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JULITTA BOROWSKA
HALINA KOZŁOWSKA

CHANGES IN ACTIVITY OF TRYPSIN INHIBITORS IN BEAN (*PHASEOLUS VULGARIS*) AND PEA SEEDS (*PISUM SATIVUM*) DURING HEAT TREATMENT

Institute of Food Engineering and Biotechnology, Agricultural and Technical University, Olsztyn

Key words: pea seeds, bean seeds, thermal inactivation of trypsin inhibitor.

The influence of the process of cooking and sterylization on changes in the activity of trypsin inhibitors in been and pea seeds, was examined. The favourable effect of inactivation of the inhibitors, expressed in a decrease of their activity to 90%, was established.

INTRODUCTION

The seeds of leguminous crops are one of the richest sources of vegetable protein [7, 12]. The content of protein in the dry seeds of leguminous plants amounts to 16-44% and it is, in the average, twice higher than in the grains of cereals [7, 10].

Among the leguminous plants, soya has become the most important on a world-wide scale. However, its cultivation in our geographic conditions, is fairly difficult and for this reason, pea and bean are a subject of growing interest. These seeds, similarly to soya, should have a wider application as a food component of a high nutritive value of protein.

They are especially rich in lysine and poor in sulphuric amino acids [7]. Besides the deficiency of sulphuric amino acids, the nutritive value of protein in these seeds is influenced by substances which are the inhibitors of trypsin [5, 6, 11].

The majority of studies on the elaboration of method for the inactivation of trypsin inhibitors concerned soya [2, 4, 5]. Much less investigations were conducted on other seeds of leguminous plants. This has become an incentive for undertaking studies on the changes which take place in these biologically active compounds, during the preparation of meals from pea and bean seeds.

MATERIAL AND METHODS

Seeds of bean variety: "Biała Wyborowa" and "Igołomska 180" and pea variety "Kujawski Wczesny", were used for the experiments.

The seeds are analysed after cooking or sterilization. They were cooked in the following ways:

— Whole seeds (10 g) or those granulated into particles 0.15 mm in size (2 g) were placed in a 250 cc beaker and poured with 50 cc distilled water at room temperature or with a solution of 1% or 2% sodium chloride. The time of heating the samples under cover till the moment of boiling was 10 minutes.

The samples were boiled for 5, 15, 30 and 60 minutes. After cooling them down to room temperature, the samples were homogenized in a mixer and supplemented with water to 100 cc.

— Whole seeds (10 g) were poured with distilled water (50 cc) and allowed to stand at room temperature (±20°C) for 10 hours. After this time, water was poured out and bean was cooked in the way described above.

— Preserves made from bean and pea seeds were prepared in the following way:

Dry seeds in an amount of 50 g were placed in 150 g cans and poured with 120 cc water or 3% NaCl solution. After closing, the cans were sterilized in an autoclave. Cans containing bean in water or salt solution were sterilized for 15, 30 and 60 minutes at 110, 120 and 130°C and those containing pea — only for 30 minutes at 120°C. Before the starting of analyses, cans were stored in a freezer at +5°C.

The sample for analysis was prepared by thoroughly granulating the whole content of the can in a mincer and then homogenizing it in a mixer.

In the prepared samples for analysis, the trypsin inhibitor activity was determined by the method of Kakade et al. [3], using N^a-Benzoyl-DL-arginin-4-nitroanilidihydrochloride (BAPA) as a substrate.

RESULTS AND DISCUSSION

The anti-trypsin activity in the seeds under investigation was distinctly differentiated. The highest activity was found in the "Igołomska" bean variety (50.37 TUI/mg DM); a smaller activity in bean variety (Biała Wyborowa" (13.97 TUI/mg DM) and lowest in pea variety "Kujawski Wczesny" (4.39 TUI/mg DM). The trypsin inhibitor activities established in the material examined, except for bean "Igołomska", were distinctly weaker than those found in soya seeds where their activity usually exceeds 60 TUI/mg DM [4, 11].

INFLUENCE OF COOKING ON CHANGES IN TRYPSIN INHIBITOR ACTIVITY

During the cooking of bean, the greatest quantitative changes were observed already after 5 minutes (Fig. 1). The decrease in activity of bean "Biała Wyborowa" was 78% and of "Igołomska" almost 90%. Longer cooking caused further decrease but it was small in comparison with the decrease observed after 5 minutes.

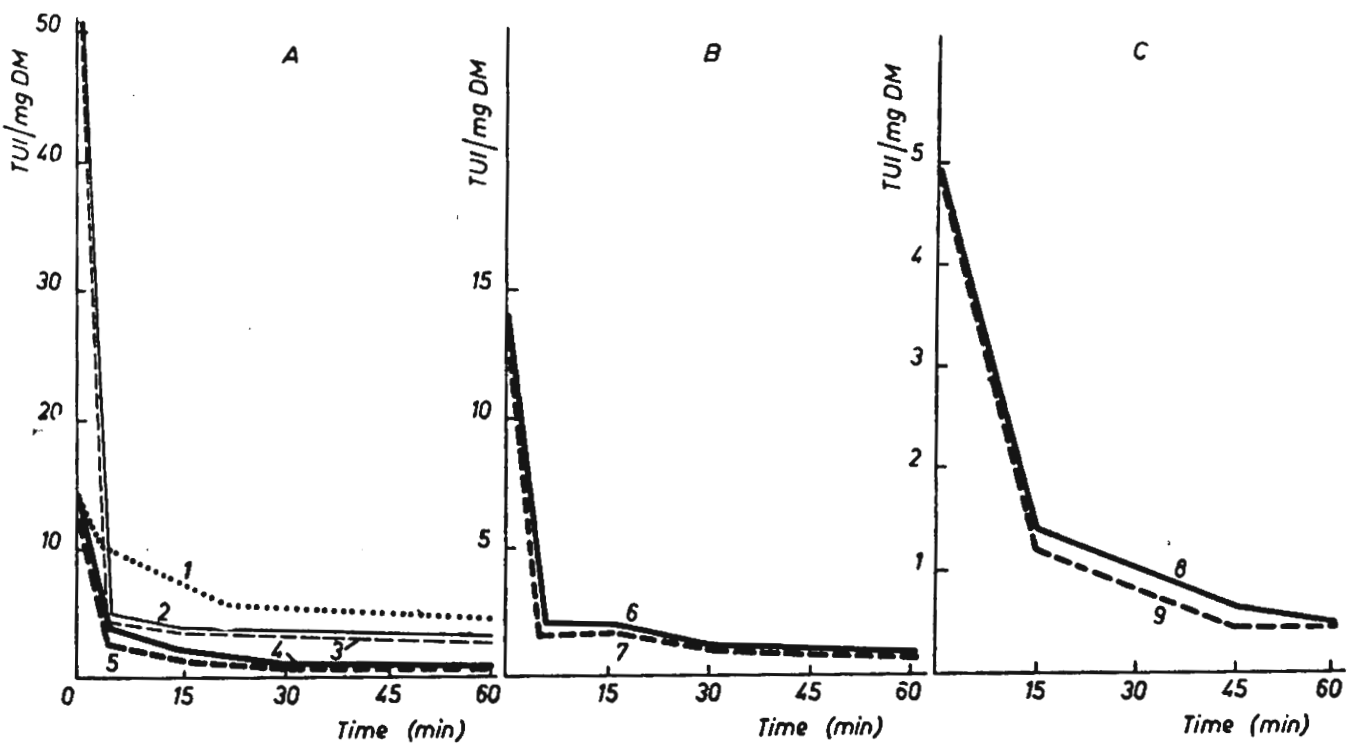

Fig. 1. Changes in trypsin inhibitor activity in the seeds of bean and pea under the influence of cooking; A: 1 — meal from Biała Wyborowa bean, 2 — seeds of Igołomska 180 bean, 3 — seeds of Igołomska 180 after soaking, 4 — seeds of Biała Wyborowa bean, 5 — seeds of Biała Wyborowa after soaking; B: 6 — Biała Wyborowa bean in 1% NaCl, 7 — Biała Wyborowa bean in 2% NaCl; C: 8 — meal from pea, 9 — seeds of pea

After 1 h cooking, in the seeds of each variety, there was about 6% activity left in spite of the fact that before cooking, the seeds had a very differentiated level of trypsin inhibitors. Soaking of seeds before cooking did not give the expected result in the form of a quicker decrease of activity during cooking in spite of such suggestion available in literature [12]. During the cooking of meal made from seeds of the "Biała Wyborowa" bean, the changes in trypsin inhibitor activity had a slightly different course (Fig. 1 A). In this case, the changes in activity were slower than in intact seeds and after 60 minutes of cooking, the activity amounted to 2.84 TUI/mg DM which was 20% of the activity of theuncooked meal. Similar observations were made by Soviet researches [1] who observed the smallest destruction of inhibitors in case of heating the meal. A separate analysis of bean alone after cooking and of bean with the decoction,

gave higher results in the latter case (Table 1). This shows that part of protein having the property of inhibiting the activity of trypsin, passed to the solution. Cooking of seeds in 1 or 2% salt solution did not cause any greater decrease in the activity of the inhibitors in comparison with the results obtained after cooking in water (Fig. 1 B). Only minimally higher results were obtained in case of cooking bean in 2% salt solution.

Table 1. Changes in trypsin inhibitor activity during the cooking of bean seeds „Biała Wyborowa" and „Igołomska 180"

Variety	Time min.	Bean without decoction		Bean with the decoction	
		TUI/mg DM	%	TUI/mg DM	%
Biała Wyborowa	0	13.97	100.00	13.97	100.00
	15	1.70	12.19	1.96	14.01
	30	0.87	6.25	1.10	7.84
	60	0.75	5.38	0.87	6.25
Igołomska 180	0	50.37	100.00	50.37	100.00
	15	3.57	7.09	3.82	7.78
	30	3.02	5.99	3.52	6.98
	60	2.83	5.62	3.18	6.32

For the sake of comparison, the seeds of pea "Kujawski Wczesny" characterized by the lowest activity and the meal obtained from it, were cooked in water. In this experiment, the first analysis was made after 15 minutes and a significant decrease in activity, amounting to 77% in whole seeds and 71% in meal, was observed. A prolongation of the cooking time caused a further lowering of activity and after 60 minutes this activity was similar both in seeds and in meal and it amounted to about 9% as compared to the material before cooking (Fig. 1 C).

Similar tendencies of decrease in trypsin-inhibitor activity during the heating of water extracts of legumious crops seeds were found by Soviet investigators [1]. They stated also that in order to obtain a similar effect, expressed in an 80% decrease of activity, the longest time was required by water extracts of bean. It has been also pointed out that the rate of inactivation depends on the physicochemical properties of inhibitors, whose character is different and is related not only to the species of the plant but also to its variety [1, 3].

THE INFLUENCE OF STERILIZATION ON CHANGES IN TRYPSIN INHIBITOR ACTIVITY

Besides cooking the studies revealed the significant influence of sterilization, on the lowering of the trypsin inhibitor activity of inhibitors present in bean seeds (Fig. 2).

Sterilization of bean in water (Fig. 2) showed that the decrease in activity was the greater, the higher the temperature applied and the longer the time of its action. The most important changes were observed after 15-30 minutes at 120°C and 130°C. A prolongation of the time of action of these temperature to 60 minutes, caused only small changes in trypsin inhibitor activity. After 15 minutes the lowering of activity depending on the temperature applied, was 45-57%. The prolongation of

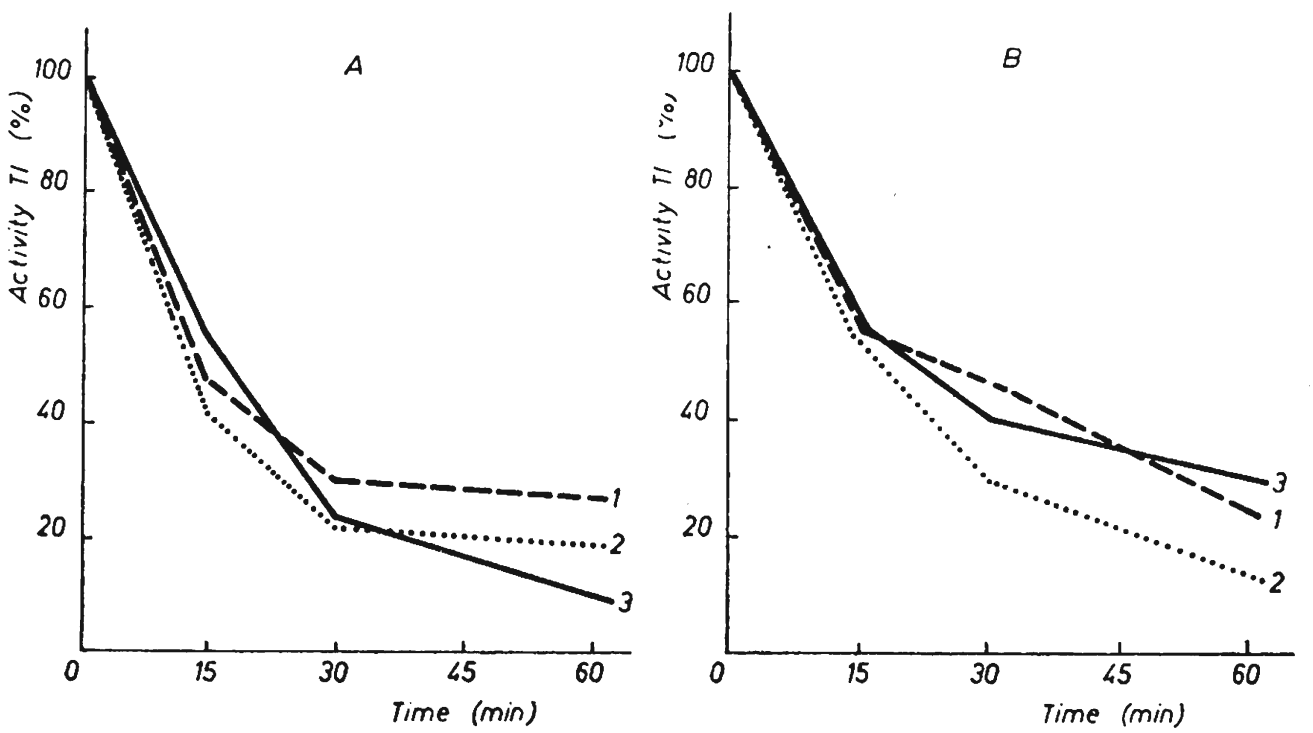

Fig. 2. Influence of the sterylization time on the trypsin inhibitor activity in bean seeds; A. Bean in water, B. Bean in salt solution; 1 — 120°C, 2 — 130°C, 3 —110°C

time from 15 to 30 minutes caused a 17-30% decrease of activity and from 30 to 60 minutes — by 2-13% only. The changes in trypsin inhibitor activity at 110°C had a different pattern: with the prolongation of sterilization time, a gradual decrease in activity was observed, and after 60 minutes, it exceede 90%.

The changes in trypsin inhibitor activity in the seeds after sterilization in salt solution (Fig. 2 B) were similar. The higher the temperature applied, the better the effect of inactivation of trypsin inhibitors. For example, after 60 minutes of sterilization at 110°C, the activity of trypsin inhibitors decreased by 71% at 120°C — by 74% and at 130°C — by 86% (Fig. 2 B).

The dependence between the time of sterilization and the level of trypsin inhibitors was slightly different. Unlike the preserves in water the trypsin inhibitor activity (Fig. 3) decreased with the prolongation of sterilization time. For example, at 130°C — a prolongation of time from 15 to 30 minutes, caused a 25% decrease in activity and from 30 to 60, by a further 16%.

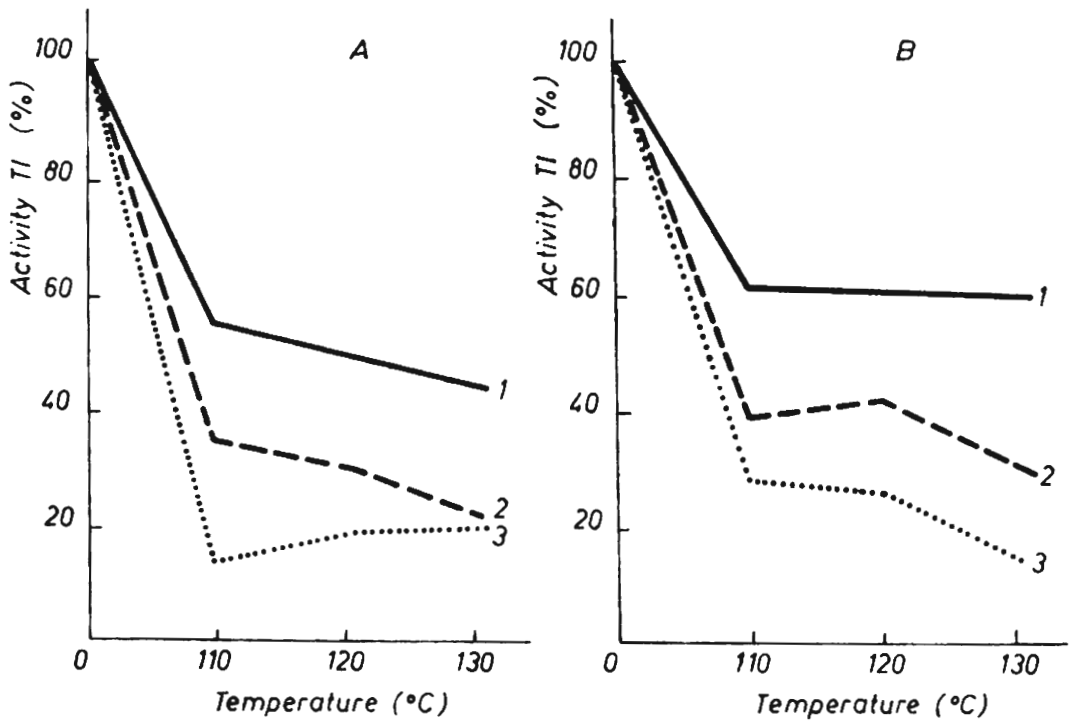

Fig. 3. Influence of the sterilization temperature on the trypsin inhibitor activity in bean seeds; A. Bean in water, B. Bean in salt solution; 1 — 15 min, 2 — 30 min, 3 — 60 min

A still smaller inactivation of trypsin inhibitors was obtained during the sterilization of cans with pea at 120°C for 30 minutes (Table 2). In this case the lowering of activity averaged 24%.

The results obtained differ much from those described in literature. After 1 h autoclave sterilization of pea, bean and horse bean seeds at 120°C, Olędzka et al. [8] observed a complete inactivation of trypsin inhibitors. Also Liener [5] reports that during 30-minute sterilization at 121°C, a complete inactivation of trypsin inhibitors in the seeds of leguminous crops takes place. Our experiments show that a complete inactivation of inhibitors is neither possible during cooking [1] nor during the sterilization proces. The reason for it may be the presence of the very temperature-resistant inhibitors of trypsin in the examined varieties of bean and pea. Taking into account the fact that the major par of pressures [9] are sterilized in industrial plants at 116°C for 25-35 minutes, it is

T a b l e 2. Changes in trypsin inhibitor activity in pea seeds under the effect of sterilization (120°C, 30 minutes)

Sample	TI Activity		
	TUI mg/DM	TUI mg/protein	%
Seeds of pea	2.10	8.99	100.00
Seeds of pea after sterilization:			
— in water	1.57	6.72	74.76
— in salt solution	1.64	7.02	78.10

possible to consider the possibility of prolongating the time of sterilization in order to obtain a better effect of inactivation of undesired trypsin inhibitors.

SUMMARY

The experiment which simulated the preparation of meals from pea and bean seeds in a traditional way pointed to a favourable effect of inactivation of trypsin inhibitors, expressed by their decrease to 90% except for sterilized pea. Such an effect, according to Liener [5] is sufficient for an optimal utilization of protein from edible crops by the human organism.

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Authors address: 10-957 Olsztyn-Kortowo

J. Borowska, H. Kozłowska

ZMIANY AKTYWNOŚCI INHIBITORÓW TRYPSYNY W NASIONACH FASOLI I GROCHU PODCZAS OBRÓBKI TERMICZNEJ

Instytut Inżynierii i Biotechnologii Żywności, AR-T, Olsztyn

Streszczenie

Przebadano wpływ procesu gotowania i sterylizacji na zmiany aktywności antytrypsynowej w nasionach fasoli Biała Wyborowa i Igołomska oraz grochu Kujawski Wczesny.

Stwierdzono, że zastosowane w doświadczeniu parametry gotowania i sterylizacji wywierają korzystny wpływ na obniżenie aktywności antytrypsynowej, aczkolwiek w żadnym wypadku nie uzyskano całkowitej inaktywacji inhibitorów.

Maksymalne obniżenie aktywności antytrypsynowej uzyskano po 60 min gotowania fasoli (pozostało 6% aktywności) i grochu (9% aktywności). Wykazano, że w czasie gotowania, część białka o właściwościach inhibujących przechodzi do roztworu. Moczenie nasion fasoli nie dało spodziewanego efektu szybszego spadku aktywności w procesie gotowania.

W przypadku sterylizacji maksymalne obniżenie aktywności (90%) uzyskano sterylizując nasiona fasoli w temp. 110°C przez 60 min.