick peas in water, acidic, and alkaline solutions could be correlated with an increment in the apparent digestibility coefficient (ADC), while dry heating caused only a 27% decrease in TIA, which was not enough to increase ADC. These results were related with high TIA levels found in raw chickpea seeds (10 TI units/mg dm). Ndzondzi-Bokuango et al. (1989) showed that cooking caused a marked TIA decrease in faba beans which improved PER in rats. Fernandez et al., (1996) reported that ADC value for rats fed raw faba beans was 82%, which decreased to 75%, 76%, and 79% when seeds were soaked in distilled water, citric acid, and sodium bicarbonate, respectively. Cooking the previously soaked faba beans and the dry-heated faba beans resulted in ADC values between 80 and 81%, data very similar to the raw faba beans. Comparing these ADC values with the results of TIA obtained here for the same set of samples did not show any relationship, since TIA content was not modified or slightly decreased only after soaking, TIAs were completely eliminated after presoaked seeds were cooked in either water or citric acid, and TIAs in dry-heated faba beans decreased to 0.81 TI units/mg dm. Fernandez et al. (1996) did not find differences in PER (calculated for 7 days) for animals fed raw or dry-heated faba beans. For this reason it was decided to continue the biological trial up to 28 days.

After 28 days the food intake decreased for rats fed raw faba bean and dry-heated faba bean diets in relation with that found for the control casein (Table 2). This could be explained by the fact that faba bean diets are poor in some essential amino acids. Several authors have described that the imbalanced amino acid composition in the diet causes a marked reduction of food intake (Leung and Rogers, 1969; Harper et al., 1970; Peter and Harper, 1985) that could be due to sharp nonspecific changes in the blood and brain amino acid profiles (Harper et al., 1970; Peng et al., 1975). However, in this study the food intake was similar for both faba bean diets.

When the biological analyses were expressed on a weekly basis (Table 3), it was found that once biological trials were extended for 21 and 28 days, higher PERs for dry-heated faba beans were obtained compared to those for raw faba beans. These facts could be justified because the lower TIA levels found in dry-heated faba beans could make the animals better utilize the diet protein, causing a greater weight gain for the same amount of food intake.

In conclusion, TIA levels in raw faba bean seeds were quite low (2.6 TI units/mg dm) and dry-heating caused a sharp drop in TIA (69%), although it was not enough to modify apparent digestibility coefficient levels in rats, but it was reflected in the improvement of PER and FTI for longer periods of time.

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